POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA BIOLOGIAE EXPERIMENTALIS

FUNDATOR

KAZIMIERZ BIAŁASZEWICZ

VOL. XIX

PAŃSTWOWE WYDAWNICTWO NAUKOWE
WARSZAWA 1959

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Nakład 975 egz. Ark. wyd. 22,5 + 9 wkładek. Ark. druk. 22. Papier druk. sat. V kl. 70 g, 70 × 100. Oddano do składania w marcu 1959.
Druk ukończono w grudniu 1959.
Zam. nr 520 W-72 Cena zł. 68.—

CONTENTS

Acta Biologiae Experimentalis, Vol. XIX, 1959

THE DIGESTIVE MECHANISM OF GREEN PLANTS IN THE INGLUVIES AND GLANDULAR STOMACH OF *ANSER ANSER* L.

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(Received 5 October 1958)

It follows from works covering the problem of food digestion by birds in the anterior section of the alimentary tract (crop and glandular stomach) that they are devoted above all to the granivorous (pigeons and hens). There have been no investigations to explain the digestive mechanism of food in geese as birds whose diet may be largely supplemented with green plants. The object of the present paper is to explain the digestive mechanism of green plants as the basic food of geese.

MATERIAL AND METHODS

The object of the experiments was two groups of *Anser anser* L. of Pomorze breed numbering 11, and the continuous experiment continued for five months (from May 15 to October 15)*.

The experimental material was divided into two series: test and control. Both series of geese stayed outdoors during their lifetime, each separately enclosed. At night they were kept indoors, also separately. At definite intervals they were weighed in the morning before they were given the first food ration.

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* The first group of very weak geese, 11 in number, were received on May 14th and only 3 of them, marked K_1, D_1 and K_5 in the tables, survived. Another 8 specimens came from the same farm somewhat later. They were 19 days old and they were divided into two lots and kept on the diets obligatory for test and control geese respectively. In all tables showing the age of the geese, the first number stands for days of life in experimental conditions, while the second (in brackets) refers to the age of the goose from the moment of hatching.

Test series D (5 specimens). These geese stayed in a grass plot enclosed with wire net and their basic food consisted of green plants: *Lolium perenne* L. till July, and later on, till the end of the experiments, *Sonchus oleraceus* L., small amounts *Mycelis muralis* L., *Taraxacum officinale* Web. and *Galinsoga parviflora* Cav., as well as water. The geese of this series were given with their morning and evening rations of green plants anything between 5 to 60 g of wheat bran depending on the age of the goose. In addition to bran the very young geese were also given eggs, and meat and bone meal (see Table I).

Table I

The amount of food eaten by test geese

Symbol of goose	Age of goose	Food					Total
		Eggs	Wheat bran	Meat and bone meal	Green plants	Water	
	Days	g	g	g	g	ml	g
1	2	3	4	5	6	7	3+4+5
D_1	19	115	45	41	120	77	201
D_2	19(38)	—	95	21	300	193	116
D_3	55(74)	—	515	404	730	469	919
D_4	81(100)	—	1315	405	1170	701	1720
D_5	101(120)	—	2165	405	1700	945	2570

Control series K (5 specimens). This series of geese stayed all day long in a space enclosed with wire net and deprived of vegetation. The composition of their diet is shown in Table II. Because of a shortage of carotene in the diet which was shown in a very pale colour of the beak and legs, the geese of this series were given 12 doses of carrot juice varying from 28 to 70 ml. The geese were fed several times a day after the weight of various food ingredients was recorded.

Alongside of the continuous experiment carried out in the conditions described above, five 24-hour quantitative experiments were made. Two geese (one from the test and another from the control series) were used in each of these experiments, in which the geese were placed in large wooden boxes with glass panes on their bottoms so that it was possible to collect exactly all faeces from them. Both geese were weighed before the experiment and fed only on green plants during it. Also weighed were the plants given to the geese and the water they drunk in that time was measured. In the 24-hour experiments the geese were given food and water for the first 12 hours, while the other 12 hours was a period of rest.

Table II

The amount of food eaten by the control geese

Symbol of goose	Age of goose	Food													Total columns 3—13
		Eggs	Barley grit	Oatmeal	Wheat bran	Potatoes	Meat and bone meal	Cheese	Bread	Milk	Oats	Wheat	Green plants	Water	
	Days	g	g	g	g	g	g	g	g	g	g	g	g	ml	g
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
K_1	19	85	365	45	220	710	84	—	—	—	—	—	120	75	1509
K_2	19(38)	200	1060	250	704	2600	156	270	130	30	—	—	320	201	5400
K_3	55(74)	340	1610	550	2021	10625	311	300	1440	—	485	360	450	282	18042
K_4	81(100)	370	1700	550	3447	20325	311	475	1965	—	535	1225	455	543	30903
K_5	154	635	3160	370	5238	27455	1077	2200	2105	—	1233	3420	100	210	46893

In 24 hours both geese ate their fill of green plants with no water and were slaughtered, the first pair being given vapour of 14% urethan and the rest having their spinal cord cut.

After the plumage was removed the alimentary tract was taken out and the pH of the ingluvies* and glandular stomach measured. Content (leaves) was taken and slices of tissue cut out from the same sections. The regions from which plants and slices of tissue were taken are indicated in figures (Plates I, II and III). Slices of animal tissue and leaves were set in Regaud's fixative. Microtomic slices of animal tissue were stained with Delafield's and Mayer's haematoxylin and tinged with eosine, while slices of plant tissue were stained with ferrous haematoxylin after Regaud and tinged with light green.

THE INFLUENCE OF FOOD ON BODY WEIGHT

In both continuous and 24-hour experiments a principle was accepted in reference to all series of geese: their willingness to eat their fill was a measure of the amount of food and the frequency of feeding. Practically, the series of test geese (fed on green plants) ate almost without stopping, while the series of control geese had rather long spans of rest. This would prove that the rate at which food moves in the alimentary tract in both series of geese varies. The amount of food taken by the test geese during their lifetime is shown in Table I.

The figures in brackets in the second column are explained by a note given in „Methods". The figures given in the last column under „Total" indicate the whole amount of food eaten by each of the test geese in its lifetime in the conditions of the continuous experiment minus the amount of plants eaten and water drunk during the 24-hour experiment (Table I, columns 6 and 7).

The composition of the diet of the control geese and the amount of various ingredients eaten during their lifetime in the conditions of the continuous experiment minus the amount of green plants and water is shown in Table II.

An important fact follows from a comparison of the data given in Tables I and II — from the first days of the life of geese a considerable part of their diet may be replaced by suitable green plants. With growth and development the change in the diet may be so profitable that if the amount of food (minus green plants)

* This part of the alimentary tract was called — unechter Kropf — by Groebbels (1932), Kropf (in pigeons and hens) by Mangold (1929), and crop by Sturkie (1954).

eaten by the test geese (D) during their lifetime is expressed in a percentage of the amount of food eaten by the corresponding control geese (K) during their lifetime in the continuous experiment, the values for D will vary as follows: D_1 — 13%, D_2 — 2%; D_3 — 5%; D_4 — 6.5% D_5 — 6%. Consequently it follows that almost 95% of the diet of geese may be replaced with green plants from their 30th day of life on the condition that they are fed on

Table III

Gross body weight of geese determined at various ages

Test geese				Control geese			
Symbol of goose	Weighing date	Age of goose (days)	Gross body weight (kg)	Symbol of goose	Weighing date	Age of goose (days)	Gross body weight (kg)
1	2	3	4	5	6	7	8
D_1	15.V	1	0.15	K_1	15.V	1	0.14
	2.VI	18	0.38		2.VI	18	0.42
D_2	7.VII	19	0.45	K_2	7.VII	19	0.50
	26.VII	38	0.61		26.VII	38	1.21
D_3	7.VII	19	0.60	K_3	7.VII	19	0.84
	26.VII	38	0.69		26.VII	38	1.63
	7.VIII	50	0.95		7.VIII	50	1.75
	21.VIII	64	1.08		21.VIII	64	1.77
	31.VIII	74	1.08		31.VIII	74	1.77
D_4	7.VII	19	0.45	K_4	7.VII	19	0.69
	26.VII	38	0.61		26.VII	38	1.64
	7.VIII	50	1.06		7.VIII	50	1.99
	21.VIII	64	1.39		21.VIII	64	2.00
	4.IX	78	1.41		4.IX	78	2.06
	18.IX	92	2.05		18.IX	92	2.20
	26.IX	100	2.14		26.IX	100	2.35
D_5	7.VII	19	0.60	K_5	15.V	1	0.15
	26.VII	38	1.09		7.VI	23	1.61
	7.VIII	50	1.28		24.VII	70	2.45
	21.VIII	64	1.58		7.VIII	84	2.65
	4.IX	78	1.82		21.VIII	98	2.70
	18.IX	92	2.32		4.IX	112	2.82
	1.X	105	2.60		18.IX	126	2.85
	16.X	120	2.60		1.X	139	3.10
					16.X	154	3.18

this food from the first days of their life. This condition is warranted by the amount of green plants eaten by each of the control geese in the 24-hour experiments (cf. Table I, column 6, and Table II, column 14).

Also interesting is the amount of green plants eaten by the geese in various periods of life both in the test and control series of the 24-hour experiments. A distinct difference may be seen in geese D_3 and K_3 at the age of 74 days. A quantitative preponderance in the consumption of green plants which becomes distinct only in these geese might suggest that the plants eaten bring about changes in the alimentary tract to enable so considerable amounts of them to be eaten perhaps at the age of two months or somewhat earlier. These must be probably fairly large changes, since goose K_4 at the age of 100 days ate 39% of the plants eaten by goose D_4 (100 days), and goose K_5 at the age of 154 days ate in one day nearly 6% of the amount of plants eaten at the same time by goose D_5 at the age of 120 days. These changes are investigated in other parts of the present paper.

The replacement of valuable food with green plants and its effect on body weight increase is shown in Table III.

If follows from a comparison of body weights of the geese of both series that in the earlier periods of growth (up to $2^1/_2$ months) the test geese have smaller body weight than the control geese (compare the body weight of D_2 and K_2 as well as D_3 and K_3; Table III). The difference in body weight in favour of the control geese is expressed in a percentage of the body weight of the test geese: K_1 — 9.5%; K_2 — 49.5%; K_3 — 39%; K_4 — 8.9%; K_5 — 18.2%. The value is smaller if we take into account the initial body weight of the geese of the test series, which in three of the geese of series D is smaller than the initial body weight of the geese of the control series. The difference here is as follows: D_2 — 50 g; D_3 — 240 g; D_4 — 240 g. When analyzing the difference in body weight between geese D_5 and K_5, we have also to bear in mind the longer life of goose K_5 (the difference being 34 days). The larger body weight of the geese of the control series seems to be negligeable in comparison with the amount of food (minus green plants) eaten by the geese of both series. The phenomenon is even clearer if the amount of food eaten (minus green plants) is related to 1 kg of body weight increase, as shown in Table IV.

A comparison of the figures of columns 4 and 9 (Table IV) proves a steady increase in food taken per 1 kg of body weight increase which reaches its maximum in the geese 74 days old (D_3 and K_3). A decrease is seen in the series of control geese at the age of 100 days (K_4) and 154 days (K_5). In the test goose 120 days old (D_5) the amount of food eaten per 1 kg of body weight increase grows by 20.8% of the amount of food eaten by goose D_4 but it is 32.9% smaller in relation to the amount food eaten by goose D_3. The decrease in the amount of food eaten by goose D_2 is due to the change in the diet, for it is the first goose of the second group (see footnote in „Methods" and the column under „Total" in Table I).

Table IV

Amounts of food taken (minus green plants) per kg of body weight increase

Symbol of goose	Age of goose (days)	Total body weight increase (g)	Quantity of food eaten per 1 kg of body weight increase (g)	Increase in % in relation to goose D_1	Symbol of goose	Age of goose (days)	Total body weight increase (g)	Quantity of food eaten per 1 kg of body weight increase (g)	Increase in % in relation to goose K_1
1	2	3	4	5	6	7	8	9	10
D_1	19	230	874	100.0	K_1	19	280	5389	100.0
D_2	19(38)	160	725	69.6	K_2	19(38)	710	7606	253.6
D_3	55(74)	480	1915	208.7	K_3	55(74)	930	19400	332.1
D_4	81(100)	1690	1018	735.0	K_4	81(100)	1660	18014	592.9
D_5	101(120)	2000	1285	869.6	K_5	154	3030	15476	1082.1

The absolute body weight increase (Table IV, columns 3 and 8) grows steadily except in goose D_2, in which it amounted only to 160 g during 19 days of life in experimental conditions. This is undoubtedly connected with the change in the diet, the decisive bulk of which in this series of goose is made up by green plants. This interpretation is suggested by a considerable body weight increase during the same time in the „parallel" control goose K_2.

The results obtained seem to suggest that the food taken by the test geese, in which green plant prevail, may be assimilated just as well as other foods used before. Body weight increase in goose D_4 (100 days) is even somewhat larger than in control goose K_4 (100 days), which also bears out the conclusion that geese may feed almost exclusively on green plants suitably selected.

THE CYTOLOGICAL EXAMINATION OF CHANGES IN CELLS OF PLANTS REMOVED FROM THE INGLUVIES AND THE GLANDULAR STOMACH

The object of this part of the research is to explain with the help of histological methods whether and to what extent cellular elements of consumed plants (plasma, chloroplasts, cellulose membranes) undergo digestion.

In the cells of the epidermis of the ingluvies of goose D_1 (Plate I, Fig. A) remnants of plasma were preserved as granulation, while in goose K_1 plasma in the cells of this tissue was preserved whole (Plate I, Fig. B). The cells of the mesophyll preserved their plasma and chloroplasts in both geese. Remnants of plasma were preserved in few cells of the epidermis of the leaves from the glandular stomach. In goose D_1 (Plate I, Fig. A_2) some cells of the mesophyll preserved dark-stained plasma and chloroplasts of a varying intensity of stain; in goose K_1 (Plate I, Fig. B_2) all cells of this

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PLATE I

D_1 — Diagram of ingluvies and glandular stomach
K_1 — Diagram of ingluvies and glandular stomach
A — Cross-section of a *Lolium perenne* leaf from ingluvies of goose D_1 (magnified ca 150×)
B — Longitudinal section of a *Lolium perenne* leaf from the ingluvies of goose K_1 (magn. ca 120×)
A_2 — Cross-section of a *Lolium perenne* leaf from the glandular stomach of goose D_1 (magn. ca 230×)
B_2 — Cross-section of a *Lolium perenne* leaf from the glandular stomach of goose K_1 (magn. ca 110×)
C — Longitudinal section of a control *Lolium perenne* leaf (magn. ca 240×)
a — Cross-section of the ingluvies of goose D_1 (magn. ca 20×)
b — Cross-section of the ingluvies of goose K_1 (magn. ca 25×)
a_2 — Cross-section of the glandular stomach of goose D_1 (magn. ca 13×)
b_2 — Cross-section of the glandular stomach of goose K_1 (magn. ca 20×).

PLATE II

tissue preserved remnants of plasma and chloroplasts few of which are lightly stained and evenedged.

In the next pairs of geese (D_2, K_2; D_3, K_3) cytological changes in the leaves from the ingluvies and the glandular stomach are ever larger and in geese D_4 and K_4 four leaf tissues (upper and lower epidermis, palisade and spongy tissues) were examined in connection with the change of the plant, while plant material was taken from two sections of the ingluvies. The changes undergone by plant cellular material are shown in Plate II, Figs. A, A_1 and A_2 as regards goose D_4 and in Figs. B, B_1 and B_2 as regards goose K_4.

The largest cytological changes are observed in the plants removed from the ingluvies I and II and the glandular stomach of geese D_5 and K_5. In the upper epidermis from the ingluvies cells are plasmolyzed, but those in the lower epidermis near the spongy tissue more strongly so. In goose D_5 remnants of plasma usually situated near the cellulose membrane were preserved in few cells

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PLATE II

D_4 — Semi-diagramatic drawing of the ingluvies and glandular stomach of goose D_4

K_4 — Semi-diagramatic drawing of the ingluvies and glandular stomach of goose K_4

A — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose D_4 (magn. ca 120×)

B — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose K_4 (magn. ca 120×)

A_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose D_4 (magn. ca 100×)

B_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose K_4 (magn. ca 130×)

A_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose D_4 (magn. ca 80×)

B_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose K_4 (magn. ca 90×)

C — Cross-section of a control *Sonchus oleraceus* leaf (magn. ca 100×)

a — Cross-section of ingluvies I of goose D_4 (magn. ca 25×)

b — Cross-section of ingluvies I of goose K_4 (magn. ca 25×)

a_1 — Cross-section of ingluvies II of goose D_4 (magn. ca 30×)

b_1 — Cross-section of ingluvies II of goose K_4 (magn. ca 25×)

a_2 — Cross-section of the glandular stomach of goose D_4 (magn. ca 15×)

b_2 — Cross-section of the glandular stomach of goose K_4 (magn. ca 10×)

of the lower epidermis. In the palisade tissue of the leaves from ingluvies I most cells are slightly plasmolyzed near the spongy tissue; chloroplasts situated near the cellulose membrane were preserved in only few cells in ingluvies II of goose D_5; they varied in colour (dark and light) and only an outline remained of some. In ingluvies II of goose K_5 dark brown plasma fills up the whole of many cells and there are also cells in which yellow granulations occupy a central position. Chloroplasts dark and distinct. Cells of the spongy tissue of the leaves from ingluvies II of goose K_5 preserved remnants of plasma as granulation, while distinct chloroplasts either take up the inside of the cell or are situated near the cellulose membrane. The cytological changes are shown in the respective drawings (Plate III, Figs. A, A_1 and B, B_1).

The largest changes in cells were observed in the leaves taken from the glandular stomach.

Glandular stomach D_5

Upper epidermis. Remnants of plasma preserved in the form of yellow-stained granulations.

Lower epidermis. The picture of cells as in the above tissue.

Palisade tissue. Remnants of plasma in the form of yellowish-stained granulations have preserved in few cells. Similarly, dark-stained chloroplasts have preserved in few cells. Most chloroplasts are colourless, light and jagged.

Spongy tissue. The picture of cells as in the tissue above.

Glandular stomach K_5

Upper epidermis. In many cells plasma completely preserved, dark-brown and usually plasmolyzed near the palisade tissue. In others plasma partly preserved, light, yellow and containing much granulation.

Lower epidermis. The picture of cells as in glandular stomach D_5.

Palisade tissue. In all cells preserved plasma fills up the whole or part of the inside of the cell. It is usually light, slightly-stained, with no clear-cut patches of colour. Borders of light-stained plasma terminating with granulations. Chloroplasts distinctly black-stained, situated usually near the cellulose membrane.

Spongy tissue. The picture of cells as in the above tissue, yet there are more cells with a light centre. Remnants of dark-stained plasma usually situated near the cellulose membrane (cf. Plate III, Fig. A_2 B_2).

The analysis of the results of cytological changes undergone by the cells of various plant cells in the ingluvies and glandular

stomach should deal first with plasmolysis and the varying colouration of plant plasma.

On the one hand the plasmolysis of cells in various tissues of the leaf proves the penetration inside the leaf of the juice secreted by the animal, while the varying degree of plasmolysis in various tissues of the leaf indicates the place of the penetration of ingluvies juice inside the leaf.

The comparison of the degree of plasmolysis of plant cells in various geese shows a certain regularity consisting in that cells of the lower epidermis undergo plasmolysis first. The plasmolysis of cells of this tissue is one-sided and occurs usually near the spongy tissue and seldom on the side of the adjoining cells. The examination of this direction of plasmolysis in many plant preparations bears out the opinion that the penetration of ingluvies juices inside the leaf is effected through the interstices in the leaf and not through the cuticle.

The spongy tissue is another tissue whose cells undergo quick and strong plasmolysis. This process naturally depends on the species of the plant, its vegetative period and the age of the goose.

The plasmolysis of cells of the upper epidermis occurs near the palisade tissue. This seems to suggest that after penetrating inside the leaf through the interstices, ingluvies juice fills up intercellular spaces in the spongy tissue, while the weaker plasmolysis of cells of the upper epidermis in comparison with that of cells of the two previous tissues would indicate a certain difficulty in the passage of juice through the layer of the palisade tissue. This interpretation seems right as regards *Lolium perenne* but not *Sonchus oleraceus* (compare the arrangement of mesophylle cells in *Lolium perenne* with that of palisade tissue cells in *Sonchus oleraceus*). The weaker plasmolysis of cells of the upper epidermis in *Sonchus oleraceus* may result from the fact that the concentration of ingluvies juice is only somewhat higher than that of the juice of cells of this tissue of the leaf. As however the plasmolysis of these cells occurs above all near the palisade tissue and only to a small extent or not at all (in older geese) near the adjoining cells, this would suggest that the cuticle of the upper epidermis too is impermeable for ingluvies juice.

Cells of the palisade tissue undergo weaker and later plasmolysis in comparison with cells of other tissue. This is particularly

distinct in older geese fed on *Sonchus oleraceus,* and would suggest that the accumulation of a considerable amount of products of photosynthesis in this leaf tissue may increase in these cells the content of osmotically active bodies which counteract the plasmolyzing effect of ingluvies juice.

It follows from the microscopic picture of leaf tissues that the plasmolysis of cells in various tissues proceeds as follows: lower epidermis and spongy tissue → upper epidermis → palisade tissue.

The varying colouration of plasma is another feature of plant cells coming from the ingluvies and glandular tissue. As one fixative and the same staining method were applied to all plants, the changes in the intensity and colouration of plasma indicate some changes taking place in them.

The plasma of cells of the upper epidermis (in *Sonchus oleraceus*) stains comparatively most intensely, almost black. The plasma of cells of the palisade tissue stains just as strongly or somewhat less so. The plasma of some cells of the lower epidermis and spongy tissue may stain just as black, depending on the section of the alimentary tract from which the leaf has been taken out. However, more often plasma is not evenly stained in the last two tissues. In many cells it is clearer at the very centre of the cell or disappears there completely. Cells may also frequently be found in which the intensity of colouration varies from very dark to ever lighter till it disappears. This proves the evanescence of plasma, which cannot be explained otherwise than by the decomposition of plasma inside the plant cell. It follows from the microscopic observation of a considerable number of plant tissues that the rate of changes in the intensity of colouration of plasma terminating with its evanescence depends on the species of the plant eaten, its vegetative period, the tissue of the leaf, its physiological condition, the degree to which the ingluvies and glandular stomach are filled with plant material and the function of the examined sections of the alimentary tract changing with the age of the goose.

All the changes observed in the plasma of leaf cells affecting the degree of plasmolysis, the intensity and evanescence of colouration become even more interesting as it was impossible to observe any changes whatsoever in the cellulose membranes with the help of the staining methods applied in the present investigation. Thus

Table V

Dimensions of various tissues and glands in the ingluvies and glandular stomach (in microns)

Symbol of goose	Section of alimentary tract	Lamina epithelialis	Lamina propria mucosae	Long axis of glands	Short axis of glands	Submucosae	Long axis of glands	Short axis of glands
	1	2	3	4	5	6	7	8
D_1	ingluvies	269.1(150.8—336.4)	132.4(46.4—203.0)	219.6(156.6—319.0)	93.1(75.4—121.8)			
	glandular stomach					377.0(220.4—510.4)	1683.9(1357.2—2204.0)	1212.2(916.4—1624.0)
D_2	ingluvies	357.1(301.6—394.4)	235.3(110.2—359.4)	223.5(121.8—522.0)	127.6(52.2—232.0)			
	glandular stomach					324.1(174.0—487.2)	2706.7(2436.0—2958.0)	803.9(719.2—928.0)
	ingluvies I	359.6(232.0—429.2)	141.4(58.0—266.8)	258.1(139.2—365.4)	130.5(58.0—208.8)			
D_3	ingluvies II	400.2(365.4—440.8)	162.2(46.4—348.0)	353.8(220.4—464.0)	130.0(92.8—208.8)			
	glandular stomach					406.0(324.8—464.0)	1317.1(568.4—2146.0)	889.2(406.0—1160.0)
	ingluvies I	426.9(290.0—487.2)	220.4(139.2—371.2)	356.7(266.8—417.6)	236.4(174.0—348.0)			
D_4	ingluvies II	311.9(243.6—452.4)	191.4(139.2—290.0)	246.6(150.8—290.0)	162.0(69.6—208.0)			
	glandular stomach					520.2(406.0—580.0)	966.4(348.0—1357.2)	829.9(266.8—1044.0)
	ingluvies I	315.1(255.2—406.0)	172.8(92.8—243.6)	256.5(197.2—301.6)	109.6(58.0—162.4)			
D_5	ingluvies II	228.8(121.8—423.4)	141.8(81.2—232.0)	246.4(220.4—313.2)	143.6(81.2—185.6)			
	glandular stomach					777.2(696.0—1044.0)	804.9(672.8—870.0)	1111.2(951.2—1600.8)
	ingluvies	279.2(208.8—348.0)	191.4(34.8—290.0)	226.2(133.4—284.2)	138.5(92.8—208.8)			
K_1	glandular stomach					404.3(290.0—696.0)	999.0(603.2—1368.8)	640.5(503.6—1009.2)
	ingluvies	458.8(371.2—580.0)	206.7(34.8—371.2)	289.0(220.4—394.4)	168.2(116.0—232.0)			
K_2	glandular stomach					492.6(371.2—580.0)	1693.3(754.0—2412.8)	887.1(394.4—1055.0)
	ingluvies I	394.1(332.6—460.2)	243.3(174.0—394.4)	327.5(208.8—406.0)	153.5(58.0—185.6)			
K_3	ingluvies II	334.1(208.8—452.4)	193.7(116.0—255.2)	258.4(162.4—336.4)	145.6(69.6—185.6)			
	glandular stomach					616.3(464.0—742.4)	1139.4(638.0—1531.2)	961.5(812.0—1102.0)
K_4	ingluvies I	192.9(116.0—255.2)	278.4(174.0—522.0)	374.1(232.0—591.6)	145.0(81.2—255.3)			
	ingluvies II	304.8(192.5—436.1)	281.9(139.2—417.6)	230.1(174.0—348.0)	135.3(116.0—174.0)			
	glandular stomach					603.2(475.6—812.0)	1697.1(1044.0—2552.0)	998.8(812.0—1160.0)
K_5	ingluvies I	200.3(155.6—266.0)	360.9(104.4—696.0)	202.7(127.6—255.2)	100.1(58.0—197.2)			
	ingluvies II	152.3(45.0—253.8)	537.1(174.0—928.0)	223.0(116.0—348.0)	116.0(58.0—174.0)			
	glandular stomach					705.0(580.0—812.0)	986.9(812.0—1123.2)	844.6(475.6—986.0)

the evanescence of plasma in cells combined with the absence of lesions or other changes in the cellulose membranes may warrant a supposition of intra-cellular digestion probably stimulated by the juice of the ingluvies or glandular stomach.

HISTOLOGICAL CHANGES IN THE INGLUVIES AND GLANDULAR STOMACH

Fragments of the ingluvies and glandular stomach were taken out of geese slaughtered at a definite time (see „Methods"). The places where tissue was cut from were marked in Plates I, II and III (small letters in white circles). One slice of tissue was cut from each of the ingluvies of the young geese D_1 and K_1 (Plate I) and D_2 and K_2, two, from two places each: ingluvies I (*anterior*) and ingluvies II (*posterior*), situated closer to the glandular stomach, of older geese, and only one from the glandular stomach of all geese.

The distribution of various tissue layers in the ingluvies and glandular stomach is illustrated by the enclosed diagrams.

It follows from the comparison of the changes undergone by the ingluvies during the development of the geese of series D and K that the corrugation of the internal layers of the ingluvies differs in both series and changes during the period of the experiment. While the folds are numerous, dense and situated near each other in goose D_1, they are lower in D_2, while in the anterior section (ingluvies I) of D_3 they are just as large as in D_1 and in the posterior section (ingluvies II) of the same goose they are larger than in the anterior section. The height of the folds and the intervals between them in the anterior section of goose D_4 are like those in the anterior section of goose D_3, while in the posterior section they are only slightly outlined and their outline is accentuated by the development of the *lamina muscularis mucosae* more prominent here. Folds of varying height may be observed in the anterior section of goose D_5; they are far higher in the posterior section of it than in the posterior section of D_4. On the whole it could be said that in the test geese the development of the folds underwent considerable changes in the posterior section only, while they were small in the anterior section in comparison with what was observed in D_1.

In control geese K_3 the folds in both sections of the ingluvies are slightly outlined; they are somewhat more developed in the

anterior section and still more in the posterior section of K_4. Distinct yet still not very high folds occur in the anterior section, and higher ones in the posterior section of K_5.

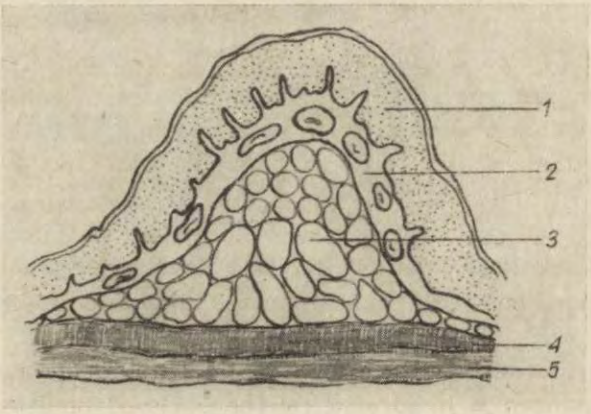


Diagram 1. Cross-section of the ingluvies

1 — *lamina epithelialis (l. ep.)*
2 — *lamina propria mucosae (l. pr. muc.)*
3 — *lamina muscularis mucosae (l. musc. muc.)*
4 — *lamina muscularis interna (circularis) (l. musc. int. circ.)*
5 — *lamina muscularis externa (longitudinalis) (l. musc. ext. long.)*

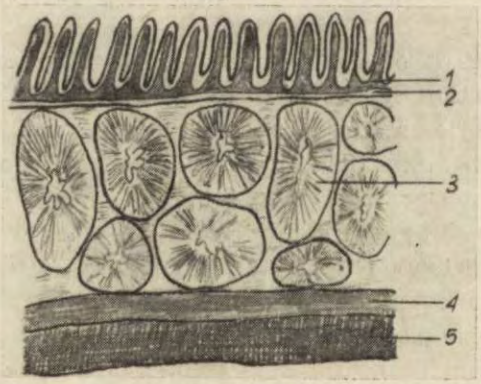


Diagram 2. Cross-section of the glandular stomach

1 — *glandulae mucosae (gl. muc.)*
2 — *muscularis mucosae (musc. muc.)*
3 — *glandulae submucosae (gl. submuc.)*
4 — *lamina muscularis interna (longitudinalis) (l. musc. int. long.)*
5 — *lamina muscularis externa (circularis) (l. musc. ext. circ.)*

On comparing the development of the folds in the ingluvies of both goose series one might say that on the whole many and fairly high folds are preserved in the anterior section in geese fed

with green plants (series D), while they are reduced in the posterior section. As regards the geese of the control series (K), they folds disappear in both sections already in goose K_3, they are underdeveloped in the anterior section and well-developed in the posterior section in older geese (K_4 and K_5).

The height of the ingluvies folds and their distance from each other (density) are reflected in the histological changes in various layers making up the ingluvies.

In order better to characterize the various layers of this section of the alimentary tract, measurements were taken of their thickness as well as the number and size of the glands. These data are shown in Table V.

The histological changes in the ingluvies affect first of all the development of the *lamina propria mucosae*, as the layer where the glands develop whose function is connected with the food taken.

With the growth of the test geese (serie D) their *lamina propria mucosae* develops more intensely for 81 days (D_4) and diminishes later on (D_5).

The development of this tissue is different in the control geese (K). It is observed to develop slightly with the growth of the geese from K_1 to K_3. In goose K_4 the tissue develops somewhat better in ingluvies II than in ingluvies I; while in goose K_5 the *lamina propria mucosae* in ingluvies I is twice, and in ingluvies II almost four times, as thick as in the anterior section of the ingluvies of goose D_5. It is true that goose K_5 was 34 days older than D_5 when slaughtered, yet the difference in the thickness of this tissue in ingluvies I and II in geese D_4 and K_4 would indicate that the changes in its development are caused by different sorts of food taken by both series of geese.

The number of glandular layers is related to the development of the *lamina propria mucosae*. Only one layer of glands is present in all test geese, while in the control geese two layers occur already in ingluvies II in K_4. Two occur in ingluvies I of K_5 and sometimes even three in ingluvies II of the same goose. The goose diet consisting of green plants reduces the number of glandular layers and the *lamina propria mucosae*.

The thickness of the *lamina propria mucosae* and the number of glandular layers in this tissue are related to the size of the glands (for the dimensions of the long and short axes of the glands see Table V, columns 4 and 5).

It follows from a comparison of the dimensions of the glands in the ingluvies of the test geese that the long axis of the glands hardly changes in the first two geese (D_1 and D_2) but their short axis becomes longer (D_2). The long axis is considerably longer in D_3 than in D_2 and D_1. The average dimensions of the long axis in ingluvies II of goose D_3 are 100 μ larger than in ingluvies I, the average dimensions of the short axes being the same (130 μ). The reverse is true of goose D_4: the average dimensions of the long and short axes in ingluvies I are considerably larger than in ingluvies II. In the oldest test goose (D_5), the average dimensions of the longer axes of the glands are almost the same in both sections of the ingluvies and only the dimensions of their short axes in ingluvies II are larger than in ingluvies I. If we assume that the average dimensions of the glands reflect their secretive function as reaction to the food taken, it follows from the present comparisons that their function in ingluvies I is reduced after 80 days (D_4) of feeding on green plants and in ingluvies II already after 55 days.

PLATE III

D_5 — Original drawing of the ingluvies and glandular stomach of goose D_5 ($^1/_3$ of the normal size)
K_5 — Original drawing of the ingluvies and glandular stomach of goose K_5 ($^1/_3$ of the normal size)
A — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose D_5 (magn. ca 110×)
B — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose K_5 (magn. ca 100×)
A_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose D_5 (magn. ca 60×)
B_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose K_5 (magn. ca 100×)
A_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose D_5 (magn. ca 80×)
B_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose K_5 (magn. ca 110×)
C — Cross-section of a control *Sonchus oleraceus* leaf (magn. ca 120×)
a — Cross-section of ingluvies I of goose D_5 (magn. ca 20×)
b — Cross-section of ingluvies I of goose K_5 (magn. ca 20×)
a_1 — Cross-section of ingluvies II of goose D_5 (magn. ca 25×)
b_1 — Cross-section of ingluvies II of goose K_5 (magn. ca 15×)
a_2 — Cross-section of the glandular stomach of goose D_5 (magn. ca 15×)
b_2 — Cross-section of the glandular stomach of goose K_5 (magn. ca 15×)

PLATE III

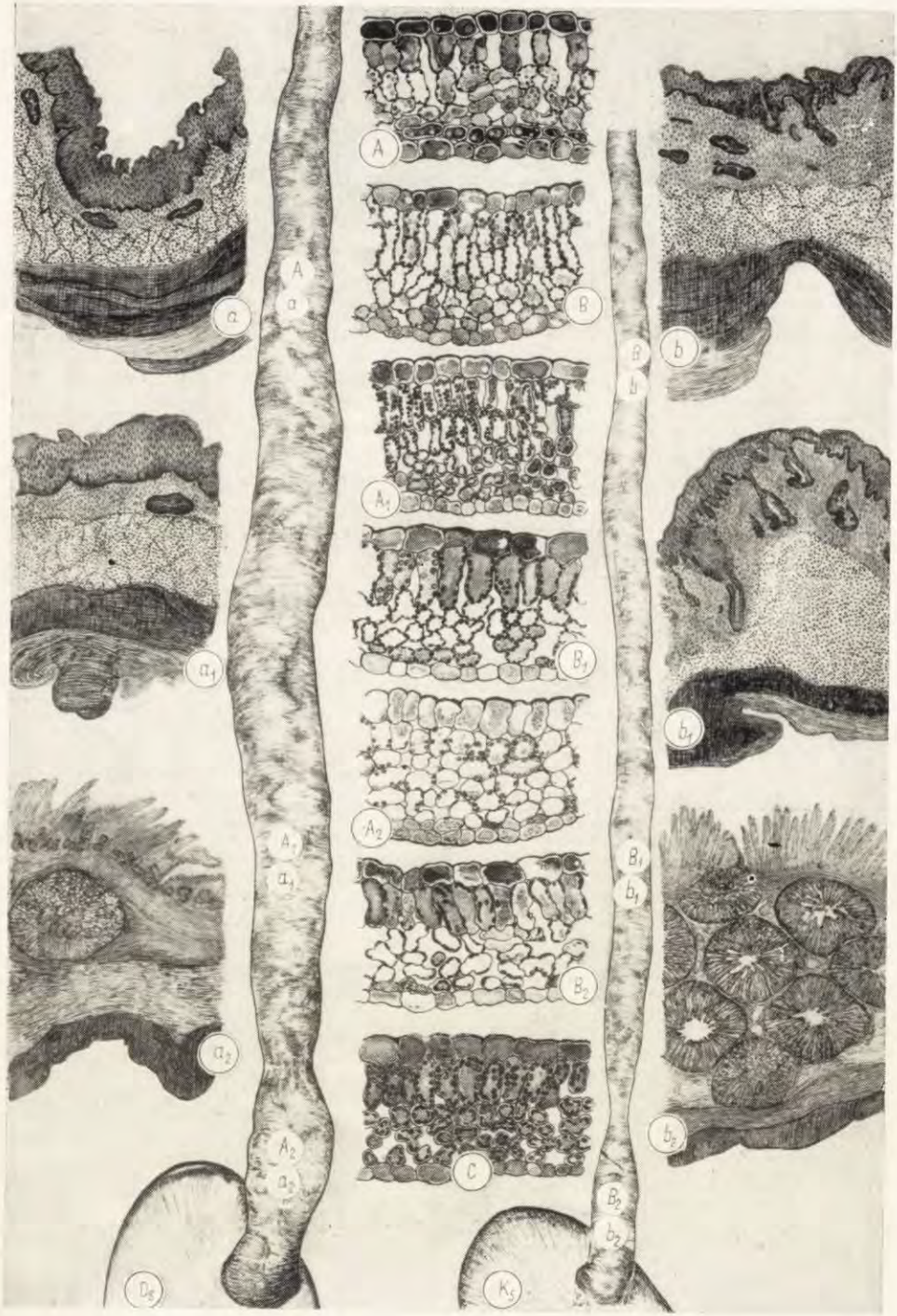
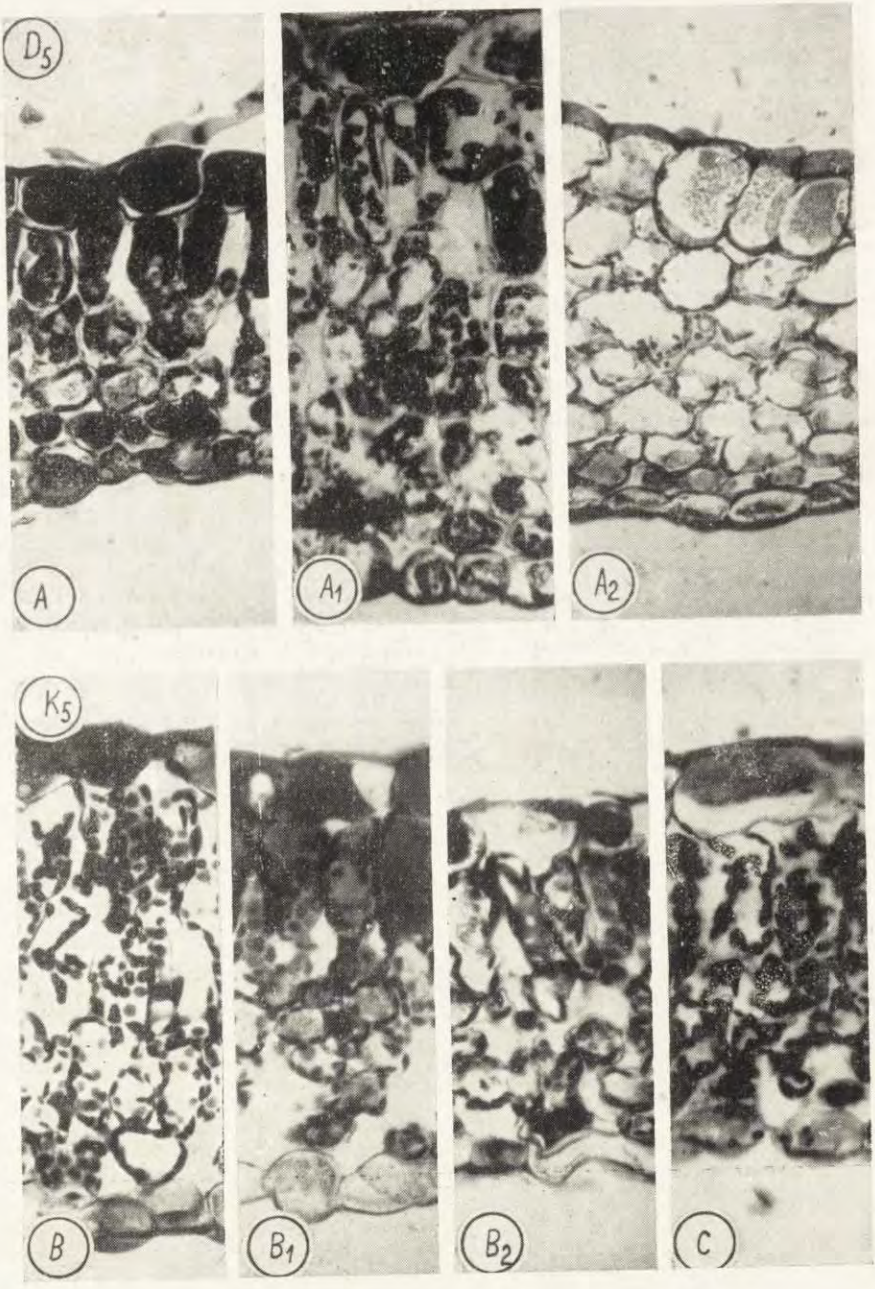


PLATE IV



The function of the ingluvies glands is indicated not only by the dimensions and number of layers but also by the percentage of glands in which efferent ducts were found in histological preparations. This figure gives some idea not only of the degree of functional development (or, more strictly, functional condition) but also of the density of pores on the surface. Calculations made in this respect show a reduction of the secretive function of the glands in ingluvies I (23%, 17%, 21%) in older geese (D_3, D_4, and D_5) in comparison with D_1 and D_2 (35%, 39%); on the other hand, a gradual increase of this function may be observed in ingluvies II in geese D_3, D_4 and D_5 (17%, 22%, 36%).

These relations are somewhat different in the geese of the control series (K). It may roughly be assumed that the number of glands with efferent ducts is almost constant in ingluvies I of geese K_3, K_4 and K_5 (11%, 18%, 15%) but far lower than in K_1 and K_2 (39%, 23%), while the number of glands with efferent ducts in ingluvies II of the control geese more than 55 days old (K_3) is almost stabilized (K_3 — 21%, K_4 — 23%, K_5 — 25%).

The development of the *lamina propria mucosae*, the number of glandular layers in that tissue, the size of ingluvies glands in the test geese all indicate that the diet consisting of green plants hampers the development of the glands more in the anterior than in the posterior section of the ingluvies.

The control geese do not show so great changes in this respect; the development of the glands in the anterior part of the ingluvies is also largely reduced after two months, while the glands of the

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PLATE IV

D_5 A — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose D_5 (magn. ca 320×)

D_5 A_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose D_5 (magn. ca 320×)

D_5 A_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose D_5 (magn. ca 320×)

K_5 B — Cross-section of a *Sonchus oleraceus* leaf from ingluvies I of goose K_5 (magn. ca 320×)

K_5 B_1 — Cross-section of a *Sonchus oleraceus* leaf from ingluvies II of goose K_5 (magn. ca 320×)

K_5 B_2 — Cross-section of a *Sonchus oleraceus* leaf from the glandular stomach of goose K_5 (magn. ca 320×)

C — Cross-section of a control *Sonchus oleraceus* leaf (magn. ca 320×)

second section show a tendency towards a certain stabilization related to a greater number of glandular layers in ingluvies II (K_4 — 2; K_5 — 3 layers) and smaller dimensions of the glands.

The changes in the glandular stomach caused by the diet of the geese affect all tissues examined in the present investigation in a varying degree.

A constant increase in the thickness of the *lamina propria mucosae* may be observed in the geese of the test series from D_2 to D_5; the same tissue develops at first more intensely in the control geese (Table V, column 6; compare D_2 with K_2, D_3 with K_3, D_4 with K_4) than in the test geese, yet the thickness of this tissue in K_3 and K_4 is almost the same. A greater increase in the thickness of this tissue is observed in goose K_5 in comparison with K_4, but it is thinner than in goose D_5.

The development of this tissue in both series of geese is related to the development of the *glandulae mucosae* between the folds and to that of the layer under it: the *muscularis mucosae*.

The *glandulae mucosae* are distinct in the geese in which the folds do not adhere to each other; in those cases their bottoms may often be observed ampoule-like extended at the bases of the folds. Glands irregular in shape (probably the bottoms of the glands) may be observed — perhaps due to the strong cohesion of the folds — in goose D_5; they are situated irregularly, sometimes in two layers on the surface, or somewhat higher above the surface of the *muscularis mucosae*, well developed in this case (distinct in Plate III, Fig. a_2, less distinct in Plate V, Phot. a_2).

It is observed that the thickness of *muscularis mucosae* is steadily growing in the test geese and reaches the largest dimensions in geese D_5. The growth of this tissue in the control geese is far slower than in the test geese, as is particularly distinctly suggested by a comparison of D_5 with K_5 (Plate III, Fig. a_2 b_2; Plate V, Phot. a_2 b_2).

The *glandulae submucosae* undergo great changes in the glandular stomach. It follows from a comparison of the figures standing for the dimensions of the long and short axes of the glands in the geese of the test series (Table V, columns 7 and 8) that from goose D_2 on the glands show a tendency for the long axis to become shorter with almost the same width. A certain increase in the length of the short axis of the glands in D_5 is due to their dif-

ferent arrangement (cf. Plate I, Fig. a_2, Plate II, Fig. a_2, Plate III, Fig. a_2).

Except for goose K_5 the *glandulae submucosae* are always larger in the „parallel" control geese (see Table V, column 7 and 8).

The most essential and important difference in the glandular stomach of the test and control geese may be seen in the number of layers of *glandulae submucosae*. Test geese D_1, D_2, and D_3 and control geese K_1, K_2 and K_3, corresponding to them, have each two layers of glands, but in goose D_4 (Plate II, Fig. a_2) many of the glands of the second layer are underdeveloped and show a tendency to evanescence, while in the „parallel" goose K_4 (Plate II, Fig. b_2) there are three layers of well-developed glands. The evanescence of the *glandulae submucosae* is most distinct in goose D_5 (Plate III, Fig. a_2; Plate V, Phot. a_2), in which the number of glandular layers has been reduced to one, while an additional fourth glandular layer (Plate III, Fig. b_2; Plate V, Phot. b_2) developed in the „parallel" control goose (K_5 — one month older than D_5).

The reduction and evanescence of the *glandulae submucosae* in the geese fed on green plants and an intense development of these glands in the control geese is probably caused by the diet; the time in which these changes occurred is noteworthy. Four months seems to be long enough for the food taken to cause so great changes in the tissue, which might appear to have been firmly established for many generations.

A comparison of the development of the various tissues and especially the glands in the ingluvies and glandular stomach points out the important fact that suitably selected green plants, which are the essential and dominant diet of *Anser anser* L., cause a more intense development of the glands in the second section of the ingluvies than in the first; the fuller development of the *glandulae mucosae* in the glandular stomach and the reduction of the *glandulae submucosae,* which would indicate the atrophy of the function.

MORPHOLOGICAL CHANGES IN THE INGLUVIES AND GLANDULAR STOMACH

The food taken by the geese in the continuous experiment (cf. Table I, column 8, and Table II, column 16) does not include green plants taken by the respective pairs of geese during the 24-hour

experiment. The amount of green plants eaten is given in figures in Table I, column 6 for the test geese, and those in Table II, column 14 for the control geese. A comparison of those figures shows that the test geese take increasing amounts of green plants as they grow, while of the control geese only K_1 and K_2 take the same amount of green plants as D_1 and D_2, and K_3 takes only 62%, K_4 39%, and K_5 6% of the amounts taken by D_3, D_4 and D_5 respectively.

The fact of eating so large amounts of green plants is accounted for on the one hand by the development of various tissues of the ingluvies and probably by the function of the glands of this section of the alimentary tract and the reduction of the *glandulae submucosae* and the development of the *glandulae mucosae*, and on the other it also causes far-reaching morphological changes of the ingluvies and glandular stomach, and probably also of other sections of the alimentary tract (cf. Plate VI, Phot. D_5 K_5), but no correlation between this fact and the development of the *laminae muscularis interna* and *externa* can be established. The final morphological changes in the oldest geese are illustrated by the measurements contained in Table VI.

The measurements of the examined sections of the alimentary tract largely supplement the histological examinations. They show that the diet composed of green plants causes not only the elongation of the ingluvies and the glandular stomach and an increase in their diameters, but also a considerable elongation (up to 30%) of the whole alimentary tract. The latter fact would not be unusual — since it is well known that higher herbivorous animals have a longer alimentary tract than carnivora and omnivora — if it were not for the very short time in which all the observed changes occurred.

PLATE V

D_5 a — Cross-section of ingluvies I of goose D_5 (magn. ca 55×)
D_5 a_1 — Cross-section of ingluvies II of goose D_5 (magn. ca 55×)
D_5 a_2 — Cross-section of the glandular stomach of goose D_5 (magn. ca 55×)
K_5 b — Cross-section of ingluvies I of goose K_5 (magn. ca 55×)
K_5 b_1 — Cross-section of ingluvies II of goose K_5 (magn. ca 55×)
K_5 b_2 — Cross-section of the glandular stomach of goose K_5 (magn. ca 40×)

PLATE V

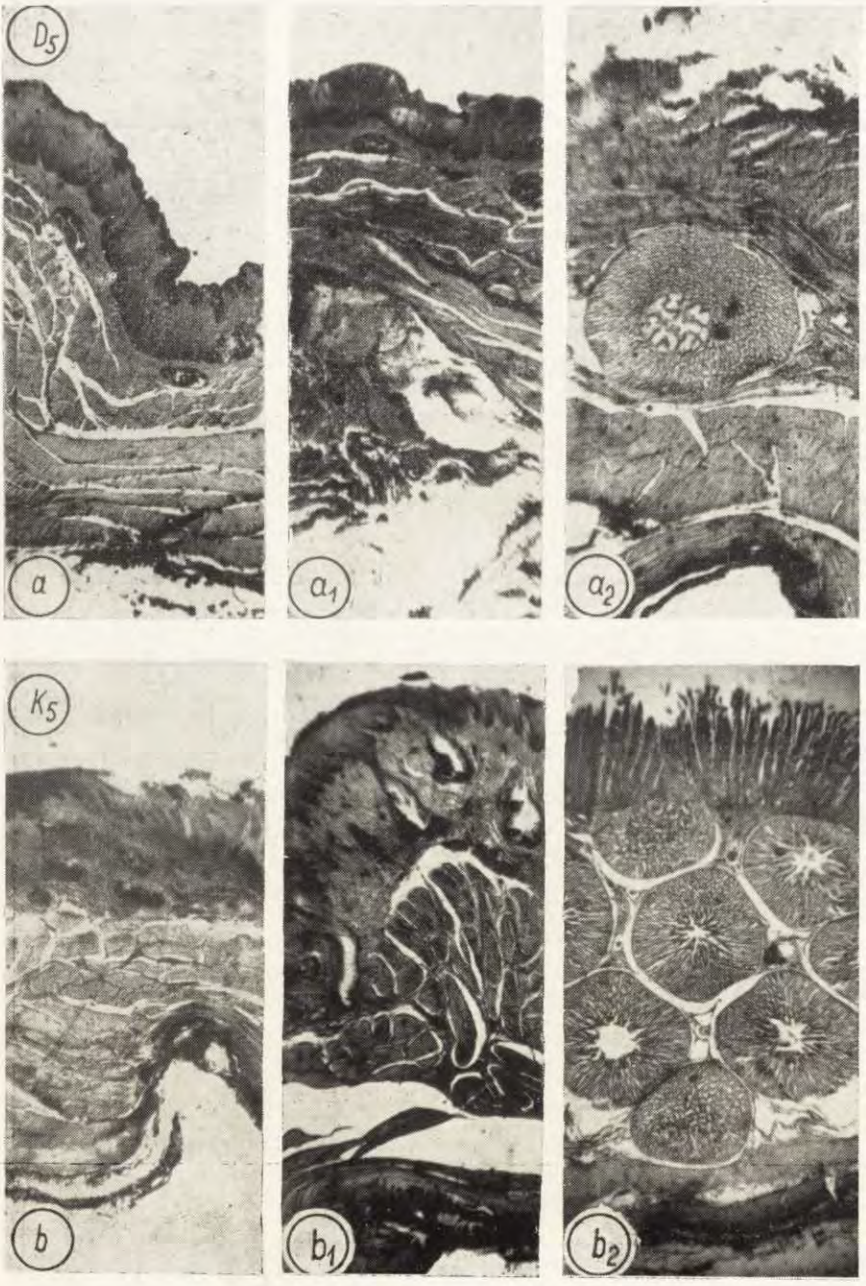

PLATE VI

DISCUSSION

The results of the experiments seem to confirm the thesis that the diet of geese may consist exclusively of green plants, but the (gross) body weight increase (Table IV, columns 3 and 4) in a given period of their life is always larger in all control geese except goose D_4. The same is illustrated by the body weight increase expressed in per cents in relation to the first geese of each series D_1 and K_1 (Table IV, columns 5 and 10). This would prove that the diet consisting of green plants is either insufficient as food or is utilized in too small a degree, particularly by the first three geese (D_1, D_2 and D_3). Only D_4 shows some increase when compared with K_4. This fact calls for the determination of the increase in individual geese not for the whole of their life in the conditions of the continuous experiment but of an average increase in every ten days. The results are illustrated by the data in Table VII.

In the light of these data a considerable body weight decrease occurs in goose D_2 and a fair increase in goose K_2, which is explained by the changed diet (see footnote in „Methods"). The considerable decrease in goose K_3 may be explained by the presence of parasite in the intestine, from goose D_4 on the decade body weight increase in this series equals that in the control geese. The results obtained would prove that the utilization of green plants is more intense in older geese (D_4 — 81 days) on the condition that green plants have been their essential diet from the first days of their life. This condition is confirmed by the results of the research carried out by E. S. Snyder and his fellow-workers (1955), who found in a 14-week experiment no distinct difference in body weight increase in geese fed on food containing no green plants and those taking mixed food (the difference in favour of the geese kept outside pasture was 0.44 kg). The experiments made show that only after 80 days of feeding on green plants the decade body weight increase is levelled in both series of geese. A slightly

PLATE VI

D_5 — In situ photograph of the glandular stomach and part of the ingluvies (dim. ca 1.6×)

K_5 — In situ photograph of the glandular stomach and part of the ingluvies (dim. ca 1.6×)

lower body weight increase in goose D_5 in the periods examined might be caused by the vegetative period of the leaves (October).

A larger body weight increase expressive of the assimilation of the plants taken is undoubtedly due to the histological changes discussed in part III of the present paper. These changes depend on the time of feeding on green plants and are distinctly revealed only in D_3 (81 days of life in the conditions of the continuous experiment).

The amount of green plants taken by geese D and K during 24-hour experiments must be considered together with the amount of water drunk at the same time. The latter ingredient of the diet must, in some way, be related to the food taken. The large

Table VI

Length of the ingluvies, glandular stomach and whole alimentary tract in the oldest geese

Symbol of goose	Section of alimentary tract	Length in cm	Width in cm	Difference in % between D_5 and K_5	
				Length	Width
D_5	ingluvies	43	1.9; 3.7*	7	32; 59
	glandular stomach	9.5	3.2	21	44
	whole tract	368.5		28	
K_5	ingluvies	40	1.3; 1.5*		
	glandular stomach	7.5	1.8		
	whole tract	265.5			

* The second measurement taken in the middle of the ingluvies.

amount of water, almost equalling body weight, taken by the test geese in a day is amazing. For better illustration the data on the amount of water drunk and taken with food are presented in Table VIII.

The figures in column 4 of the above Table are noteworthy. It may be said that, in general, the test geese, irrespective of age, take an almost equal amount of water per 100 g of fresh plants, but from goose D_3 on a decrease is observed this series. Even with respect to the water drunk goose D_3 indicates those

changes which are closely connected with the functioning of the whole organism. The same is observed in the control geese as the reaction to the green plants eaten. Goose K_4 drinks 98% more water per 100 g of green plants eaten than goose D_4, and goose K_5 275% more than goose D_5, while it eats only 6% of the amount of green plants eaten by goose D_5. The amount of water drunk and taken with food during the 24-hour experiments is no less noteworthy. The first two geese both of the test and control series took almost the same amounts while from the third pair of geese (D_3 and K_3) on a distinct difference appear. Constant and very intense demand for water, which almost equals (gross) body weight in goose D_5, is observed in the test series. An increased amount of water is also observed to be taken by the control geese, but it is much smaller than that taken by the test geese; goose K_3 takes 39% less than goose D_3, K_4 33% less than D_4, K_5 87% less than D_5. The difference in the utilization of water by the two series is so great — from the third pair of geese on — that to explain this phenomenon only by histological changes due to a different diet

Table VII

Average body weight increase of the geese (in kg) every 10 days (based on the data contained in Table III and IV)

Symbol of goose	Average increase every 10 days (kg)	Symbol of goose	Average increase every 10 days (kg)
D_1	0.12	K_1	0.15
D_2	0.08	K_2	0.37
D_3	0.09	K_3	0.17
D_4	0.21	K_4	0.21
D_5	0.20	K_5	0.21** (20*)

* During the whole lifetime in the conditions of the continuous experiment.
** During 126 days in the conditions of the continuous experiment.

does not seem satisfactory. It might be supposed that the great amount of water needed by geese fed on green plants should be related to the different utilization of minerals by the two series of geese. The results obtained through the experiment cannot be compared with others as the author has failed to come across experiments of a similar type.

In addition to the changes which may occur in the examined sections of the alimentary tract of geese as a reaction to green plants attention was paid to the pH of the ingluvies and the glandular stomach. Measurements were taken after the geese had been slaughtered and so the pH of the sections of the alimentary tract of the control geese was the result of the action of the green plants taken at that time. The measurements of pH are contained in Table IX.

Table VIII

Amount of water drunk and taken with plants during the 24 hour experiments

Symbol of goose	Amount of leaves eaten (g)	Amount of water drunk (ml)	Amount of water drunk per 100 g of fresh plants (ml)	Amount of water taken in plants (g)	Total*
1	2	3	4	5	3+5
D_1	120	77	64	92**	169
D_2	300	193	64	244	437
D_3	730	469	64	568	1037
D_4	1170	701	60	978	1679
D_5	1700	945	56	1390	2335
K_1	120	75	62	92	167
K_2	320	201	63	260	461
K_3	450	282	63	350	632
K_4	455	543	119	380	923
K_5	100	210	210	82	292

* The weight of 1 ml of water being accepted as equal to 1 g.

** The average percentage of water in eaten plants amounts to 76.97% in D_1 K_1; 81.33% in D_2 K_2; 77.77% in D_3 K_3; 83.59% in D_4 K_4; 81.74% in D_5 K_5.

The pH of the ingluvies of the test geese increases with age fairly regularly with the exception of goose D_2. This may be a result of the changed diet (the first goose of the second group — see footnote in „Methods"). The value of pH in goose D_4 in relation to goose D_3 slightly deviates from this regularity. From goose D_3 on three or four measurements (D_5) were made going from the front backwards in the direction of the glandular stomach. Goose D_5 is an exception here as its pH in the second and third sections turns

Table IX

The pH of the ingluvies and glandular stomach of the test and control geese

Symbol of goose	Section of alimentary tract	pH	Average	Symbol of goose	Section of alimentary tract	pH	Average
D_1	ingluvies	5.0	5.0	K_1	ingluvies	8.3	8.3
	glandular stomach	2.2	2.2		glandular stomach	2.5	2.5
D_2	ingluvies	4.8	4.8	K_2	ingluvies	4.8	4.8
	glandular stomach	4.9	4.9		glandular stomach	4.6	4.6
	ingluvies I	6.0; 5.1; 5.7	5.6		ingluvies I	5.2; 4.8; 4.1	4.7
D_3	ingluvies II			K_3	ingluvies II		
	glandular stomach	5.9; 5.1	5.5		glandular stomach	4.5	4.5
	ingluvies I	5.6; 5.5; 4.9	5.3		ingluvies I	5.5; 5.7; 5.9	5.7
D_4	ingluvies II			K_4	ingluvies II		
	glandular stomach	5.4; 5.4	5.4		glandular stomach	5.4; 5.5	5.5
	ingluvies I I	5.6; 7.0; 7.1;	6.6		ingluvies I	6.7; 6.5; 7.0;	6.8
D_5	ingluvies II	6.8;		K_5	ingluvies II	7.1;	
	glandular stomach	6.1	6.1		glandular stomach	6.9; 6.7	6.8

on the alcalic side and only near the glandular stomach turns slightly on to the acid side.

As regards the control geese the high pH of the ingluvies of goose K_1 cannot be taken into account, as only a little juice was used for measurements because of lack of solid pieces of food in this section of the tract (the food had moved to the stomach and small parts of plants were preserved on the walls). An almost constant increase of pH from 4.8 in goose K_2 to 6.8 in goose K_5 is observed in the other geese of this series (with the exception of a slight deviation in goose K_3). An exceptional regularity in pH increase is revealed by the measurements of various sections of the ingluvies of geese K_4 and K_5, from more acid at the start to less acid and even basic (K_5) near the glandular stomach.

The pH of the glandular stomach of the test geese increases with age from 2.2 (D_1) to 6.1 (D_5), with the exception of a slight deviation in D_4, while in the control geese it increases depending on age from 2.5 (K_1) to 6.8 (K_5). The results of these experiments approximate those obtained by V o n k and his fellow-workers (1946) from their examinations of young chickens kept on a diet rich in proteins.

The pH of the ingluvies of the test and control geese may be compared only in the last two pairs of geese in which a certain stabilization has taken place. The pH of both the ingluvies and glandular stomach of geese K_4 and K_5 is higher than in the „parallel" test geese.

Smaller body weight increases of the test geese in early stages of their growth (Table VII), the levelling of these increases in later stages (D_4 and D_5), the histological changes caused by the action of food in the ingluvies and glandular stomach at this time, the varying pH in the sections examined and great cytoplasmatic differences in plants cells coming from the ingluvies and glandular stomach are all connected with the utilization of plant protein contained in the cells of the leaves eaten.

The histological examinations of the leaves coming from the ingluvies and glandular stomach supplied no proof of any changes or evanescence of the cellulose membranes. A leaf swallowed whole remains undamaged and is moved from the ingluvies to the glandular stomach. Great changes depending on the plant tissue and the age of geese occur at that time in the plasma of plant cells.

The evanescence of plasma in plant cells removed from the ingluvies and glandular stomach of the geese closely resembles the evanescence of plasma in cells of pieces of leaves coming from the intestine of *Lepidoptera* caterpillars (R y b i c k i 1957). The difference consists in the size of the pieces swallowed. Caterpillars cut out pieces of leaves while geese swallow whole leaves, and so the access of digestive juices secreted by the ingluvies and glandular stomach is more difficult in geese.

In spite of this it seems likely that the mechanism of the evanescence of plasma in plant cells is the same in both cases, but if differs insofar that it is stabilized in caterpillars, while it develops after some time (about 80 days) during an individual goose's lifetime.

CONCLUSIONS

1. Average decade body weight increases in older geese fed exclusively on green plants are the same as those in geese fed on other food.

2. The goose diet composed of green plants results in the fact that daily water demand (the water drunk and taken with food) almost equals body weight.

3. In geese fed on green plants an almost complete evanescence of plasma in plant cells occurs, while the cellulose membranes do not change.

4. Histological changes in the ingluvies of geese fed on green plants consists in the reduced development and probably also reduced function of the glands, particularly in the anterior section (I) of the ingluvies; the function of these glands is taken over by the glands of the posterior section (II).

5. Changes in the glandular stomach of geese fed on green plants consist in a more intense development of the *glandulae mucosae* and in the reduction of the layers of the *glandulae submucosae*, probably accompanied by the atrophy of their functions.

6. The goose diet composed of green plants causes morphological changes in the whole alimentary tract consisting in its elongation.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

DIFFERENCES IN THE HISTOLOGICAL STRUCTURE OF THE INGLUVIES AND THE GLANDULAR STOMACH OF DOMESTIC AND WILD GEESE

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(Received 1 December 1958)

It was found in a previous study that a diet consisting of green plants prevents the development of glands in the anterior section of the goose ingluvies while an intense development of the *glandulae mucosae* and a reduction in the number of *glandulae submucosae* was observed in the glandular stomach. The object of the present paper is to compare the histological structure of the ingluvies and the glandular stomach of a domestic goose fed on a mixed diet in a household and of a wild goose with the structure of the same sections in a goose examined in the previous study.

MATERIAL AND METHODS

The material for comparison consisted in the 120 and 154 day-old test geese discussed in the previous study* and denoted in the present paper as the first series (D — test, K — control).

Test goose D (120 days) — its chief food were green plants supplemented with 2.165 g of wheat bran and 405 g of meat and bone meal taken throughout its lifetime in the conditions of a continuous experiment (see Methods in the previous study).

Control goose K (154 days) — there were no green plants in its diet and during its lifetime it ate 635 g of egg, 3.160 g of barley grit, 370 g of oatmeal, 5.238 g of wheat bran, 27.455 g of potatoes, 1.077 g of meat and bone meal, 2.200 g of cheese, 2.105 g of bread, 1.233 g of oats, 3.420 g of wheat.

* M. Rybicki and L. Lubańska, Acta Biol. Exper. 1959. Vol. XIX.

Anser anser L. (4 specimens) of an indefinite variety, 160 days old, denoted as the second series, kept in a household and fed on a mixed diet.

Anser fabalis Latham (2 specimens) of uncertain age but probably hatched in the same year, shot down on their migration in Jezioro-Łebskie on Oct. 27-th.

Two sections of the ingluvies were taken into account: anterior and posterior, the first being denoted as ingluvies I and the second, ingluvies II, cut out near the glandular stomach. One piece was cut out from the mid part of the glandular stomach.

The pieces of tissue were preserved in Regaud's fixative while the slices were stained with Delafield's stain.

THE HISTOLOGICAL STRUCTURE OF THE INGLUVIES AND THE GLANDULAR STOMACH OF THE GEESE OF THE SECOND SERIES FED ON MIXED DIET

Folds in ingluvies I not very high, with broad bases and even surfaces. Epithelium thick, with numerous foldings at the border of the *lamina propria mucosae*. The *l.pr.muc.* thicker in the folds and thinner at their bases and between the folds, usually containing one layer of glands but single glands in another layer occur very seldom. Shape of glands varying. Elongated glands, 24% of which have efferent ducts (Plate I, a), prevailing in the folds. The *lamina muscularis mucosae* well developed in the folds, not so well at their bases and between the folds. The *lam. musc. interna* (circ.) not so well developed as the *l. musc. externa* (long.).

Folds in ingluvies II fairly high and at intervals longer than in ingluvies I. The surface of the folds smooth and even. Epithelium better developed than in ingluvies I, strongly folded at the border of the *l. pr. muc.* The *l. pr. muc.* fairly well developed, but thinner than in ingluvies I. A single layer with glands less numerous than in ingluvies I is found in this tissue. The distribution of the glands varies: they are more numerous in the folds and less numerous between the folds (Plate I, b). Only a small number of glands in the folds have efferent ducts (12%). The dimensions of the glands are almost the same as in ingluvies I. The *l. musc. muc.* better developed in the folds and *lam. musc. int.* (circ.), not so well developed as the *l. musc. ext.* (long.), just as in ingluvies I.

Glandular stomach has fairly well developed folds. Glands fairly distinct at the bases of the folds. The *muscularis mucosae* well developed. The *submucosa* contains two layers of glands with long

axes vertical to the lumen of the stomach. Large, very well developed glands prevail, 11% of which open outside. The *lam. musc. ext.* (long.), better developed than the *l. musc. int.* (circ.), (Plate I, c).

THE HISTOLOGICAL STRUCTURE OF THE INGLUVIES AND THE GLANDULAR STOMACH OF *ANSER FABALIS* LATHAM

Folds in ingluvies I very high, narrow, lying close to each other, with not very even surfaces (Plate II, a). Epithelium thin, forming folds and inlets going into the *l. pr. muc.* near the *l. pr. muc.* This layer is rather thin but thicker in the folds and thinner in the hollowings. One layer of lenticular glands is found in it, a few of which (4%) have efferent ducts. The *l. musc. muc.* well developed in the folds and the *l. musc. int.* (circ.), less well developed than the *l. musc. ext.* (long.).

Folds in ingluvies II varying in height alternating from high to low (Plate II, b). Epithelium much thinner in the folds, thicker in the hollowings, forming inlets and folds at the border of the *l. pr. muc.* just as in ingluvies I. *L. pr. muc.* somewhat thinner than in ingluvies I, thicker in the folds, thinner between the folds, containing one layer of glands varying in shape. About 12% of the glands with efferent ducts. *L. musc. muc., l. musc. int.* (circ.), and *ext.* (long.), as in ingluvies I.

Glandular stomach with fairly well developed folds but not so high as in the geese of the previous series. Glands at the bases of the folds well distinct. *L. musc. muc.* very thin, hardly distinct. The *submucosae* contains two layers of well developed glands, while small and poorly developed glands prevail in the third layer. Long axes of the glands usually vertical to the lumen of the stomach. About 17% of the glands open outside. *L. musc. ext.* (long.), less well developed than *l. musc. int.* (circ.).

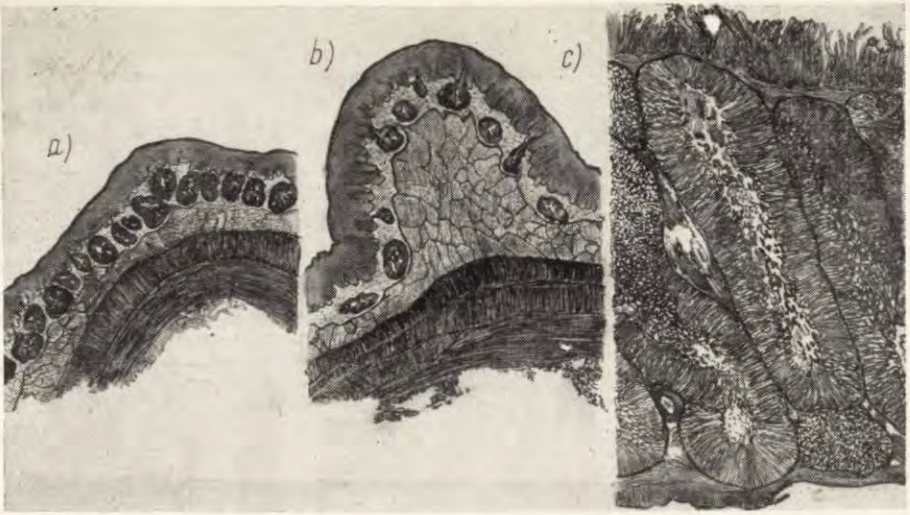
DISCUSSION

A comparison of the material concerning the geese of the first series (D and K fed on the diet described in the chapter on Material and methods) shows that the diet of the geese brings about several changes, which are also expressed in the formation of folds in the ingluvies, higher and narrower in *Anser fabalis* than in the other goose series. The epithelium in the geese of the first series

is always thicker in ingluvies I but this layer is better developed in ingluvies I and II in goose D than in goose K (measurements shown in Table V in the former study). However the epithelium is best developed in the geese of the second series — 415.2 μ (290.0—1060.0 μ) in ingluvies I and 462.1 μ (359.6—580.0 μ) in ingluvies II, while in *Anser fabalis* it measures 271.3 μ (174.0—394.4 μ) in ingluvies I and 248.7 μ (162.4—452.4 μ) in ingluvies II, and thus it is thinner than in goose D but thicker than in K (the first series). The measurements of the epithelium show that in ingluvies II of the geese of the first series this layer becomes thinner by 27% in goose D, by 24% in goose K and by 8% in *Anser fabalis* as compared with the thickness of this layer in ingluvies I of the same geese. The epithelium in ingluvies II is 10% thicker as compared with ingluvies I only in the geese of the second series. It seems that this slight increase in the thickness of the epithelium in ingluvies II of the geese of the second series, and slight decrease (8%) in *Anser fabalis,* is within the limits of accuracy of the method of measurements and consequently the thickness of this layer could be approximately regarded as the same in all three goose series.

Of all the geese examined the *l. pr. muc.* is least developed in goose D of the first series (measurements shown in Table V of the previous study) and the difference in the thickness of this layer between ingluvies I and II of this goose is 18% (thinner in ingluvies II). This layer in ingluvies II of goose K of the first series is 33% thicker in comparison with ingluvies I of the same goose. As the *l. pr. muc.* in ingluvies I of the control geese is by 52%, and in ingluvies II, by 74% thicker in comparison with the layer in ingluvies I and II of goose D, the conclusion may be drawn that green plants being the main diet of geese hamper the development of this layer. The *l. pr. muc.* is always thicker in ingluvies I and thinner in ingluvies II in the other goose series. This layer is 45% thinner in ingluvies II of the geese of the first series and 26% thinner in ingluvies II of *Anser fabalis.* The poorer development of the *l. pr. muc.* in ingluvies II than in ingluvies I is thus a characteristic bringing the geese of the second series and *Anser fabalis* closer to goose D of the first series.

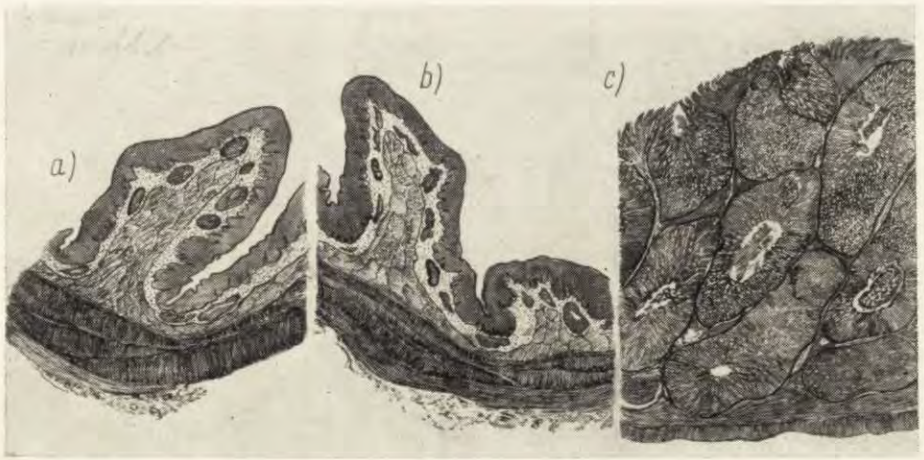
The development of the *l. pr. muc.* can be properly interpreted in connection with the development of the glands, their size and



a — Ingluvies I, goose of the second series* fed on a mixed diet in a household; cross section
b — Ingluvies II, goose of the second series fed on a mixed diet in a household; cross section
c — Glandular stomach, goose of the second series fed on a mixed diet in a household; cross section

* All drawings magnified ca 20 times.

PLATE II



a — Ingluvies I, *A. fabalis;* cross section
b — Ingluvies II, *A. fabalis;* cross section
c — Glandular stomach, *A. fabalis;* cross section*

* All drawings magnified ca 10 times.

Table I

Characteristics of glands of ingluvies and glandular stomach in geese

	Anser anser L.		*Anser anser* L. fed on a mixed diet in a household	*Anser fabalis* Latham
	D — test	K — control		
	ingluvies I			
No of layers of glands	1	2	1 to 2	1
Long axis of gland	256.5μ(197.2—301.6μ)	202.7μ(127.6—255.2μ)	376.3μ(248.0—527.0μ)	341.2μ(232.0—510.4μ)
Short axis of gland	109.6μ(58.0 —162.4μ)	100.1μ(58.0 —197.2μ)	233.2μ(176.7—313.2μ)	141.1μ(69.6 —197.2μ)
No of glands with efferent ducts (%)	21	15	24	4
	ingluvies II			
No of layers of glands	1	2 to 3	1 to 2	1
Long axis of gland	246.4μ(220.4—313.2μ)	223.0μ(116.0—348.0μ)	375.7μ(232.0—487.2μ)	333.8μ(232.0—522.0μ)
Short axis of gland	143.6μ(81.2 —185.6μ)	116.0μ(58.0 —174.0μ)	223.2μ(150.8—268.8μ)	110.8μ(46.4 —208.8μ)
No of glands with efferent ducts (%)	36	25	12	12
	glandular stomach			
No of layers of glands	1	4	2	2 to 3
Long axis of gland	804.9μ(672.8— 870.0μ)	986.9μ(812.0—1123.2μ)	2889.5μ(812.0—4292.0μ)	1856.9μ(603.2—2610.0μ)
Short axis of gland	1111.2μ(951.2—1600.8μ)	844.6μ(475.6— 986.0μ)	1124.4μ(696.0—1392.0μ)	804.4μ(371.2—1044.0μ)
Number of open glands %	—	5	11	17

the number of glandular layers, as it is illustrated by the data given in Table I. The thickness of the *l. pr. mucosae* is related first of all with the number of glandular layers. Goose K of the first series has more layers than any other goose. The intermediate position between goose K and the other series is occupied by the geese of the second series, in which another layer of glands sometimes occur in both of the examined sections of the ingluvies. The appearance in this (second) series of another layer of gland in some cases in ingluvies I with the simultaneous slight increase in the thickness of the *l. pr. muc.* (4%) as against the thickness of this layer in ingluvies I of the control goose, is not so striking as in ingluvies II, where the thickness of the *l. pr. mucosae* is 61% smaller in comparison with the same layer in ingluvies II of the control goose. It appears the diet taken by the geese affects the development of the *l. pr. mucosae,* while the development of the glandular layers in it is probably conditioned by the character of the diet in terms of its physico-chemical properties.

The influence of the diet of the goose is clearly shown in the size of ingluvies I and II. A comparison of the size of the glands (Table I) in the geese examined during the experiments with the size of the glands as shown in the previous study (see Table V) shows that the glands in ingluvies I in goose D are larger than those in goose K. All other goose series have larger glands than goose D in this part of the alimentary tract. The same applies to the glands in ingluvies II. It follows from the comparison of the size of the glands that the number of glandular layers in the *l. pr. muc.* is related to the reduction of their size. As the development of the glands is related to their function, which is always conditioned by the quality and the physico-chemical properties of the food taken, their functional condition is to some extent accounted by the figures for the proportion of glands with efferent ducts. There are very large differences in this respect. In ingluvies I most of such glands are found in the geese of the second series (24%), somewhat fewer in goose D of the first series (21%); an intermediate position is occupied by goose K of the first series (15%), while *A. fabalis* closes the list (4%). In ingluvies II the number of gland with efferent ducts grows in all geese with the exception of the second series. In the light of the descriptions of geese D and K of the series the increase in the number of glands with

efferent ducts in ingluvies II seems pretty clear and is accounted for both by the definite food and by the function of this section of ingluvies, where food stays somewhat longer. The growing proportion of glands with efferent ducts in ingluvies II in *A. fabalis* can also be interpreted in a similar manner. What is difficult explain however is a decrease in the number of glands with efferent ducts in ingluvies II in the geese of the second series. It is perhaps quite plausible to suppose that the lower proportion of glands with efferent ducts is to some extent compensated by the size of the gland itself, as follows from the comparison of the dimensions of their long and short axes with the dimensions of the long and short axes of the glands in ingluvies II of geese D and K of the first series (Table I).

The tissues of the glandular stomach of the three goose series undergo no smaller changes. The size of the folds on the surface of the stomach is the largest in goose D of the first series —777.2 μ (696.0—1044.0 μ), it is 705.0 μ (580.0—812.0 μ) in goose K, 590.3 μ (406.0—696.0 μ) in the geese of the second series and 230.6 μ (150.8—394.4 μ) in *Anser fabalis*. The height of the folds on the surface of the stomach is related to the development of the *glandulae mucosae*, the appearance of which in goose D of the first series is different than in the geese of the other series (these changes having been described in the previous study).

The greatest changes were however undergone by the *glandulae submucosae*. These changes refer to the number of glandular layers. An extreme position is occupied in this respect by goose D of the first series which has only one glandular layer. Although they are large their functions are probably strongly reduced as no glands opening into the lumen of the stomach were found in this goose. The reverse is observed in goose K of the same series, which has four layers of well-developed glands (see Plate III in the previous study), only 5% of their total opening into the lumen of the stomach. These two geese of the first series supply the basis of comparison with others, as so large histological changes in them can be ascribed only to food. The geese of the second series have two layers of glands of which 11% open into the lumen of the stomach. There are already two or three layers of glands present in *A. fabalis*. Although their dimensions are smaller than those of glands in the geese of the second series, but of all geese it is in *A. fabalis* that

has the largest number of glands opening into the lumen of the stomach (17%). The following conclusions follow from the comparison of the histological structure of the glandular stomach and the ingluvies.

1. The number of glands with efferent ducts in ingluvies II is higher than in all geese examined except the second series.

2. A different structure of glands in ingluvies I and II in *A. fabalis* is probably a characteristic of the species.

3. A diet entirely without green plants (goose K of the first series) or only partly supplemented with green plants (the geese of the second series and *A. fabalis*) has a stimulating effect on the numerical development of glandular layers in the glandular stomach.

Acta Biologiae Experimentalis, Vol. XIX, 1959

A POLAROGRAPHIC METHOD OF MEASURING THE INTENSITY OF RESPIRATION IN AQUATIC ANIMALS

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(Received 1 November 1958)

In the course of investigations on respiration in small aquatic animals the necessity arose to work out a sufficiently sensitive method for measuring the oxygen concentration in small amounts of water.

In such analyses good results are obtained by the polarographic method (Bielawski 1958). This method consists in measuring the current intensity in the circuit during electrolysis of substances becoming reduced or oxidized on a dropping mercury electrode (Kolthoff and Lingane 1952).

The O_2 molecules dissolved in water have no electric charge and do not migrate in the electric field; they reach the mercury catode only by way of diffusion. The current in the circuit depends therefore on the speed of oxygen diffusion and for that reason it bears the name diffusion current.

The oxygen concentration of flowing water may be measured polarographically by immersing electrodes into it. In that case it is not necessary to take samples. Owing to the insignificant intensity of the diffusion current practically no oxygen escapes from the examined water and therefore the amount of water flowing near the electrode may be very small.

Another advantage of the polarographic method is that continuous measurements of oxygen concentration in the water can be automatically recorded (Manning 1940) and that they may be made for several days provided an appropriately constructed anode is used (Spoor 1948, Wood 1953).

The form of the polarographic cell plays a considerable role in investigations on respiration. Convenient is a cell in which the

water escaping from the vessel with the examined animal flows near the electrodes.

Wood (1953) constructed such a cell from a plastic block by drilling in it suitable channels for the flowing water and for the electrodes. Zagórski (1956) used for the construction of a polarographic cell polymethyl methacrylate known as plexiglass. Polymethyl methacrylate is chemically resistant to the majority of solvents applied in polarography and is easily processed mechanically. The cell used in the method described below is made of the same material.

DESCRIPTION OF THE APPARATUS

The measurements of the O_2 diffusion current were made by means of a Heyrowski polarograph produced in Czechoslovakia. The other elements are schematically represented in Fig. 1. They com-

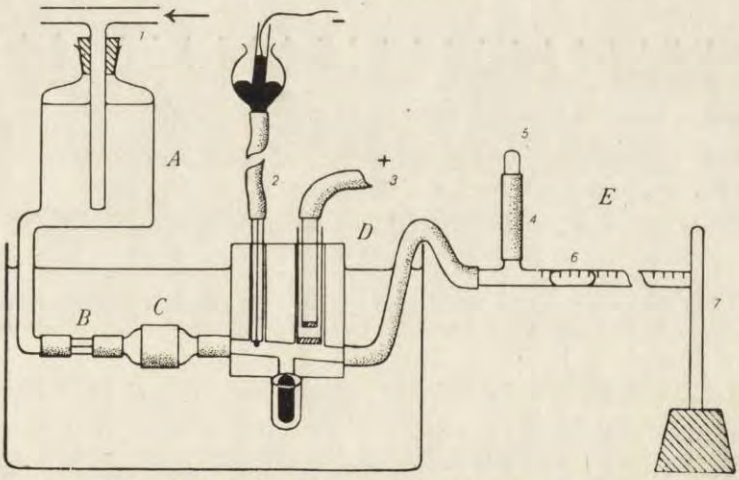

Fig. 1. Schematic diagram of the apparatus

A — Mariotte flask; 1 — T-shaped tube; B — capillary limiting the speed of the flow of water; C — vessel containing the examined animal; D — polarographic cell, 2 — dropping mercury catode, 3 — end of flexible salt bridge; E — instrument for measuring the speed of flowing water, 4 — rubber tube, 5 — glass plug, 6 — air bubble, 7 — glass rod

prise a Mariotte flask maintaining a constant hydrostatic pressure, a capillary tube limiting the speed of the flowing water, a vessel with the examined animal, a polarographic cell and a device for measuring the speed of the flowing water.

The Mariotte flask is provided with a T-shaped tube. If the oxygen concentration of the water in the flask is to differ from that in water saturated with air we pass through the water in the flask a mixture of gases with a known oxygen content. To separate that water from the air we pass a slow stream of the said mixture through the horizontal branch of tube T.

From the Mariotte flask the water runs through a glass tube into the capillary that regulates the speed of the flow. The glass tube cannot be replaced here by a rubber tube because rubber is permeable to oxygen and consequently when the flow of water is slow, of the order of 5 ml per hour, the oxygen concentration of the water may be changed to a considerable extent.

The water then flows through a vessel formed of two glass parts lying close to each other and joined by a rubber ring. In this vessel we place the examined animal.

The polarographic cell is made of a thick plate of plastic into which four channels are drilled (Fig. 2). The water flows through channel a) having a diameter of 4.5 mm and being slightly inclined

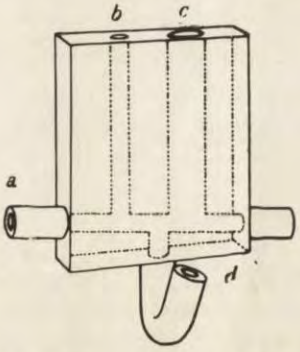

Fig. 2. Polarographic cell

a — channel through which the water flows, b — channel to hold the dropping mercury electrode. c — channel to hold the end of the flexible salt bridge, d — channel from which the mercury dropping from the mercury electrode pours outside

to level. Into that channel enters a vertically placed channel b) which contains the dropping catode with a drop rate of one drop in 1.5 sec. and channel; c) containing the terminal part of the flexible salt bridge. This salt bridge is made of a rubber tube filled with a saturated KCl solution. The tube is connected on one side

with a saturated calomel electrode and on the other with a sintered glass plug (Hume and Harris 1943). Owing to the inclination of channel a) the mercury from the catode enters channel d) which is bent upwards and overflows outside.

Having passed through the polarographic cell the water passed into an instrument for measuring the speed of flow. This instrument is made of a pipette with a glass tube fused to it vertically. On that glass tube a rubber tube is fixed which is closed at the other end by a glass stopper. When compressing the rubber tube a bubble of air enters the pipette and moves to its end.

If the time of the movement of the air bubble between the lines of the calibrated pipette is known it is possible to calculate the speed of the water flow, of the order of 5 ml per hour, with an accuracy of 1%. A glass rod placed at the end of the pipette prevents the water from dripping and the air bubble from moving irregularly.

In order to avoid the influence of the surrounding temperature on respiration and on the oxygen diffusion current, a part of the apparatus is placed in a water bath. The water in the water bath is mixed with air because the usually applied electric mixers may distort the results in consequence of shocks.

The analysed water is in electric contact with the water bath through the mercury escaping from the polarographic cell. This is the reason why the electric heater of the water bath must be well isolated or grounded.

The above described apparatus has been adapted to serial analyses. For that purpose the water from the Mariotte flask flows into a glass tube with 6 lateral tubes fused to it. To each of the lateral tubes are successively added all the remaining elements illustrated in Fig. 1, i.e. the capillary limiting the speed of the flow, the vessel containing the animal, the polarographic cell together with the electrodes and the instrument for measuring the speed of the flow. Of the six parallel devices one is left for control without the animal and it is here that the oxygen concentration of the water flowing from the Mariotte flask is measured.

When the oxygen consumption is relatively small there is only a slight difference between the oxygen concentration in the water flowing from the vessel with the animal inside and that in the water from the control cell. In order to increase the accuracy of

measurement of this difference an additional electric arrangement is introduced (Lingane and Kerlinger 1940) which compensates a part of the oxygen diffusion current and allows to apply a greater sensitivity of the galvanometer.

CALIBRATION

The calibration of the above mentioned apparatus consists in determining the relationship between the dissolved oxygen concentration and the diffusion current. When using a greater number of catodes for serial analyses the calibration is made as follows: The oxygen diffusion current is measured in the control cell (control catode). Subsequently about 2/3 of the diffusion current is compensated by means of the above arrangement and the difference of intensity between the diffusion current in the control cell (control catode) and in the remaining cells (remaining catodes) is measured with a greater sensitivity of the galvanometer. In all calibration work the Winkler method was used a standard reference.

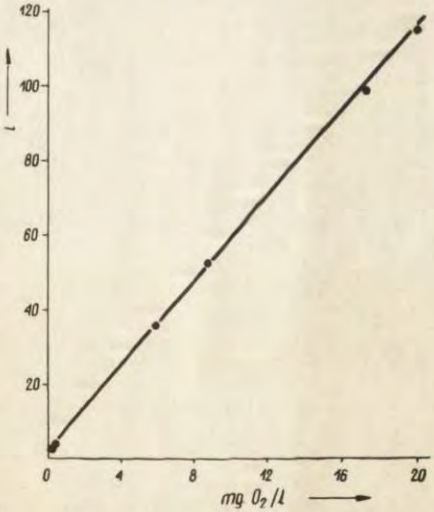

Fig. 3. Calibration curve
i — current intensity

More exact are the measurements of the intensity of the diffusion current made by plotting the whole polarographic wave and measuring its height. Simpler is the measurement at only one potential following the waves (Ingols 1941, Moore, Morris

and Okun 1948, Busch and Sawyer 1952). To avoid maximum suppression the diffusion current was measured at a potential following a wave of hydrogen peroxide.

In tap water there occurs a decrease of tension in consequence of considerable electric resistance. In this connection the polarographic wave, in particular the wave of hydrogen peroxide, appears in higher tensions applied to the electrodes (Giguère and Lauzier 1945). The change of the wave becomes greater with an increase in the oxygen concentration of the water (Moore, Morris and Okun 1948, Busch and Sawyer 1952). To avoid an error caused by the decrease of tension in the water, the author plotted in each case the entire horizontal section of the current voltage curve following the wave of hydrogen peroxide. From the mean height of that section the intensity of the diffusion current was

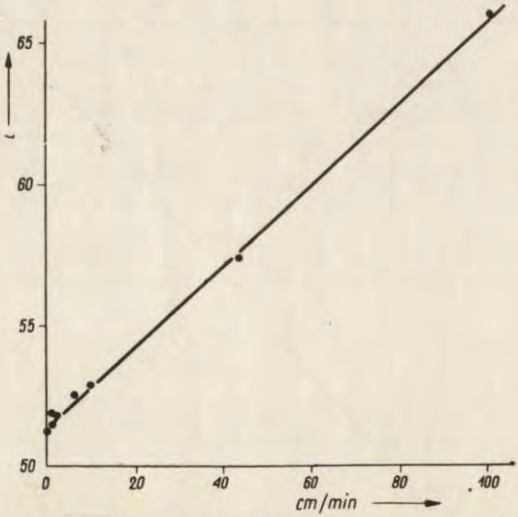

Fig. 4. Influence of the speed of flowing water near the electrodes on the diffusion current

read. The diffusion current is a linear function of the dissolved oxygen content within the range of the investigated dissolved oxygen content (0—20 mg O_2/l, Fig. 3).

The salts dissolved in water bear no influence on the value of the O_2 diffusion current unless they become reduced at a poten-

tial more negative than that of oxygen. In the majority of natural waters the concentration of substances forming an obstacle in analyses is so small that their influence can well be neglected (Moore, Morris and Okun 1948).

The speed of oxygen diffusion and thus also the value of the diffusion current depends on the temperature. The temperature coefficient defining the magnitude of changes in the intensity of the diffusion current when the temperature changes by 1°C and is expressed in percent of the diffusion current at 20°C, is in given conditions a constant magnitude. The values of that coefficient obtained for the particular catodes amount at an average to 1.25%/1°C (1.18 to 1.32%/1°C). Similar results were obtained by Busch and Sawyer (1952).

Another factor affecting the intensity of the oxygen diffusion current is the movement of the water near the catode. A linear relationship is obtained if the oxygen diffusion current is plotted against the flow of the water, as shown in Fig. 4. If the flow is very slow, of an order of 1 cm/min., its influence on the measurements may be neglected.

The above described method permits to examine the oxygen consumption of an order of 0.01 mg O_2 per hour. Owing to the flow of water and to placing a part of the apparatus in a water bath, it is easy to keep the examined animal in constant conditions as regards temperature, oxygen concentration, salinity and just as easy to change these conditions.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

SUCCINIC ACID DEHYDROGENASE ACTIVITY IN TISSUES OF *VIVIPARUS VIVIPARUS* L. AND *VIVIPARUS FASCIATUS* O. F. MÜLL

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(Received 2 October 1958)

INTRODUCTION

The subject of this work is to determine whether differences in oxygen consumption in two species of *Viviparus* (*V. viviparus* L. and *V. fasciatus*. O. F. Müll.) observed by several authors (Kreczko and Michejda 1956, Obuchowicz 1958, Krywieńczyk 1952) are related to, or caused by similar differences in the activity of respiratory enzymes. It is also an attempt to find whether there exists an enzymatic differentiation between males and females.

For investigation the succinic acid dehydrogenase (SDH) was chosen, which is considered as an enzyme intimately bound with the structure of tissues and seems also to be wholly synthesised by tissues of the given organism (Precht — Carlsen 1953). The tissues used were the foot muscle (as tissue chosen for general biochemical characterisation Kirk and al 1953, Michejda 1955), and hepatopancreas (as being an organ of intense metabolism (Baldwin 1938, Rees 1953).

MATERIAL AND METHOD

The investigations were carried out on water snails, *V. viviparus* L. and *V. fasciatus* O. F. Müll. (*Prosobranchia*). The snails were caught at low temperature of water. *V. viviparus* were found in water pools near the river Warta, and *V. fasciatus* in the river Warta.

After brushing the algae off the shells the snails were kept for two days in tap water in order to remove foodstuffs. Only individuals with shells 30—32 mm high were used for the experiments.

The foot muscle without skin and the two last whirls of the body containing chiefly the hepatopancreas were dried on filter paper. The tissues were weighed with an accuracy of up to 5%, then dropped into the tube of a homogenizer and the phosphate buffer* (pH 7.4) was added, at a ratio of tissue/bufer = 1 : 100.

Glass pestles without teeth but with sicklelike, sharpedged cuttings were employed (M i c h e j d a 1957). Such a pestle mashes both the foot and the hepatopancreas tissue.

The SDH activity was investigated by the Thunberg method. 3 ml of homogenate and a glassbead (to enable better mixing) were introduced into the tube; 1 ml of methylene blue 0.005% and 2 ml of sodium succinate (pH 7.3) were added to the sidearm. After closing the tube with the sidearm cap, the air was evacuated for three minutes by means of a vacuum oil pump. In order to avoid the mixing of fluids the pump tap was opened gradually and the tube was shaken slightly and kept in a slanting position. Then the tube was put into a water bath at 35°C. After reaching temperature equilibrium (ab. 2 min.) the content of the sidearm was poured into the tube and from this moment the methylene blue decoloration was observed and the time noted. The suspension of the snails tissue precipitates easily, therefore the tubes had to be shaken every 5 minutes.

The work was done in white scattered light to avoid the acceleration of reduction of methylen blue by strong light. The decoloration lasted usually more than 15 minutes. A control test was prepared with boiled tissue and methylene blue diluted 10 times no substrate being added. As many tests proved nonenzymatic decoloration was practically none.

The constancy of test colours was controlled photometrically. At first the same homogenate was examined twice. After satisfying myself of the methodical accuracy ($\pm$ 1 min.) each measurement was made only once.

The nitrogen content was determined by the Kjeldahl micromethod in 3 ml of homogenate corresponding to 30 mg of fresh tissue.

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* Natrium and potassium phosphates (8:2) 0.02 M.

RESULTS

The activity of succinic acid dehydrogenase was related to the wet weight or to the N content of the examined tissue and calculated according to the formula 1 and 2:

$$(1) \qquad \text{SDH activity} = \frac{100}{t \times \text{wet weight}},$$

$$(2) \qquad \text{SDH activity} = \frac{100}{t \times \text{N content}},$$

where t is time of decoloration in minutes. Wet weight of examined tissue samples in all experiments was equal to 30 mg.

According to the data of Tables I and II one may draw following conclusions.

1. There are no differences in the SDH activity in both tissues between males and females within one species.

2. There are, however, differences between tissues (foot muscle and hepatopancreas) within one species.

3. In general, the variability of SDH activity in individuals of the same species referred to wet weigt is relatively small and it becomes still smaller if referred to N content. The N measurements show a marked constancy in the percentage of N content in the tissues. We assume therefore that the percentage of N content in the tissues of all individuals of the same species is rather identical. Therefore the results referred to wet weight are well comparable. Comparing both species and both tissues with each other one see a distinct difference in N content related to wet weight. The muscle in *V. fasciatus* has 13% more N than in *V. viviparus*. The percentage content of N in the hepatopancreas exceeds that in the muscles: in *V. viviparus* — 19%, and in *V. fasciatus* — 11%.

4. Tests of blank showed that the rate of methylene blue reduction, without the participation of SDH, was very small for the muscle. It caused only a very small decoloration after 3 hours. The value for hepatopancreas, however, was higher and amounted to 100 — 115 min., for *V. fasciatus* (only in two cases 35 min!), and to a much longer time for *V. viviparus*.

Corrections resulting from blank were not considered as they did not exceed the accuracy of the readings i.e. 1 min.

Table I

SDH activity in the footmuscle and hepatopancreas of *Viviparus viviparus*

	Footmuscle								Hepatopancreas							
	♂				♀				♂				♀			
	Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to	
			Wet weight	N content			Wet weight	N content			Wet weight	N content			Wet weight	N content
	26	0.490	0.13	7.84	26	0.517	0.13	7.43	13	0.591	0.26	12.9	13	0.598	0.26	12.8
	31	0.476	0.11	6.77	29	0.490	0.11	7.02	14	0.571	0.24	12.5	17	0.598	0.20	9.8
	31	0.476	0.11	6.77	32	0.462	0.10	6.75	16	0.585	0.21	10.7	16	0.585	0.21	10.7
	30	0.449	0.11	7.42	27	0.490	0.12	7.55	17	0.529	0.20	11.1	13	0.598	0.26	12.8
	33	0.462	0.10	6.57	29	0.480	0.11	7.17	15	0.598	0.22	11.1	13	0.585	0.26	13.2
	30	0.476	0.11	7.19	31	0.462	0.11	6.98	15	0.639	0.22	10.4	16	0.571	0.21	10.9
	29	0.476	0.11	7.22	32	0.449	0.10	6.95	13	0.598	0.26	12.8	15	0.598	0.22	11.1
	29	0.462	0.11	7.46	30	0.476	0.11	7.19	15	0.571	0.22	11.7	17	0.598	0.20	9.9
	27	0.503	0.12	7.35	31	0.462	0.11	6.97	16	0.639	0.21	9.8	17	0.585	0.20	10.1
	27	0.497	0.12	7.45	25	0.503	0.13	7.87	17	0.612	0.20	9.6	13	0.612	0.26	12.5
					26	0.503	0.13	7.56								
Mean	29.5	0.477	0.11	7.20	29.0	0.481	0.11	7.22	15	0.593	0.22	11.3	15	0.594	0.23	11.4

Table II

SDH activity in the footmuscle and hepatopancreas of *Viviparus fasciatus*

	Footmuscle								Hepatopancreas							
	♂				♀				♂				♀			
	Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to		Decoloration time in min.	N content in 30 mg of tissue mg	SDH activity related to	
			Wet weight	N content			Wet weight	N content			Wet weight	N content			Wet weight	N content
	20	0.558	0.16	9.0	15	0.558	0.22	11.9	10	0.623	0.33	16.1	9	0.598	0.37	19.0
	20	0.544	0.16	9.2	20	0.544	0.16	9.1	9	0.639	0.37	17.4	12	0.612	0.28	13.6
	20	0.544	0.16	9.2	17	0.544	0.20	10.8	8	0.626	0.41	20.0	14	0.639	0.24	11.2
	20	0.517	0.16	9.7	18	0.558	0.18	10.0	12	0.598	0.28	14.0	11	0.598	0.30	15.2
	19	0.558	0.17	9.4	16	0.558	0.21	11.2	12	0.612	0.28	13.6	8	0.598	0.41	20.5
	19	0.558	0.17	9.4	20	0.529	0.16	9.5	8	0.653	0.41	19.1	10	0.653	0.33	15.3
	17	0.585	0.20	10.1	16	0.544	0.21	11.5	10	0.639	0.33	15.6	9	0.612	0.37	18.1
	15	0.558	0.22	11.9	21	0.529	0.16	9.0	9	0.639	0.37	17.4	11	0.629	0.30	17.2
	18	0.544	0.18	10.2	18	0.558	0.18	9.9	9	0.598	0.37	18.6	10	0.612	0.33	16.3
	15	0.544	0.22	12.2	21	0.544	0.16	8.7	10	0.626	0.33	16.0	12	0.612	0.28	13.6
	17	0.544	0.20	10.8					9	0.585	0.37	19.0				
Mean	18	0.550	0.18	10.1	18	0.547	0.18	10.2	9.5	0.621	0.35	16.9	10.5	0.606	0.32	16.0

DISCUSSION

It was found that there exist differences in the SDH activity between the examined species of *Viviparus*. The SDH activity both in muscle and hepatopancreas is higher in *V. fasciatus* than in *V. viviparus* (40% and 32% respectively).

Some authors find similar differences in oxygen consumption of the same species of *Viviparus*. The results of investigations by Kreczko and Michejda (1957) show almost twice as high the oxygen consumption in *V. fasciatus* as compared with *V. viviparus*. This agrees, as the authors state, with the ecological demands of both species.

Similar differences in oxygen consumption (30%) in the same two species of snails are given by Obuchowicz (1957). The investigations mentioned above were carried out in various seasons. Snails were taken from waters in the environs of Poznań.

Krywieńczyk (1952) carried out similar studies on *Viviparus* from West Germany and the differences in oxygen consumption (30%) were in favour of *V. fasciatus*.

The similarity of results shows that the differences in oxygen consumption is a characteristic value for the examined species. The results of this paper indicate that these differences are parallel to the differences in the activity of respiratory enzymes. There are no publications dealing with SDH activity of *Prosobranchia*. There are, however, papers dealing with other snails.

Kirberger (1953) determined the SDH activity of *Helix pomatia* L. Analysing 100 mg of tissue she obtained the decoloration time for muscle after 22 min. and for hepatopancreas after 17 min. In reference to wet weight the values of SDH activity are 0.045 for muscle and 0.059 for hepatopancreas, i.e. they are 3 times lower than the values obtained by myself.

Baldwin (1938) studied the enzyme activity in *Helix pomatia* L. Although he examined 500 mg of tissue he obtained a much longer decoloration time (to 80 min.). His results, however, cannot by compared with mine as he used slices which yield no good results in Thunberg's method.

The similarity of the SDH activity in tissues of both sexes coincides with their great biochemical similarity, shown by chromatographic investigations (Michejda 1955). In insects differences of the activity of oxidases in males and females were found

in certain tissues, e.g. in the muscle and nervous tissue in males of *Periplaneta americana* L. the succinic acid oxydase (SOX) activity (also DPN, cytochrome c and iron content) is higher than in females (Sactor B. and Bodenstein D. 1952). These differences do not, however, exist in other tissues e.g. in the midgut and hindgut.

The oxygen consumption of hemogenate and SOX activity in mature males of *Bombyx mori* are much higher than in females (Wojtczak L. 1955).

The differences in SDH activity, shown in this paper, between muscle and hepatopancreas are distinct in both species. The higher enzyme activity in hepatopancreas, as compared with that of the muscle, is quite clear by due to its role in metabolism. It is „responsible for the digestion, absorption and storage of food" (Baldwin E. 1938). Similarly, Rees (1953) and Weinbach (1953) state that succinates are the most important substrate of Krebs cycle in the metabolism of hepatopancreas of snails. The comparison of values obtained for the SDH activity related to N content shows their great importance for the interpretation of results. It must be noted that neither the reference to wet weight nor to the total N content is an optimal method. The question whether it is right to refer activities of life processes to various bases (wet weight, dry weight, delipidated mass, protein N, whole N content) is still unsolved (Michejda J. 1958).

SUMMARY

The measurements of the succinic acid dehydrogenase (SDH) activity in the homogenates of muscles and hepatopancreas in *Viviparus viviparus* and *Viviparus fasciatus* have proved:

1. SDH activity in hepatopancreas tissue is greater than that in muscles of both examined species.

2. No differences were found between SDH activity in examined tissues of males and females in both species.

3. SDH activity of the examined tissues is greater in *Viviparus fasciatus* than in *V. Viviparus*. This difference corresponds to the difference in oxygen consumption found by other authors in both examined species.

The author wishes to thank Dr Jan Michejda and Mr Józef Bielawski for advice kindly given during this work.

Reported investigations were sponsored by the Biochemical Committee of the Polish Academy of Sciences.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

STUDIES ON THE DIVISION OF LABOUR IN ANTS GENUS FORMICA*

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The biology of social insects demonstrates a series of analogous phenomena. The most characteristic of these is the existence of an asexual caste and the division of labour within it.

Social insects belong to separate systematic groups — sometimes very distant from each other — and any biological analogies could only arise in them as a result of convergence; thus the genesis of these phenomena may be very various and depend on the path of evolutionary development and biology of the given animal. For this reason in examining the genesis of the labour division in ants the present work attempts to take into consideration the specific biology of the whole family Formicidae as well as of the particular species.

This work was carried out in 1954—1956.

METHOD

Field experiments

The object of the field experiments was studing the division of labour in the functions outside the nest. The objects of study were several species of the genus Formica: *F. rufa, F. pratensis, F. truncicola.*

The first group of experiments was conducted according to the Ökland (1931) and Kiil (1934) method based on mass markings of workers while fulfilling specific functions.

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* This paper forms part of a thesis presented 17.I.1957 at the Nencki Institute of Experimental Biology.

The second group of experiments was a continuation of previous studies (1958) on one specific function, — collection of the aphides excreta, — using the method of mass marking of workers fulfilling that function. The method of the present work is based on marking individual and further more prelonged observation of individuals attending to the aphides. The experiments summarized in Tables I, II and III, were carried out on one small (about in diameter 20 cm) nest of *F. rufa*. The workers from this nest cultivated the aphides on a small tuft of *Melampyrum pratense* L. Each ant found on one stalk (length 5 cm) of *Melampyrum* was individualy marked and observed for a period of four weeks. In all, 27 individuals were marked.

In all field experiments marking was executed on the run avoiding in this way catching the ants. After several attempts most often skin dyes were used. Unfortunately no dye was found which could be guarranteed to last longer than two months. The question of dyes remains the most troublesome and undoubtedly holds up the course of the experiments.

Laboratory experiments

The laboratory experiments mainly concerned the division of labour within the nest and were conducted on species of *Formica sanguinea* and partly on *F. fusca*.

The most troublesome problem was finding suitable methods of the cultivation which would allow free observation of the interior of the nest. Simple observations show, that contrary to the current opinion, light is not an intrusive factor in the cultivation of ants. Very frequently ants, kept in glass containers with earth, choose a site next to the glass for cells with larvae, pupae and even eggs, in fact, even though there exist full opportunities for building the cell within the earth. Obviously this is done by ants in nests which are acclimatized and conditioned to the new situations. If we heat such a nest from the side next to the glass there is a good chance that the workers near it will place the young there paying no attention to even bright light.

On the basis of these observations and the generally known fact of the considerable plasticity and adaptability of *F. sanguinea* the cultivation of this species was tried in glass containers without earth, completely illuminated. The nest consisted of two or three pieces of plane glass placed one on top of the other in a glass container so that the space between them served as cells. Such a colony was very successful, the workers cared for the young in a usual way and, in some cases, the female even laid eggs. „Slaves" of the species *F. fusca* respond well to such conditions. This method was next tried on the relatively non-adaptable species *Componotus herculeanus ligniperdus* Latr. This colony also prospered very well as proved by female laying eggs and breeding a new generation.

A culture of this type has considerable drawbacks. Firstly the very important and work absorbing function of building, and conserving the nest completely stops to be performed. In order to reveal individuals capable of fulfilling this function from time to time a little clod of humid soil was introduced into the nest. Each time a few workers immediately attemp-

ted to build. However the normal course of life is from this point of view completely disturbed, young workers are reared in a colony, where the problem of building a nest does not exist; besides, due to the adaptation the full light probably one of the stimuli to building a nest has disappeared. Thus in these experiments this function is at least not presented in its normal course.

The unnatural of life conditions in the experimental colonies probably alter the course of ants life and function also in other aspects; it is not possible to accept all results literally; for example the quantitative ratio of workers fulfilling particular functions is undoubtedly out of relation to normal conditions. Apart from this such a colony is necessarily small.

However from a very general point of view if we consider the most important functions, (with the above exception — building the nest) colony life is on the whole reproduced and is completely observable. Using individual marking of each ant in such a colony, it is possible to observe all functions performed by each individual for several months.

An important difficulty is the lack of fast dyes. In order not to waste several months of experimental work it is necessary to repeat marking of all individuals before the dye disappears. This was done every two months on the average. Minimal doubt as to the identity of the individual, causes the necessity of elimination of the ant from the experiment, for sake of accuracy, since its whole personal history becomes uncertain.

16 such colonies of *F. sanguinea* were set up among them several with „slaves" of *F. fusca*. The initial number of workers in a particular colony varied from 30—80. In a well prospering colony the number of inhabitants increased afterwards due to the hatching of the young.

3 colonies were of a special type: colony No 14, in which all workers were selected while fulfilling one function — collecting of the excreta from aphides — and colonies 15 and 16 for which only young individuals were chosen whose maximal age was two days at the time of setting up the colony.

In setting up the latter colonies the suggestions of K. Heyde (1924) were followed that young ants have not yet developed a defence instinct and that it is possible tu unite them with young individuals of a foreign nest without arousing antagonisms. In the present work, young individuals from several nests were successfully brought together.

Two foreign colonies were also successfully united. As yet no results of this experiment may be given as to the division of labour but it yet is mentioned, in the present paper because the method of a successful union of two nests is in itself of considerable importance. Until now uniting of foreign nests has not been successful with the exception of the K. Heyde's work where, as mentioned above, it was only attempted with young individuals. In the present work however, the combination of normal colonies previously observed for a series of months, is very important.

It was assumed that in a new site impregnated with foreign odours, the ants might pay less attention to the difference between the odours of nests. Thus, gradually, workers from two nests were introduced to a third

clean container. Cases of assault were few and above all not violent and short. There was no one case of death, which proves that the mixed culture was a success. Already on the first day the reaction towards their own and foreign pupae was the same (which is besides characteristic for *F. sanguinea*), they were arranged and cared for together. Similarly after short and feeble attacks a fertile female from a third foreign nest was accepted.

It is not to be excluded that this method would not work for all species. *F. sanguinea* is accustomed to foreign odours in the nest since it continually abducts foreign pupae. However, a simple introduction of one colony to another always leads to a death struggle in this species as well.

All colonies except the last were observed for three to six months. The ants were observed every one or two hours for 24 hours a day. Each time the observer attempted to note the occupation and behaviour of each individual, in so far as its position allowed a certain indentification of the marking.

Functions were registered as follows: 1) sitting on the larvae or pupae (possibly also beside them); 2) staying (passively?) in the nest, outside the cells with the young generation; 3) transferring larvae or pupae; 4) transferring other workers; 5) assisting the hatching of the pupae (active); 6) staying immobile at the hatching pupae; 7) caring for the newly hatched ants; 8) feeding the larvae: 9) feeding the female; 10) feeding other workers or males; 11) removal of waste products from the nest; 12) walking about the territory (foraging); 13) attacking enemies; 14) eating; 15) drinking; 16) carrying buildings materials; 17) digging earth.

For further analysis of the results it is necessary to establish the size of each individual. In general among investigators the width and length of the head is taken as the criterion of the size of an ant in a given polimorphic caste (Adlerz 1886, Pricer 1908, Buckingham 1911, Alpatov and Palenitschko 1925, Palenitschko 1927, Arnoldi 1927, Kiil 1934). Although from the point of view of a biometry this criterion is not sufficient, as shown by Raignier and v. Boven (1955); however here relative sizes are concerned only. In Formica the factor most correlated with size of the ant is the width of the head. According to this criterion it is possible, in general terms, to talk of large, medium and small ants. As these dimensions were made on living individuals they were done by eye; however this was not a single measurement but each worker was observed for several months so that it was possible to correct possible error in determining size.

As to the estimation of the ants age (physiological factors) only ants, hatched in the colonies were analysed or those which, when introduced, were so young that it was possible to estimate their age within an error of one day.

Looking for further possible factors influencing on the development of the labour division in the nest, an attempt was made to analyse the type of behaviour of the workers fulfilling particular functions. The evaluation of behaviour characteristics in these experiments is only approximate. As criteria excitability, mobility and activity of a given individual were taken.

Table I

Attendance on stalk A

Observations		No of marked individuals												Number of marked non-identified individuals	Remarks
Date	O'clock	1	3	4	6	9	10	11	13	16	17	18	29		
28.VIII	12.40	+												4	
	15.40	+	+	+										5	
	19.30	+				+	+							3	
29.VIII	10.30	+	+	+	+				+					2	Poor visibility
	11.20	+	+	+	+			+	+					1	
	15.15	+	+	+	+			+							
	16.00	+		+	+			+						1	
	18.35		+	+		+		+				+		2	
	20.30	+	+			+		+						1	
30.VIII	7.00	+	+	+			+	+			+			1	
	8.00	+	+	+				+			+			1	
	11.30			+					+	+	+				
31.VIII	6.30	+	+	+			+		+		+		+	1	
	9.00	+	+	+			+	+			+			5	
	12.00	+	+	+					+		+			1	
	14.40	+		+					+		+			2	
	15.05	+		+					+		+		+	2	
	18.00	+	+	+		+	+				+			2	
1.IX	2.00	+		+		+					+				
	10.40	+	+	+			+			+				3	Poor visibility
	12.00			+			+		+	+				1	
	13.10	+		+			+		+	+				1	
2.IX	10.40	+							+	+		+		1	
	13.20	+							+	+		+		1	
3.IX	10.40	+							+	+			+		
	13.20	+										+		2	
4.IX	1.20	+							+					1	Poor visibility
	10.40	+							+					1	
6.IX	14.15	+							+						From September 6th observations were carried out on No 1 and No 13 only
	24.30	+													
7.IX	15.15	+							+						
8.IX	7.30	+													
9.IX	14.15	+												6	
11.IX	19.30								+					5	

Table II

Attendance of individual No 1 on stalk A

Month	Day	Hour of observations																						Remarks
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
August	28												+			+				+	+					
	29										+	+				+						+				
	30							+	+			−														
	31	+					+			+			+		+	+			+							
September	1		+			+	+				+		−	+								+	+			
	2							+			+	+	+	+	+											
	3				+				+								+						+			
	4				+						+	+	+	+	+					+						
	5	+									+	+	+	+	+											Plant began to dry up
	6														+										+	
	7																									
	8																									Heavy rain
	9																									
	10									+						+										
	11																			+						
	12										+	−										+				
	13										+	+	+					+								
	17								+						+		+									
	18										−					−										

Table III

Attendance of individual No 1 through 24 hours on stalk A

	Hour of observations																								Sum
	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24		
Number of observations	2	1	2	1	2	2	2	1	5	5	6	4	2	3	2	1	2	2	1	2	1	1	2		51
Presence of individual No 1	2	1	2	1	2	2	2	1	5	4	5	4	2	3	2	1	1	2	1	2	1	1	2		48
Absence of individual No 1										1	1						1								3

Three kinds of reactions to stimuli were differentiated: aggressiveness, indifference, and timidity. In order to get as much such data as possible on each individual, artificial disturbance of peace in the colony was created

Table IV

Attendance of individual No 29

Date of observation	Hour	Presence on stalk A	Presence on other stalks of *Melampyrum*	Presence on the oak
4.IX	10.40	+		
5.IX	10.30		+	
11.IX	19.30	+		
14.IX	9.35		+	
	10.17		+	
	10.45		+	
16.IX	7.15		+	
17.IX	15.50		+	
	16.20			+
18.IX	16.00			+
19.IX	10.30			+
	13.25			+
20.IX	18.25			+
	18.45			+
21.IX	11.00			+
	12.00			+
22.IX	6.25			+
	8.20		+	
24.IX	10.30			+
	10.55			+
25.IX	7.30			+

in many varied ways in order to prevent adaptation. This was done for example by a slight shacking of the whole vessel containing the colony, throwing a foreign body into it, introducing a live insect (large fly, caterpillar, etc.) introducing foreign ants, spraying the nest with water, slight shifting or even complete removal of the glass under which the cell was sited.

The criteria for determining ethological characteristics used in this work are not precize. The work is limited to very general conclusion which do not require great exactness but only the most wide and relative qualifications of specific individuals: excitability and mobility is considered.

RESULTS

The first group of experiments were carried out according to the Ökland and Kiil method with the only difference that besides the species *F. rufa* — *F. pratensis* and *F. truncicola* were studied. The results of the present work are so far in accordance with that of these authors that it is not necessary to describe them. It may be concluded, that specific groups of workers fulfil as a rule the same functions outside the nest.

The second group of experiments was a series of individual observations on ants collecting excreta from aphides.

Table I shows that several individuals continuously for two weeks returned to one stalk of *Melampyrum pratense* 5 cm in length. On the 6th of September rainfall lasting three days interrupted the action of ants and afterwards the plant began to dry up. Besides up from September the 6th, the observer was replaced by another person and as a result this part of the observations concerns only three individuals, with certain markings and could be read by the new observer without possibility of error.

Table II shows the results of observations on one individual, No 1, showing the greatest stability of all those observed. On the 9th of September the *Malampyrum* began to dry up, on the 11th of September there were already no aphids present, however No 1 continued to remain for hours on the deserted stalk. Till the 17th of September, that is six days after complete desolation of the plants, No 1 continued to return to the dried up stalk.

Table III shows the frequentation of individual No 1 during 24 hours. Individuals show considerable differences also in this matter. Only a few workers as No 1 show stability independently of the hour of the day; they could be found before dawn beside the

aphids numbed from the cold and a slight shaking of the stalk knocked them to the ground.

Results of the observations on individual No 29 (Table IV) are very interesting. From the 4th of September this individual was observed several times on a couple of already dessiccating *Melampyrum* stalks. On the 17th it was observed to leave the nest and go in the opposite to usual direction; for a few minutes it was seen wandering round the terrain then was lost from sight. After an hour it was found beside aphids on a young oak plant. From that day No 29 remained on his site till 25th of September when the observations became interrupted.

The next group of experiments to be considered were mainly performed on the species *F. sanguinea* where the colonies lived in artificial nests without earth; these experiments concern the division of labour within and outside the nest.

For several months all workers and their functions in 14 such colonies were individually observed. The sum of the findings are analysed from several points of view and are given in the tables.

Relation between function and size

Out of 13 normal colonies of about 1000 workers only 323 individuals could be used for experiment, because of their functions and their morphological characters could be distinctly defined. Table V shows the quantitative and percentage relation between the dimentions of workers and their functions. Three of the most essential functions have been chosen for the table: 1) care in any forms for the young (sitting on the larvae and pupae, feeding them, transferring, helping in hatching and giving first aid to the young immediately after hatching); 2) inactive presence in the nest (this state has been observed by many authors and its meaning is as yet unknown); 3) active walking about the territory i.e. foraging.

These three groups of functions were chosen as they are the most fundamental: they exclude one another. The result of the experiments shows that this division is the most stable. Within these groups there may be a shifting of function; a worker who carried building material may start to attend to the food or vice-versa; it is also possible to change the terrain of foraging; on the other hand an individual sitting on larvae may start to help in the hatching of the young. It is necessary however to stress that

Table V

Division of size groups according to function (*F. sanguinea*)

Groups of size	Number of individuals	Functions performed					
		Nursing progeny		Immobile presence in the nest		Foraging on terrain	
		Number of individuals	% related to all of the given size	Number of individuals	% related to all of the given size	Number of individuals	% related to all of the given size
Large	174	113	**65**	51	**29**	10	**6**
Medium	65	26	**40**	19	**29**	20	**31**
Small	84	24	**28.5**	13	**15.5**	47	**56**
	323						

Table VI

Division of size groups according to function in colonies No 15 and 16

Groups of size	Number of individuals	Functions performed					
		Nursing progeny		Immobile presence in the nest		Foraging on terrain	
		Number of individuals	% related to all of the given size	Number of individuals	% related to all of the given size	Number of individuals	% related to all of the given size
Large	59	30	**51**	12	**20**	17	**29**
Medium	51	29	**57**	7	**14**	15	**29**
Small	28	14	**50**	5	**18**	9	**32**
	138						

the care for the young and particularly for the larvae and pupae is least liable to be shifted from one individual to another; workers occupied with these functions exhibit the greatest stability (it is possible from this point of view to compare them with workers attending to the aphides).

However with all these shifting within the three groups of functions mentioned above the individual composition of the group remains fundamentally the same. It must be noted that young individuals are not taken into account here, but will be mentioned later.

Returning to Table V, it is at once striking that workers of different size do not take equal parts in fulfilling particular functions. Most of the large workers are occupied with the young, they are also the bulk of the nurses. With small workers apparently, there is a tendency to work on the territory; a smaller number of these remain in the nest, however a few of the smaller individuals are in the nest inactive.

This phenomenon disappears in colonies No 15 and 16 (composed of young individuals). In these colonies, as is seen from Table VI, ants from all size groups take part in the fulfilling of particular functions in equal proportions.

Relation between function and age

From observations on young workers in normal colonies it is found that for the first period of life they remain within the nest, where they mainly perform the functions of nursing the larvae and pupae. For those individuals who afterwards go into the terrain this period in general lasts 4, 5 to 8 days. However there are also individuals who remain permanently in the nest and never venture into the terrain; and there are those who only after two or three weeks go into the terrain. On the other hand workers can be found who already on the first or second day of life leave the nest.

It is also possible to observe the phenomenon described by Goetsch (1953) when a young worker who leaves the nest because of some temporary danger — does not return to fulfil functions within the nest but remains on the terrain. Simultaneously there are other individuals, of the same age and of similar dimensions, who in similar circumstances, return to the nest and take up their previous functions. There are also some who do not react to panic, but stay constantly with the young; others however flee away holding the larvae or pupae in their jaws and after the danger

has passed, return with them to their former places. The latter two types are obviously nurses and it is to be expected, that they will not leave the nest but remain for good beside the young.

All these observations were made as well in colony No 14, as in all other normal colonies.

The connection between function fulfilled and individual behaviour

In search for further causes having a possible influence on the formation of the division of labour in the nest, an attempt was made to analyze the behaviour of workers fulfilling specific functions.

In Fig. 1 all observed workers are again segregated into three groups, this time however not according directly to the character of the function fulfilled, but to the mode of life required to fulfill that function. The criterion was whether in connection with ful-

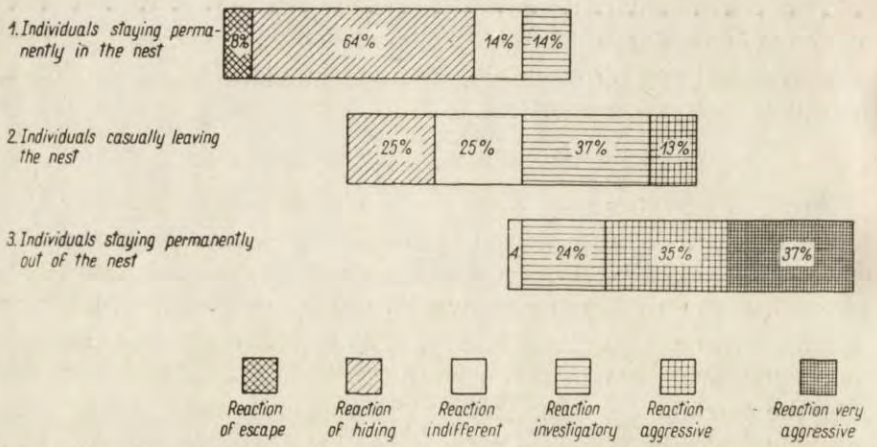

Fig. 1. Reaction types of *F. sanguinea* workers in coordination with the character of performed functions

filling its function a given worker remained permanently within the nest, outside the nest, or whether it divided its time between the nest and the terrain (leaves the nest occasionally). For clarity of results, obtained on the reaction type of each group (which are obviously not numerically equal) is given in percentages.

The table illustrates 6 different types of reaction. A specific behaviour is taken as the criterion for placing each type: an escape reaction means that the animal flees in panic as response to

all disturbances; a hiding reaction means that in a similar situation the animal seeks shelter; an indifferent reaction — that the animal does not react at all to the stimulus and continues its present occupation undisturbed; an ivestigatory reaction is one where the worker is interested in any strange object, (hand, stick etc.), but shows no sings of hostility; an aggressive reaction is when examination of a strange object is accompanied by hostile actions, biting, an aggressive attitude; and finally a very aggressive reaction — when the individual attacks each source of disturbance with extreme hostility. F.e. individuals were observed who, — independently of how long they had been in the colony, — when putting of a watch glass with food was a stimulus for other ants to evok for food in it — each time regularly flung themselves on the watch glass and bit in obstinately for 10—15 minutes (while others were peacefully eating from it). This same very aggressive group also includes those workers, who when somewhat excited, attack even their fellows.

Here is an example of a typical neutral reaction. In a nest artificially flooded with water a nurse sits immobile on a pupa which is already immersed in water; this nurse did not change its position even if, when another worker pulled the same pupa with the nurse sitting on it out of the nest.

Such a division of reaction types clearly shows skiffing of a reaction type according to what functional group it belongs.

It is necessary to describe separately the behaviour of workers in colony 14; they were all taken up with one function, collection of the aphides excreta. This colony, as a whole, was exceptionally passive and neutral, not reacting to any external stimulus including feeding. For a period of 74 days food was given nine times; 5 times no one ant participated in feeding, for the remaining 4 times one, two, or, in one case, three ants ate together. The death rate in this colony was higher than in any other: after 30 days out of 82 workers only 13 remained alive and 7 young who hatched meanwhile. It is necessary to mention that care of the young was the only function fulfilled to a certain extent, owing to this fact some of the pupae hatched out.

DISCUSSION

Many authors claim that workers in the early period of life nurse the young (Forel 1874, Lubbock 1882, Perez 1900,

Wheeler 1907, et all.). However, in general, those who studied the effect of age on the division of labour in ants more closely, like Buckingham (1911), Goetsch and Eisner (1930) state that this is not an absolute rule. That is why the latter two authors taking age as the basis of the division of labour in the genus *Messor,* introduce a correction based on morphological differences. In the same way Ehrhardt (1931) supplements the role of age by what he terms the individuality („Individualität") of a given individual but does not give a satisfactory explanation of what is meant by this.

Observations in the present work confirm that young individuals mainly nurse the young, but not all. On the other hand in colonies 15 and 16 where all workers were young, a normal division of labour took place. This does not provide a decisive proof of the lack of influence of age on performing the function; in bees for example, dependence of function on age has been definitely established, the possibility of functional transference (as necessary for the family) which in bees demands concrete changes in physiological functions has been nevertheless experimentally demonstrated (Rösch 1925, 1930; Lindauer 1952).

Further facts seem to support the opinion of Lubbock (1882) and K. Heyde (1924), that the inclination of young workers to nursing does not came from a physiological predisposition to fulfil that function, but from the need of the young individual to remain in the nest for the initial period of its life. Above all, some of the young ants passively present in the nest, as stated previously in the present experiments, do not fulfil any function. Staying in the nest at an young age was also observed in those species where the young do not nurse at all. Ledoux (1949) claims that *Oecophylla longinoda* for the first 10—15 days of its life stays beside the female completely passive and afterwards leaves the nest at once for food. An interesting observation was made by Goetsch, that young ants before leaving the nest, first gather together near the exit as if some sort of inhibition was acting. Finally an observation by Goetsch confirmed by the present work is most suggestive; this is that often a young ant, who in face of danger deserts the nest, — remains on the terrain and does not return to its previous functions in the nest (it is mainly nursing). All the evidence suggests that a young worker has a certain type of in-

hibition before leaving the nest for the first time. It is also possible to associate this sort of behaviour with the softness of the chitin in young ants (Lubbock), with its underdeveloped sight (Goetsch), the gradual development of certain reflexes (Heyde, Kiil) and consequently with the necessary gradual adaptation of the young organism first to the nest and then later to the outside world.

Thus the fact that in certain species young workers begin their role with the function of nursing, cannot be taken as proving that this function is physiologically determined. The fulfilling of this function remains connected with the remaining during the early period of life in the nest.

The next question to be discussed is that of the morphological differences and their influence on the division of labour in ants, which has been considered as decisive by many observers. At the beginning it is worth while remembering that polimorphism is not in the least limited to animals living in communities and exhibiting so called division of labour. On the other hand there are social insects (bees) which have a division of labour but do not however exhibit the second degree polimorphism within the caste of workers. Thus, it would appear to be a misunderstanding to look for the genesis of the division of labour in the polimorphism of the worker caste.

The present work was carried out only on a species of the genus *Formica*, therefore it can only deal with incomplete polimorphism, where the worker caste, independently of the range of internal fluctuations, is however a single caste (that is why it was possible to consider only the size of the workers, not taking into account the difference of form).

In the light of the observations of Westwood (1841), Bates (1863), Reh (1897), Vosseler (1905), Kiil (1934), Bernard (1951), Raignier and v. Boven (1955), Morley (1946) it is necessary to give up the supposition that morphological variations within a polimorphic worker caste have a direct simple connection with the division of labour, that morphological and functional division are correlated. On the other hand however the results of the observations and experimental work of Adlerz (1886), Pricer (1908), Buckingham (1911), Goetsch and Eisner (1930), Erhardt (1931), Ökland (1931), Kiil (1934) together

with those of the present work point out to the existence of a certain connection between morphology and function. Undoubtedly it cannot be regarded as accidental that the distribution of workers of various dimensions is not the same for all functions. It is obvious however, that these relations are very unstable and ambiguous. These authors claim that fulfilling specific functions within a nest is prevalent, but only prevalent, in workers with certain morphological character. At the same time there exists a discordance in the relation between the range of size and functions in various genera and even species of one genus. For example, according to the data of Forel (1874) in *Atta sexdens* the function of cutting leaves is performed mainly by small workers whereas in *Atta cephalotes* the same function is performed by large workers. A second example: data on this type of relations in different species of the genus *Pheidole* from such workers as Heer (1852), Lespes (1863), Forel (1874), Büchner (1876), Wheeler (1902 and 1907), Weismann (1894), Bernard (1951) are completely different. This considerable variation in the relations between morphology and function in very closely related species suggests that this correlation was not at the basis of the philogenesis of the division of function. Otherwise we would have to assume that the labour division developed independently and afresh in each individual genus and even species. It is impossible to hold this view because of the lack of correlation between the range of sizes and function fulfilled is often found in very closely related species which must have had a common origin in a relatively recent period. Whereas the social life in ants and therefore their division of labour, is according to general opinion, of much earlier origin.

It is pertinent to inquire whether a certain correlation between morphological differentiation and division of labour is necessarily an evidence of their interdependence? In fact, such a correlation may result from a common origin of both phenomena, or a common dependence on some third factor.

The origin of the division of labour should therefore be sought in some other factors. These factors must simultaneously exert an influence on the morphological differentiation of worker ants and determine a certain correlation between the two phenomena under discussion.

Analysis of Fig. 1 discloses a connection between the division of labour and a new factor: there exists an obvious correlation between the function performed and the individual behaviour characteristics of the workers.

Lubbock (1882), K. Heyde (1924), Stäger (1924—25), Natzmer (1915), Ehrhardt (1931), Kiil (1934), Morley (1946), Schneirla (1950) have all indicated wide individual variations in the behaviour of ants. The present work has confirmed the wide range of these variations. While some workers react to every external stimulus, even to the slightest change in the situation and readily interrupt the carrying out of some function, others continue their occupation in spite of all changes in the situation. The type of reactions to some danger threatening the nest are complately varied: some individuals jump to defence the nest, others hasten to escape carrying the young away, some seek shelter in the nest, and finally others, particularly indifferent (mainly nurses), simply stay with the young and react in no visible fashion to the danger.

One can therefore speak of different types of reaction among workers from the same nest.

Now Fig. 1 shows that depending on the type of reaction exhibited, some individuals carry out functions which do not demand mobility and which are to the least extent affected by external factors; while others on the contrary perform tasks demanding constant movement. The latter individuals are workers which are constantly working, their energetic temperament expressing itself in a frequent change of task, unmethodical performance of these tasks, rapid reaction to stimuli and finally great agressiveness.

To that group of functions demanding great mobility and excitability belongs the task of carrying the prey and building materials to the nest. An observation of Stäger is of interest. Studying the division of labour he found in a *F. rufa* carrying out these tasks, that the workers of this species are incapable of performing a given task for any length of time, reacting strongly to the slightest stimulus. This is in complete accordance with the present findings. Stäger, however, did not take into account the fact that these characteristics are true only for those workers, performing the functions which he was studying, and he unjustifiably generalised his findings to all the workers of this species. Observations

of workers engaged in the collection of the excreta from aphides would have led to completely different conclusions about the workers of *F. rufa*.

A number of workers have shown (Natzmer, Stäger, Ökland 1931, Kiil) that the individual composition of groups of ants performing the two above mentioned functions is variable. This fluctuation however considerably decreases if we accept the suggestion of Ökland and Kiil that these two functions (transporting building materials and prey to the nest) should considered as one which can be called foraging. It follows from this that although foraging workers are not permanently employed they change their function for another of a similar character, since just such a character of function suits their type of reactions. Thus we can list the individual characteristics of ants fulfilling these functions as follows: instability, submitting to incidental stimuli, which give a picture of a mobile, excitable animal. Undoubtedly such characteristics are necessary for foraging which exposes the workers to all sorts of stimuli and dangers from the outside world. This gives the common character to all functions connected with foraging.

Quite in contrast with this group of functions are those connected with the interior of the nest (nursing, passive presence in the nest). We also have data which indicates that the function of collection the excreta from aphides is similarly a passive function. Despite appearances it demands minimum of mobility (carrying the excreta to the nest two or three times in 24 hours), keeping to a fixed trail which in general is not exposed to change or any dangers (due to the constant movement along it); it is as if such a path were a continuation of the nest on the outside. Experimental verification of the correctness of such an evaluation of the function of collection the excreta from aphides is to be found in the results obtained from colony No 15. This colony was unable to survive and perished, the only function fulfilled however was nursing the young (in the absence of aphids). It was observed that in *F. sanguinea* this function is for the most part fulfilled by large ants, just as within the nest. There exist also an opinion (Natzmer, Stäger, Ökland, Eidmann 1927, Kiil, Dobrzańska 1958), that the individual composition of the group of ants fulfilling this function is very stable.

In view of this the theory put forward by Buckingham (1911), that individuals sitting inactive in the nest are a defence reserve, appears untenable. The ability to defend is directly associated with excitability and mobility of the ant. Passive immobile individuals are also not warlike. The present work shows that in *Formica* small ants are the most warlike and for the generally they are the most mobile and excitable specimen which agrees with of Buckingham data (work on *Camponotus americanus* and *C. herculeanus*).

Building (digging) and rubbish removal can be included among intermediary functions requiring mobility but less subject to external influences. The present work however, did not give close attention to these functions.

Thus, if we divide all functions within the ant nest into three groups (given in Fig. 1) according to their character, it is seen that with the fulfilling of a specific characteristic function, the individual characteristics of each ant are linked. One must conclude therefore that the actual division of labour within a given nest results from the individual variations in the behaviour of the workers.

This formulation however does not in itself solve the problem. It is necessary to find the causes, which involve individual variations in the behaviour within a nest; at the same time these same cause must — as stated previously — have an influence on the morphological differentiation in the workers.

Wesson (1940) and Goetsch (1953, 1955) have substantiated experimentally the hypothesis of Emery (1894, 1915), that the polimorphism of the worker caste is determined by nutrition in the larval stage. If this is so, even in dimorphic species, then the immediate influence of nutrition is even more probable in species with an incomplete polimorphism. Besides direct observations also substantiate this: there arises in each nest generations of various dimensions, depending on the prevalent factors, acting their embryonic development. This phenomenon can be very successfully brought about in an artificial nest. But in natural conditions it is also possible to observe for example, that in a young nest workers are on the whole small since, due to lack of labour power, they were less fed during their embryonic development. The season of the year should also have a similar effect; one can

expect for example that in the hatching period of the sexual generation, when the demand for food is increased, while the number of working members does not increase — the next generation of workers feeding would be poorer. Therefore the period in which a given generation of workers comes into being is decisive as far as the morphological characteristics are concerned. Periodicity of the nest development is qualified by the history of the nest and by the external factors. Even casual occurrences may play some part here: sudden drop in temperature, rain etc. This is of course only a general rule: one can imagine that a particular larva might by chance get more food, or that a larger than usual prey might temporarily increase the food supplies of the nest at a decisive period in the development of some of the larvae.

It would seem that the above argumention may account for the fact that a number of authors (Alpatov and Palenitschko 1925, Palenitschko 1927, Kiil 1934) have obtained a multipeaked curve of variance of the asexual castes for various ant species. The morphological variance of the worker castes is not a result of ordinary individual variability involved by casual factors; besides certain regularities of the nest development play a part here, and do not affect the individuals but the whole generations. These relations are therefore more complex and cannot be expressed as a simple single peaked variance curve.

We therefore have the factors which exert an influence on the morphological differentiation of the worker caste. In the author's opinion these same factors determine the behaviour characteristics of a given generation.

In conditions, difficult for the colony, causing the development of a stunted generation, an increased activity of the workers is demanded. The smaller (younger) the colony is the sooner must the worker leave the nest in search of food, and has necessarily to fulfil an ever-changing variety of tasks. A similar role is played by the season of the year: according to the season there will be in the nest a greater or smaller number of the young in need of food, which would be more or less difficult to be procured, so that there may or not be demanded an immediate activity of the workers being hatched. In other words all these factors which influence the size of a given generation, also affect its behaviour characteristics. Workers born in difficult conditions (i.e. mainly small) have to

undertake a number of functions and leave the nest at an early age; they therefore become active and energetic. On the other hand workers hatched in a period of prosperity (and therefore of larger size) may succumb a longer time to a natural inclination to stay in the nest for since food is plentiful and all the needs of the colony are easily satisfied by the older generation; in other words there is no reason for leaving the nest too early and there is nothing to stimulate the activity in these individuals. Such a generation remains therefore slow, indifferent to stimuli and inactive.

The effect of breeding i.e. the development conditions in the post-embryonic period, upon the character of an individual, upon the types its reaction are demonstrated by the data from colonies No 14, 15 and 16. In colonies 15 and 16 all the workers, independently of their size, were bred while young in similar conditions, they did not exhibit the normal tendency of workers of a specific size to carry out certain functions, and the division of labour developed independently of morphological differences. In colony 14, mature workers, with fully formed individual characters failed to change their usual functions for some new tasks: passive and unexcitable individuals which had been engaged in work with the aphides were only fit for nursing the young, and for this reason the colony perished. Such a colony in which all the individuals are passive is an unnatural and artificial union. In natural conditions, in order that some of the workers could passive, others must be active.

This confirms an observation by B u c k i n g h a m that large workers, which in her opinion of are lazy and of minor activity, are more active when the colony is small. According to B u c k i n g h a m's also the enhanced activity of smaller workers results from the fact that they appear first in a young colony and the female burdens them with all possible work.

As is seen, there is here a certain consistency: much activity is demanded of a stunted generation, whereas a generation of larger sized individuals is born into a world of better conditions, in which enhanced activity is not necessary for the survival of the colony.

It should be emphasised once again, that the correlation between individual characteristics and size is, and should only be, relative. Apart from the role of casual effects, of which mention

was made above, one must also take into account the fact, that the pupae stage in ants lasts several weeks. Thus it may often happen that generations, which had poor conditions at the larval stage (and which therefore are of small size), may after hatching be faced with better conditions in which their behaviour characteristics (reaction types) will not correspond to their morphological feature. These two phenomena will therefore never be in a perfect harmony; according to Adlerz the maximal correlation between the function and the size of workers amounts to 60—75%. The exceptions mentioned in the present work would account for the remaining 25—40%. Thus the author's hypothesis explains the existence of a certain, but not complete convergence between morphological features and the division of labour, which could not be accounted for on the basis of the previous theories.

It seems that this hypothesis may apply in broad outline to all species exhibiting incomplete polymorphism. Differences between species may consist in the fact, that for given species another functions are of prime importance, and that a different activity is necessary in performing this or other functions. For example, one could imagine, that for the genus *Atta* (the author knows this genus only from the reports), the function demanding great activity and vitally necessary for the survival of the nest, is the culture of fungi. This might explain why in this genus the smaller individuals generally remain in the nest, which is in contradistinction to the case of *F. sanguinea*.

An analysis of this kind carried out for other species, and taking into account their actual biology, might explain many conflicting data in this field.

After having sent the present paper for print I read the publication of Otto (1958*), which unfortunately was not available to me earlier. We have before us a case, when two different experiments, carried out by partly different methods lead te the same authors' conclusions.

The slight differences in results are to be accounted for by

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* OTTO Dieter 1958 — Über die Arbeitsteilung im Staate von *Formica rufa rufo-pratensis* Gössw. und ihre verhaltensphysiologischen Grundlagen. Wissenschaftliche Abhandlungen Nr. 30, Deutsche Akademie der Landwirtschaftwiss. zu Berlin.

modifications in the experiment method. The species characters seem to be also responsible for the majority of differences in our results.

The consistency of our results is as more valuable to me as Otto aplied much more extensive research, which enabled him to precise his conclusions in those cases, where my own method allowed to formulate my results only in a hypothetical form.

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Acta Biologiae Experimentalis. Vol. XIX, 1959

EFFECTS OF ADAPTATION TO ENVIRONMENT ON CHEMOTAXIS OF *PARAMECIUM CAUDATUM*

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(Received 20 September 1958)

The modifying effects exerted on protozoan motor reactions by adaptation to a stimulus are a rather common phenomenon. Known examples include adaptation of protists to light (Mast and Pusch 1924, Mast 1932, 1937), mechanical stimuli (Jennings 1905), and temperature (Mendelsohn 1902). Other data relate to motor reaction modifications due to adaptation to chemical stimuli (Oliphant 1938, 1942, Okajima 1954).

As regards chemotaxis, changes in such reactions are well known in *Paramecium aurelia*, but their origins are obscure (Jennings 1905). An earlier report (Dryl 1952) covered my own observations concerned with the effects of adaptation of *Paramecium caudatum* to KCl solutions on chemotactic reactions. In absence of more accurate experimental methods, I was unable to explore the phenomenon of adaptation in more detail. A new photographic method for recording protozoan locomotion (Dryl 1958) and a quantitative method for investigating chemotaxis (Dryl 1959) have offered new opportunities of experimental research concerned with the subject.

MATERIAL AND METHODS

The investigations concerned a pure strain of *Paramecium caudatum* isolated in 1955 in a laboratory of the Department of Experimental Biology of the M. Nencki Institute of Experimental Biology, Warsaw. The Infusoria were grown in a nutrient prepared from 2 or 3 g. of triturated dry hen-egg yolk in 1 litre of tap-water. The nutrient medium was changed every 6—8 days.

In investigating the effects of 24-hours adaptation of Infusoria to solutions of $MgCl_2$ (30 mM), $CaCl_2$ (30 mM), NaCl (40 mM), and KCl (20 mM), on chemotaxis, use was made of the quantitative method described in the preceding report (Dryl 1959), except that the Infusoria were not washed in pure water prior to the experiment, as this would defeat the purpose I had in view. The Infusoria were collected with a micro-pipette directly from the thigmotactic accumulations invariably found in the medium to which they were being adapted, and a thick portion was transferred into the pure water spread over a glass plate and cautiously but thoroughly stirred. Subsequently, as quickly as possible, pure tap-water was added dropwise as control, and the four solutions investigated were instilled likewise in succession.

The results of adaptation of Infusoria to a 40 mM solution of KCl were examined in a somewhat modified manner involving photographic registration of protozoan locomotion (Dryl 1958). Here use was made of the fact that Infusoria adapted to a 40 mM solution of KCl are largely immune to chemical stimuli and therefore spontaneously enter the area occupied by pure tap-water after they were deposited with a pipette on the periphery of the water on the glass plate used for observation of chemotactic reactions. The control solution (pure tap-water) and the solutions investigated were instilled on the areas outlined by the suitable marked squares immediately after the Infusoria have passed into the medium, and 2-second macrophotographs were made 10—15 seconds after the last drop of the solutions examined had been instilled. This made possible quantitative evaluation of chemotaxis after an interval of only 15 seconds since the moment when the Infusoria left their former environment, which is of considerable practical importance in view of the fact that the changes revealed in the reactions of „adapted" Infusoria to high concentrations of the substances involved are rapidly disappearing.

RESULTS

Tables I and II record the results of 24-hours adaptation of Infusoria to solutions of $MgCl_2$ (30 mM), $CaCl_2$ (30 mM), NaCl (40 mM), and KCl (20 mM). Adaptation to solutions of $MgCl_2$ and KCl is non-specific, since chemotactic reaction to solutions of quinine is in either case distinctly reduced as compared to controls. Adaptation to solutions of NaCl diminishes chemotactic sensitivity to this substance, but appears to have no major effect on reactions to solutions of quinine. Infusoria adapted to $CaCl_2$ however, exhibit slightly increased chemotactic sensitivity to quinine, but this must be considered as an exceptional phenomenon. The data shown in Tables I and II refer to chemotactic sensitivity exhibited by adapted Infusoria after they were kept for roughly 1 minute in pure tap-water, as this is about the time needed for transferring

and stirring the Infusoria and instilling the solutions under investigation on the areas of the four squares on the glass plate.

Phot. 1 (2-second macrophotograph) shows the effects exerted on chemotactic reaction to quinine by adaptation of Infusoria to a 40 mM solution of KCl. The Infusoria stop at the boundary of 1.14 and 0.759‰ solutions of quinine and die almost instantly as is shown by the white dots around the squares in the photograph. Occasional Infusoria enter the 0.506‰ solution of quinine, although some of them also die almost instantly. The Infusoria enter a 0.337‰ solution of quinine without any reaction, although this concentration is about 10 times the threshold value (0.03‰) for non-adapted Infusoria. Consequently 0.506‰ must be looked upon as the threshold value for quinine concentrations with regard to *Paramecium caudatum,* adapted to 40 mM KCl, since the infusoria show at the boundary of higher quinine concentrations a ne-

Phot. 1. Exposure time = 2 seconds. Chemotactic response of *Paramecium caudatum* to quinine solutions after 24hs adaptation to 40 mM KCL

gative chemotactic response, or die in such concentrations, whereas they freely enter lower concentrations without exhibiting any chemotactic reactions. Analogical threshold concentrations with regard to Infusoria adapted to 40 mM KCl are: for $MgCl_2$ and NaCl, 225 mM, and for ethanol 759 mM. On the border of higher concentrations the Infusoria died similarly as in the case of 1.14‰

Table I

Changes in the chemotactic response of *Paramecium caudatum* to $MgCl_2$ and NaCl solutions caused by adaptation over 24 hours to 30 and 40 mM solutions of $MgCl_2$ and NaCl respectively; m = average number of Infusoria calculated from 10 successive photographic records; s = standard deviation; % = percentage of Infusoria calculated with reference to control as 100

Concentration in mM	NaCl (Infusoria non adapted)			NaCl (Infusoria adapted 24 hs to 40 mM NaCl)			$MgCl_2$ (Infusoria non adapted)			$MgCl_2$ (Infusoria adapted 24 hs to 30 mM $MgCl_2$)		
	m	s	%	m	s	%	m	s	%	m	s	%
60	—	—	—	7.1	±1.3	8.1	—	—	—	—	—	—
45	8.1	±1.84	6.9	17.8	±1.76	20.2	—	—	—	—	—	—
30	14.5	±1.92	12.3	40.2	±3.66	45.7	—	—	—	8.0	±1.55	6.3
20	63.6	±6.43	53.9	72.1	±3.52	81.7	5.1	±1.23	5.8	21.5	±2.66	17.1
13.5	84.4	±5.75	71.6	—	—	—	31.3	±3.36	35.6	30.8	±3.44	24.4
9	—	—	—	—	—	—	42.2	±3.92	48.0	95.3	±7.08	75.6
6	—	—	—	—	—	—	54.3	±6.55	61.7	—	—	—
Control	118.1	±4.39	100	88.2	±4.96	100	87.9	±5.57	100	125.6	±6.91	100

Table II

Changes in the chemotactic response of *Paramecium caudatum* to quinine solutions caused by adaptation over 24 hours to media of various chemical compositions

Concentration of quinine in %	Infusoria non adapted			Infusoria adapted 24 hours to 30 mM $CaCl_2$			Infusoria adapted 24 hs to 30 mM $MgCl_2$			Infusoria adapted 24 hs to 40 mM NaCl			Infusoria adapted 24 hs to 20 mM KCl		
	m	s	%	m	s	%	m	s	%	m	s	%	m	s	%
0.045	—	—	—	—	—	—	5.9	±1.22	6.5	—	—	—	11.9	±1.45	8.3
0.03	1.4	—	1.4	0.7	—	0.6	27.5	±2.51	30.2	9.2	±1.34	6	30.4	±2.00	21.3
0.02	23.5	±2.17	24.2	7.2	±1.26	6.4	46.0	±3.58	50.6	16.0	±1.84	10.4	48.4	±3.08	33.8
0.0135	45.8	±4.67	47.2	31.3	±2.26	27.7	75.9	±4.74	83.4	114.4	±7.09	74.8	95.6	±3.94	66.9
0.009	85.7	±4.72	88.3	88.8	±3.32	78.6	—	—	—	118.2	±8.55	77.2	—	—	—
Control	97.1	±3.54	100	112.6	±4.75	100	91.0	±5.53	100	152.9	±7.12	100	143.2	±4.73	100

quinine, whereas they entered the lower concentrations without any signs of locomotor disturbances.

Table III

Chemotaxis of *Paramecium caudatum* in relation to different pH of the environment. The Infusoria were placed in a buffered mixture NaH_2PO_4/ /Na_2HPO_4 showing a pH of 6.4. Buffered solutions of a concentration of about 10 mM, with an addition of 1.5 mM $CaCl_2$. Notations: m, s, and % as in Tables I and II

pH of buffered solution	Number of Infusoria			pH of buffered solution	Number of Infusoria		
	m	s	%		m	s	%
4.5	7.1	—	5.2	8.2	0.3	—	0.2
5.22	186.5	±7.57	136	7.68	3.4	—	2.5
5.78	184.5	±7.58	135	7.17	14.5	±2.21	10.6
6.17	190.1	±8.84	139	6.72	129.4	±5.48	94.4
Control	137.0	±6.40	100	Control	137.0	±7.77	100

The results referred to show almost complete inhibition of chemotactic response in Infusoria adapted to 40 mM solutions of KCl, since the residual chemotactic reaction, if any, fails to protect

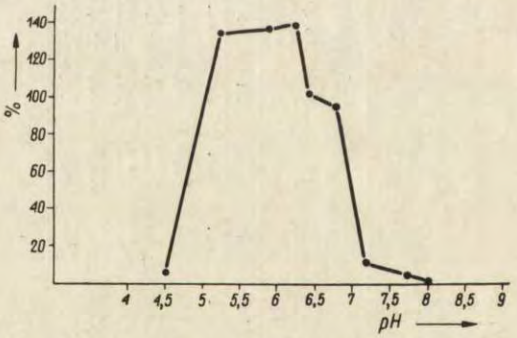

Fig. 1. Chemotaxis of *P. caudatum* in relation to pH of environment expressed in percentage of Infusoria in the solution investigated with reference to control = 100

the Infusoria against contact with concentrations lethal within 1 or 2 seconds. The results also confirm the thesis claiming chemotactic adaptation to KCl solutions to be non-specific.

Table III shows numerical data relating to chemotactic response of *Paramecium caudatum* to various pH values of the medium. The experiments involved buffered solutions of NaH_2PO_4 Na_2HPO_4 used in various ratios and a total concentration of 10 mM, with an addition of 1.5 mM $CaCl_2$. The solution was spread in a 0.4—0.5 mm. layer on the glass plate, and portions of experimental Infusoria were deposited with a pipette in the corners of the plate. The Infusoria spontaneously passed into the buffered solution since it had positive chemotactic properties (pH = 6.4) with reference to the medium in which the Infusoria were kept before the experiment (pH = 7.7—8.0). After a few minutes, solutions of various specified pH were instilled on the areas of the four suitably marked squares. The successive close-range photographs were made at 5 seconds intervals as required by the quantitative method for investigating chemotaxis in protozoans. The results are recorded in Table III which shows the arithmetic mean (m) from 10 determinations, standard deviation (s), and percentage calculated with reference to control = 100. Graphically, the results are shown in Fig. 1.

Optimum chemotactic pH ranged between 5.2 and 6.2, which is in agreement with the data reported by Johnson (1929) and myself in an earlier paper (Dryl 1952).

Infusoria adapted over 24 hours to a 40 mM solution of KCl failed to show chemotactic response to the buffered solutions referred to above. In a solution whose pH was 4.5 a number of infusoria died within some ten or slightly more seconds.

Changes induced in the chemotactic reactions of Infusoria by adaptation are of short duration and, therefore, the experiments concerning effects of adaptation have to be performed as quickly as possible since sensitivity to higher concentrations of the substances involved becomes fairly rapidly normal in pure tap-water. Investigations concerned with the duration (time of extinction) of changes in the chemotactic reactions were run with Infusoria adapted for 24 hours to 40 mM KCl, with a testing solution of 0.03‰ quinine, known to elicit in non adapted Infusoria a negative chemotactic response. In these experiments a glass plate (8 × 12 cm.) was covered with a thin (0.4—0.5 mm.) layer of water similarly as in other experiments, and placed on a black background with two 1 cm. squares drawn in white ink. The squares were

marked, one — 0.03‰ quinine, and other — control. Adapted infusoria were placed on the four corners of the plate and this marked the beginning of the experiment. Within 30 seconds the infusoria were evenly distributed in the layer of tap-water, whereafter a 0.03‰ solution of quinine was added drop-wise on the area outlined by the corresponding square. The first and subsequent

Table IV

Duration of chemotactic adaptation of *Paramecium caudatum*. The Infusoria were adapted over 24 hours in a 40 mM solution of KCl. The figures in the columns indicate the number of Infusoria in pure tap-water (Control) and in 0.03‰ quinine in relation to the interval since the moment when they were transferred into the pure tap-water on the glass plate

Time in minutes since transfer to tap-water	2	4	6	8	10	12	14	16	18	20	25	30
0.03‰ quinine	50	44	32	33	24	20	15	14	12	4	4	2
Control (tap-water)	56	50	53	52	47	49	46	50	53	52	56	57

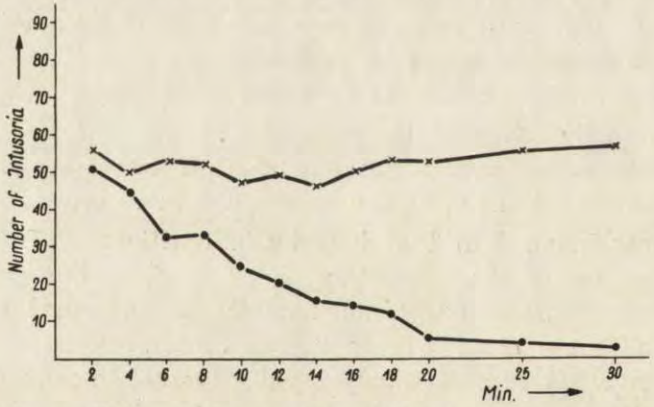

Fig. 2. Time of disappearance of chemotactic adaptation. Infusoria adapted to 40 mM solution of KCl over 24 hours
× — number of Infusoria in pure tap water;
• number of Infusoria in 0.03‰ solution of quinine

macrophotographs were made at 2-minutes intervals. Extinction of the effects of adaptation was judged by the diminution of the number of Infusoria within the square occupied by the 0.03‰

solution of quinine as compared to the control square. The results are recorded in Table IV and Fig. 2.

As will be seen from the data recorded, the number of Infusoria within the area occupied by the 0.03‰ solution of quinine diminished continuously over an interval of 20 minutes since the beginning of the experiment, whereas in the control square there were small numerical fluctuations. The effects of 24 hours adaptation of Infusoria to 40 mM KCl may thus be assumed to vanish within approximately 20 minutes.

DISCUSSION

Effects of adaptation on chemotactic reactions of *Paramecium caudatum* have been successfully demonstrated with regard to solutions of KCl, $MgCl_2$, and NaCl. Adaptation was most conspicuous in the case of KCl, whereas the effects of the other two substances were less marked. The phenomenon is non-specific in character as was demonstrated most strikingly in the case of 40 mM KCl which for a time completely suppressed chemotactic response to substances widely different in physiological and chemical respects, such as NaCl, $MgCl_2$, quinine, and alcohol. Infusoria adapted to 40 mM KCl also failed to react to differences in pH, which lends further support to the thesis claiming adaptation to be non-specific.

Biologically, diminution or suppression of the chemotactic response is harmful since it eliminates the natural defence mechanism protecting the animal against numerous injurious agents present in the environment. Complete inhibition of chemotactic response to differences in pH makes it impossible for the Infusoria to collect in places abounding in bacteria and nutritive substances which slightly acidify the environment where present. The eminent role of potassium in chemotactic adaptation is worth emphasizing for two reasons: 1) potassium salts are notably effective in modifying chemotactic response, and 2) potassium salts may play an important role in the life of Infusoria since they are present in substantial amounts in all the components of organic origin present in the environment.

In experiments run with *Paramecium multimicronucleatum* (Oliphant 1938), adaptation to media containing salts of sodium, lithium, or potassium, involved inhibition of ciliary

movement reversal in lower concentrations, and reduced duration of reversal in concentrations higher than that to which they had been adapted. Also in *Opalina* (Okajima 1954), adaptation to a medium containing KCl was noted to reduce sensitivity to chemical and electrical stimuli. As may be seen, the inhibiting effects of potassium salts on protozoan motor reactions are rather common, though still little explored.

By experiments run with 0.03‰ quinine, the chemotactic response of *Paramecium caudatum*, modified by adaptation to KCl, was demonstrated to be restored to normal within roughly 20 minutes. Hence the conclusion incident to laboratory practice that *Paramecium* should be thoroughly washed for at least half an hour in pure water prior to any experiments concerned with motor reactions, if the genuine reaction to chemical stimuli is to be explored.

Chemotactic adaptation in Infusoria explains numerous facts associated with variations in motor reactions. The possibility of such adaptations ought to be reckoned with in all experiments of an ethological character, and especially in those concerned with processes of „learning" in protozoans.

SUMMARY

Paramecium caudatum adapted over 24 hours to a medium containing $MgCl_2$, NaCl, or KCl, displays reduced chemotactic sensitivity towards quinine solutions. The diminution is most notable in the case of KCl, and successively less so in the cases of $MgCl_2$ and NaCl.

Adaptation to KCl and $MgCl_2$ is non-specific in nature. Adaptation to 40-mM KCl was noted to modify chemotactic reactions with regard to a wide variety of chemical compounds, such as salts (NaCl, $MgCl_2$), quinine, and ethanol. Adapted Infusoria were also found to be completely unable to react to differences in pH. Chemotactic sensitivity is diminshed by adaptation to 40 mM KCl to such an extent that it fails to protect the Infusoria against almost instantaneous death on the borderline of high concentrations of the substances investigated.

The changes caused by adaptation to 40 mM KCl in chemotactic reactions last about 20 minutes.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

CHEMOTACTIC AND TOXIC EFFECTS OF LOWER ALCOHOLS ON *PARAMECIUM CAUDATUM*

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(Received 20 September 1958)

Depending on concentration, alcohols may have varied effects in biological processes in protozoa. Some research workers (Daniel 1909, Loefer and Hall 1936) believe non-toxic concentrations of ethyl alcohol to accelerate the rate of division of protists. In media devoid of nutrition, survival of *Paramecium caudatum* was prolonged by addition of ethyl alcohol (Bills 1924a). However higher concentrations of alcohol and of other narcotics gave rise to conspicuous toxic symptoms, such as slowing of movements, reduced rate of formation of food vacuoles, and slower pulsation of the contractile vacuole (A. Goldschmied-Hermann 1935a). Comparative studies on the narcotic and toxic effects of lower alcohols on *Paramecium caudatum* have shown these to be in direct proportion to the molecular weight of the alcohol in question (Bills 1924b).

A stimulus for the research reported in this paper was given by the interesting observation of A. Goldschmied-Hermann (1935b) who noted that *Paramecium caudatum* introduced into a drop of a 1,2 or 3 per cent. solution of ethyl alcohol gather after a few minutes on the periphery of the drop where they move very slowly, if at all. The author referred to suggested that this may be due to evaporation of ethanol on the periphery and consequent relative diminution of concentration as compared to the central area of the drop. Under that conditions, the Infusoria avoided the higher concentration in the central area. This suggested

a negative chemotactic reaction in protozoa with regard to alcohol, and the surmise was confirmed in my subsequent experiments which revealed in protozoa a distinct negative chemotactic reaction with regard to all lower alcohols. This created an opportunity for comparative studies on chemotaxis and toxic effects of alcohols from the viewpoint of correlation between the two phenomena which is the subject of the work under report.

MATERIAL AND METHODS

A pure stain of *Paramecium caudatum* was isolated in 1955 in the Biological Department's laboratory, M. Nencki Institute of Experimental Biology, and subsequently grown in a mass culture in a nutrient prepared from 2 or 3 g. of triturated dry hen-egg yolk in 1 l. of tap-water. Thick and well developing cultures of infusoria were grown in wide-bottom flasks with free access of atmospheric air. A day before the experiment, thick portions of the infusoria were collected with a pipette from the thigmotactic ring formed by the protozoa beneath the surface of the liquid in the 1st or 2nd day after addition of the nutrient. This first portion of Infusoria was diluted between 10- and 20-fold with tap-water and subsequently transferred into a flask with a long narrow neck. Within 2 or 3 minutes, the Infusoria produced a thick geotactic accumulation in the neck. This portion was decanted into a small beaker and diluted with a small volume of water. After some time, the infusoria collected on the bottom producing a kind of white sediment which was again transferred with the aid of a micropipette into a flask with pure water. They collected in the neck, were decanted into a small beaker, and left for 24 hours. On the day of the experiment, the *Paramecium caudatum* specimens were again washed in water in order to have them in the purest possible aqueous medium. The animalcules so treated will be referred to further below as "experimental Infusoria".

The experiments were run in Warsaw tap-water containing roughly 150 mg. of $CaCO_3$ in 1 litre, pH being 7.8—8.0, and temperature 21—23°C. Aqueous solutions of alcohols were also prepared with tap-water. Determinations of pH were carried out with the aid of a Cambridge Instrument Co potentiometer of an accuracy of up to 0,01 pH.

Investigations concerning chemotaxis were carried out in a dark-room. Detailed procedure in conducting experiments was as follows.

A glass plate, roughly 8 by 12 cm., with a rough matted margin 1 cm. wide is placed strictly horizontally on the stage of a magnifying glass. A definite volume of water (about 5 ml.) is spread over the surface of the glass plate until it reaches the ground margin which prevents it from covering an area larger than originally intended. The thickness of the aqueous layer should not exceed 0.5—0.6 mm. The glass plate is put on a black background and a slip of celluloid with the required drawings or inscriptions in white ink is inserted between the two.

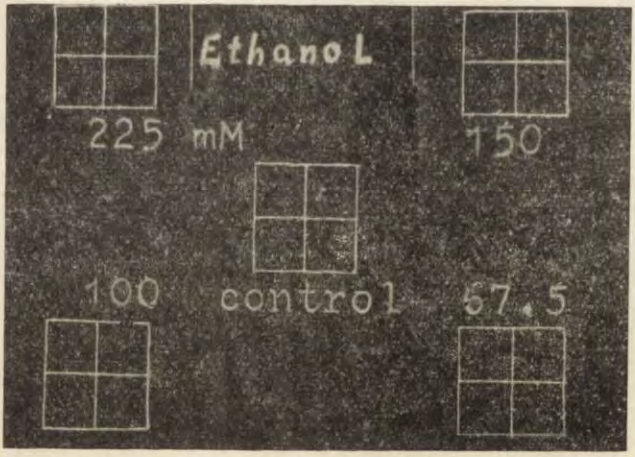
Beams of light from two projectors on either side of the glass plate, are directed through heat filters parallel to the plate whereby a dark-field illumination effect is obtained. A camera suitably adapted for close-range photographs is fixed vertically above the plate in a stand which makes possible easy regulation of distance between camera and object.

When all preliminary arrangements are made, a thick portion of experimental Infusoria (roughly 0.5 ml.) are pipetted into the aqueous layer on the plate and gently stirred with the pipette. After a brief interval Infusoria are evenly distributed and the experiment may be started.

In the experiments covered by this report, chemotaxis of Infusoria was investigated for the first time by the quantitative method, the essence of which consists in simultaneous observation of chemotactic reactions due to four different concentrations of the substance concerned.

Five 1 cm. squares are drawn on the black background and a celluloid slip with the name and concentration of the alcohol recorded in white ink is inserted between the glass plate and the background.

When beginning the experiment, 10—12 drops of pure tap-water are added drop-wise with a micro-pipette in the central square marked „control". The Infusoria should freely enter the area occupied by the water freshly added. Then, the solutions under investigation are deposited with the aid of a micropipette in portions of 10—12 drops on the areas outlined by the squares suitably marked, as shown in phot. 1. This operation should



Phot. 1. Chemotactic action of ethanol on *P. caudatum* investigated with aid of quantitative method

be carried out within 30 or 40 seconds, and should begin with higher concentrations and end with the lower ones. The Infusoria show a chemotactic reaction, its intensity is in direct relation to the concentration of the solution added. After an interval of 30 seconds since the last drop of solution was added, the first photograph is made, with the shutter set at 1/10 or 1/25 second. Subsequent photographs are made at 5 sec. intervals.

Table I

The chemotactic effects of alcohols on *Paramecium caudatum;* m = average number of Infusoria calculated from 10 successive photographic recordings, s = standard deviation, % = percentage of Infusoria calculated with reference to control = 100

Concentration in mM	Methanol			Ethanol			Isopropanol			1—propanol			2—Methyl—2—propanol		
	m	s	%	m	s	%	m	s	%	m	s	%	m	s	%
759	9.6	±1.92	6.1	—	—	—	—	—	—	—	—	—	—	—	—
506	55.2	±4.31	35.2	—	—	—	—	—	—	—	—	—	—	—	—
337	106.9	±12.2	68.0	—	—	—	—	—	—	—	—	—	—	—	—
225	154.7	±9.36	98.5	11.8	±1.82	10.1	—	—	—	—	—	—	—	—	—
150	—	—	—	50.7	±3.86	43.3	—	—	—	—	—	—	—	—	—
100	—	—	—	86.5	±8.16	73.9	16.3	±1.90	9.9	—	—	—	3.9	±1.58	3
67,5	—	—	—	109.5	±5.21	93.6	51.8	±4.17	31.4	6.5	±1.14	5.2	25.5	±3.13	19.5
45	—	—	—	—	—	—	99.7	±5.15	60.4	20.4	±1.84	16.3	57.0	±4.9	43.5
30	—	—	—	—	—	—	147.6	±7.39	89.5	65.8	±3.87	52.6	106.9	±5.63	81.6
20	—	—	—	—	—	—	—	—	—	110.2	±4.29	88.2	—	—	—
Control	157	±7.23	100	117.4	±4.65	100	164.6	±8.91	100	124.9	±6.64	100	131.1	±6.85	100

Concentration in mM	2—Methyl—1—propanol (Isobutanol)			1—Butanol			Amylene hydrate			2—Pentanol			1—Pentanol		
	m	s	%	m	s	%	m	s	%	m	s	%	m	s	%
45	1	—	—	—	—	—	5.7	±2.63	4.8	—	—	—	—	—	—
30	—	—	—	—	—	—	16.0	±2.90	13.6	11.9	±2.02	9	—	—	—
20	—	—	—	3.5	±1.10	5.7	64.0	±4.58	54.2	32.1	±4.89	24	—	—	—
13,5	13.9	±2.39	9	11.4	±1.67	18.5	97.5	±7.75	82.6	81.9	±6.95	61.2	0.7	±0.71	0.65
9	23.4	±2.86	15.1	34.8	±4.54	56.4	—	—	—	104.3	±5.47	77.8	12.8	±1.18	12
6	64.8	±4.40	41.8	60.7	±5.28	96.8	—	—	—	—	—	—	38.9	±4.48	36.4
4	134.8	±8.35	86.4	—	—	—	—	—	—	—	—	—	70.1	±6.17	65.5
Control	154.8	±6.59	100	61.7	±4.28	100	117.9	±7.66	100	134.4	±8.25	100	107.4	±5.48	100

A total of 10 photographs afford a complete record of the experiment to be analysed quantitatively at a later convenience. The experiment ought to be repeated all over in case of technical errors, such as faulty instil-

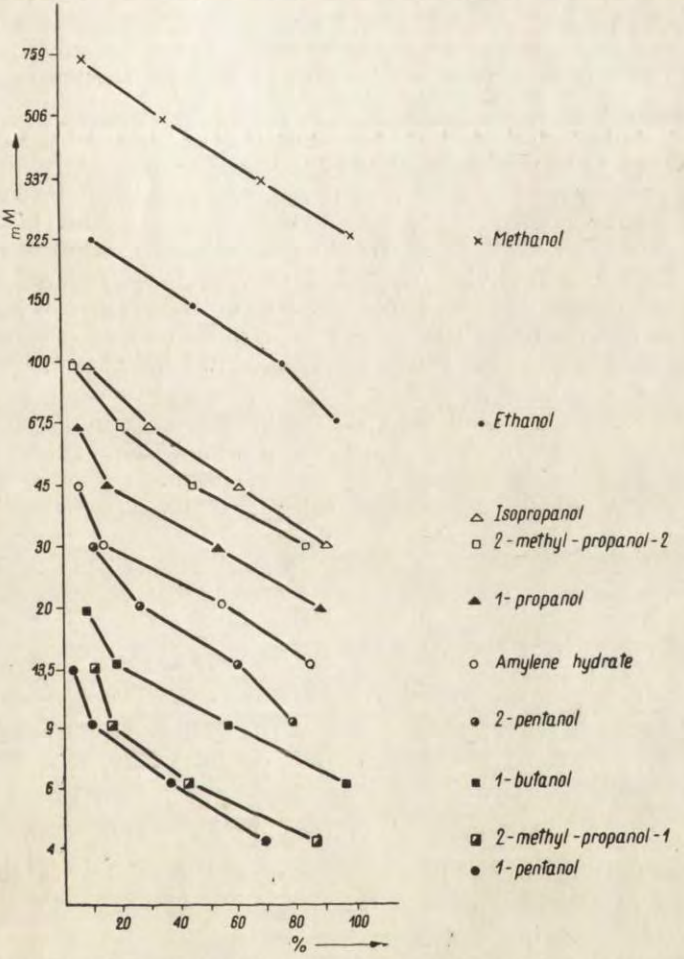


Fig. 1. Chemotactic effects of lower alcohols on *P. caudatum* expressed in % of individuals stated in squares with alcohols solution related to the control square covered with water

ling of the solution in question, or when addition of pure tap-water at the beginning (control) gives rise to a negative chemotactic reaction. The latter would indicate that the medium with the Infusoria contained some agent capable of producing a chemotactic response of sufficient intensity to show subsequently a negative reaction of the potozoa to pure water. In such cases acidification of the medium is most likely to be involved.

In experiments designed to determine the toxicity of alcohols, these were diluted with water to make 9 ml. in such proportions as to obtain the required concentration on addition of 1 ml. of water with Infusoria. For instance, 6 ml. of 1 M ethanol + 3 ml. of water + 1 ml. of water with Infusoria, gave 0.6 M ethanol with Infusoria. In this manner a more or less uniform concentration of Infusoria was obtained in all samples and core was taken to keep it at the level of roughly 200 specimens in 1 ml., that is, not too high. This reduced the chances of possible errors due to adsorption of the toxic substance on the surface of successively dying specimens (Grębecki and Kuźnicki 1956).

After preliminary tests, the experiments concerned with toxicity, were run simultaneously with 5 or 6 concentrations of the alcohol in question. The Infusoria were kept for 24 hours in the respective alcohol solutions, and then the dead-to-living specimens ratio was determined for each sample. Each sample was judiciously but thoroughly stirred and 3 or 4 ml. were spread in a uniform layer (about 0.5 mm thick) on a glass plate resting on a black background with 20 1 cm. squares drawn in white ink. The dead cells easily distinguished by their distorted shape and complete immobility, were counted under a magnifying glass and the figures from all the squares were added. It ought to be emphasized that within the range of concentrations covered by the experiments, complete disintegration of cells was never noted; otherwise, quantitative analysis of the toxic effects would obviously be impossible.

When counting was finished, macro-photographs of the entire plate were made, from which the total number of cells was counted.

RESULTS

In experiments concerned with chemotaxis, the alcohols were used in concentrations consecutively increasing by 50 per cent. with reference to the preceding one. The following concentrations (in mM) were used: 4, 6, 9, 13.5, 20, 30, 45, 67.5, 100, 150, 225, 337, 506, 759. The alcohols examined in chemotactic and toxic respects included: methanol, ethanol, 1-propanol, isopropanol, 1-butanol, 2-methyl-2-propanol, 1-pentanol, 2-pentanol, and amylene hydrate (2-methyl-2-butanol).

The results of experiments on chemotaxis are shown in table I, and in fig. 1.

The toxic effects of various alcohols are shown in table II, and in fig. 2.

The experimental data obtained show the following sequence of alcohols when arranged in the order of increasing chemotactic effects: methanol ⟨ethanol ⟨isopropanol ⟨2-methyl-2-propanol ⟨1-propanol ⟨amylene hydrate ⟨2-pentanol ⟨1-butanol ⟨2-methyl--1-propanol ⟨1-pentanol.

Table II

The toxic effects of lower alcohols on *Paramecium caudatum*. The figures given in the columns indicate the ratio of living Infusoria per cent

Concentration in mM	Methanol	Ethanol	Isopropanol	2-methyl-2-propanol	1-propanol	2-methyl-1-propanol (isobutanol)	Amylene hydrate	1-Butanol	2-Pentanol	1-Pentanol
	%	%	%	%	%	%	%	%	%	%
1000	17.8									
900	39									
800	62.5									
700	81.8	22								
600	94.7	63.2								
500	100	90.5								
400		100								
350			17.1	13.8						
300			72.5	36.6.						
250			88.9	86.1						
220			100	100						
200					5					
180					43.2					
160					84.2					
140					100					
90						11.4	13.9			
80						35.7	53.7			
70						78.9	77.3			
60						100	100			
40								27.3		
35								47.4	14	
30								75	69.1	
25								100	85	
20									100	
10										5.1
8										45.5
6										82.4
4										100

When arranged in the order of increasing toxic properties, the following sequence was obtained:
methanol ⟨ethanol ⟨isopropanol ⟨2-methyl-2-propanol ⟨1-propanol ⟨2-methyl-1-propanol ⟨amylene hydrate ⟨1-butanol ⟨2-pentanol ⟨1-pentanol.

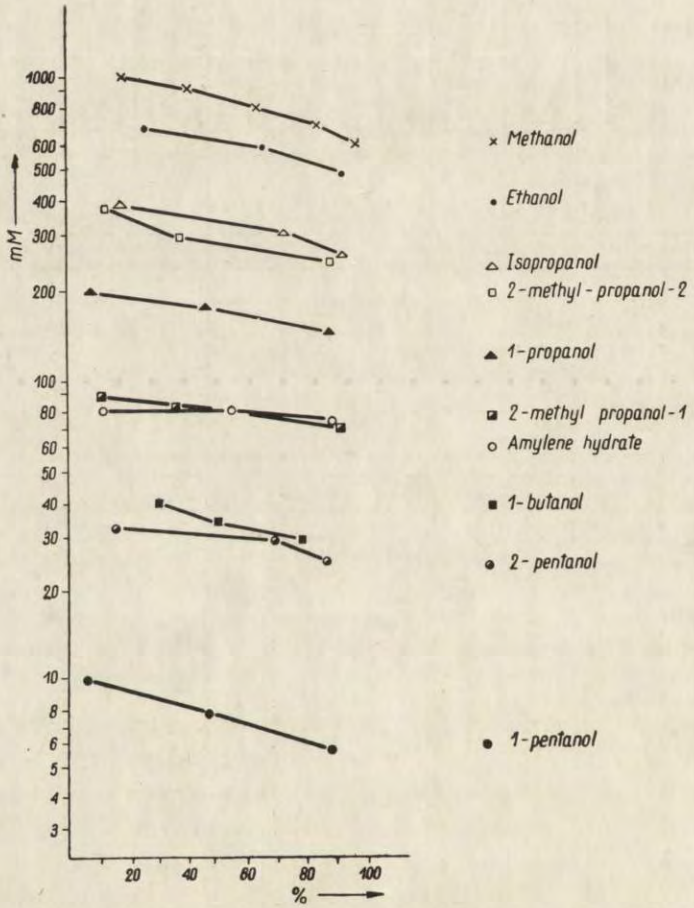


Fig. 2. Toxic effects of lower alcohols on *P. caudatum*. Percentage of living Infusoria after 24 hours treatment with alcohols.

DISCUSSION

Correlation between toxic and chemotactic properties is not a new subject in research. On evidence of comparative studies involving *Paramecium aurelia*, as early as 1899, Jennings con-

cluded that there is no such correlation. He supported his thesis by several pertinent arguments: 1) there are a number of substances completely devoid of chemotactic properties (sugars, glycerol, and urea), 2) some substances eliciting notable positive chemotactic response are at the same time very toxic (HCl, H_2SO_4, picric acid, CrO_3, etc.), 3) a number of substances giving rise to negative chemotactic reaction fail to exhibit highly toxic properties (KCl, NaCl, $MgCl_2$, etc). This evidence clearly shows Jennings's thesis to be correct in general, but it does not refute the possibility of correlation between chemotactic and toxic properties in compounds similar in chemical structure. The investigations reported in this paper were concerned with the chemotactic and toxic properties of a number of members of a homologous series of organic compounds, viz., 1-hydroxy alcohols with a chain length of 1—5 C. Within this group, correlation between chemotactic and toxic properties proved to be quite evident, and the two properties were shown to increase with the molecular weight of the alcohols, toxicity more so than chemotactic qualities. The properties concerned alcohols of the 1st, 2nd, and 3d orders diminish in that succession. This is especially conspicuous in amyl alcohols: amylene hydrate (3d order) acts weaker than 2-pentanol (2nd order) whose action is inferior to that of 1-pentanol (1st order).

When alcohols are arranged in two sequences with reference to their toxic and chemotactic properties, a certain departure from the general rule may be revealed, viz., a difference between the sequences in the position of isobutyl alcohol (2-methyl-1-propanol), which is indeed less toxic than 1-butanol but gives rise to a more pronounced negative chemotactic reaction than the latter. Here it is worthwhile to observe that Bills (1924) noted the same difference in his investigations concerned with six lower alcohols when he compared these with regard to toxic and narcotic effects on *Paramecium* (unfortunately he failed to indicate the species). The sequence in which Bills arranged the alcohols with regard to their toxic effects was as follows: methanol ⟨ethanol ⟨isopropanol ⟨n-propanol ⟨isobutanol ⟨n-butanol.

As regards narcotic effects, the arrangement was analogical except for the last two alcohols: isobutanol proved more potent as a narcotic than n-butanol. The results of my investigations are thus in agreement with Bills's conclusions, which may be taken

to indicate a correlation between the narcotic and chemotactic effects of alcohols on *Paramecium*. Final conclusions as to differences between the toxic and chemotactic effects of isoalcohols and normal alcohols would call for investigations covering a wider range of isomers, especially in amyl alcohols.

SUMMARY

The toxic and chemotactic effects of 10 lower alcohols on *Paramecium caudatum* were explored and compared. Studies on chemotaxis in protozoa involved a quantitative method and photographic recording. The toxic and chemotactic effects of the alcohols were found to increase with the molecular weight of the compounds and were noted to diminish in alcohols of the 1st, 2nd, and 3d orders in that succession. The only significant difference between two sequences of alcohols arranged according to their toxic and chemotactic properties respectively concerned the positions of isobutanol (2-methyl-1-propanol) and 1-butanol. The former, though less toxic than the other, caused in *Paramecium caudatum* a more pronounced negative chemotactic reaction.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

KINAESTHETIC TASKS IN RELEARNING ALBINO RATS

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(Received 5 July 1958)

Relearning in white rats has since long been a point of great interest. The main problems were the following: 1) the factors responsible for the positive or negative nature of transfer of learning, and 2) the role played in the transfer by adaptation to such functions as are common to both tasks.

Tolman, Ritchie and Kalish (1947) investigated the factors which guided the animals in choosing the correct path through a T-maze, and sought to determine the elements of learning which were transferred to control tests. They found that when conditions were essentially the same in both, the first task and in the control test, a turning of the entire maze through a certain angle did not upset the regular course of the control test. The situation was different when there were two factors, both leading in the given case to a solution, for instance a definite turn to the left and the recognition of the direction of the food. In the control test only one of the two factors was leading to correct solutions, for instance only a suitable turn, if the direction was changed. If the animals used to solve the first task by remembering the turn, they continued to run correctly, but animals which were solving the first task by following the direction failed to solve the control task, which was to them more difficult than to animals that had not been trained to the first task. Consequently, the authors referred to concluded that when animals find a principle to be reliable under certain conditions, they continue to follow it in somewhat modified conditions.

There is substantial agreement between a number of investigators that the learning of one task influence the mastering of

the next one. Dashiell (1920, 1930) found general adaptation to laboratory conditions to promote more rapid mastery of the first and subsequent tasks in a series. Both factors, adaptation and training help the rat to learn the direction it has to follow for getting to the food. Hunter (1922) went still further in his conclusions and claimed the mastery of one type of maze to promote the mastering of another. Training in a circular maze cuts the time of learning in a straight one. In all such cases the mastery of one task has a positive influence on the mastering of another where these involve the learning of a definite path, in a straight or circular maze, with the aid of the kinaesthetic sense. The type of the task is the same and the differences concern only secondary details.

Things are different when it comes to selecting a path (or door) in response to a positive stimulus which becomes negative in the next task. A number of American research workers have obtained a negative transfer of learning. Hunter and Yarbrough (1917), Peace (1917), Hunter (1922), trained rats to select one or the other of two paths in a maze according to presence or absence of an auditory, or optic, stimulus. When the task was fully mastered, the path-to-stimulus relation was reversed. For the trained rats the new task proved very difficult, whereas for control groups it was not. Fritz (1930) carried out 2000 and 8000 tests in which he used light as a positive or negative stimulus alternately. He considered a task as mastered when 45 of 50 tests were solved correctly.

He found that, when the path-to-stimulus relation was reversed the rats ceased to select the path to which they had been formerly trained, only after some 100 trials. The negative transfer of learning was here conspicuous. Munn (1932) applied the same method and confirmed Fritz's results. Fiedorov (1951), who worked with mice, applied the conditioned reflex method and obtained different results. He found that repeated transformation of reflexes increased the rate of that transformation. Fiedorov's results are thus in disagreement with those of other authors.

Buytendijk (1930) carried out experiments with rats in mazes with two and four paths, and retrained the animals repeatedly. He noted positive transfer of learning although the animals were unable to master completely some of the paths of the quad-

ruple maze. Bunch (1936, 1939, 1939 a, 1941), Bunch and Lange (1939), Bunch and Rogers (1936), and Bunch and Mc Craven (1938) studied in much detail the relationship between amount of transfer and interval of time. They found the intervals to have a notable effect on learning, an their length to determine the nature — positive or negative — of transfer.

The results so far available show that more rapid mastery of a maze after prior training to a maze of the same or similar type is essentially attributable to nothing more but a familiarity with a general lay out of mazes and general position of the goal, and to an association of maze running with satisfaction of hunger. Mastery of mazes though, has no effect whatever on rope ladder climbing (Brockbank — 1919) since this is an altogether different task.

Over the recent decade or two, numerous psychologists have studied this problem also in men, and their research have many points in common with those involving animals. For instance, Montgomery (1953) claims the existence of positive transfer in men, and a positive influence of intervals between tests on the rate of learning. The latter is confirmed also by Brigs, Thompson, and Brodgen (1954). Also the effect of one task on other similar has been studied; Malcolm and Arnoult (1953), Duncan, Underwood and Benton (1954), Duncan (1933), and Duncan and Underwood (1953) found that longer preliminary training is conducive to more rapid and improved learning of the consecutive task. The results of these experiments indicate that learning involves in men and animals similar processes.

The experiments reported further below were carried out with view to explore the changes attending multiple relearning in albino rats.

METHODS

The experiments were carried out with the aid a straight mazes which was put on a stand and consisted of a straight 4 m. long board with four evenly spaced transverse partitions. Each partition was fitted with four doors opening one way only. Early in the experiment the maze was fitted with eight additional four one-door partitions placed mid-way between every two four-door partitions, the first one facing directly the starting platform. The rat was always placed on the starting platform before the first door, and when it got through the maze to the end platform it entered the cage where it was usually kept and fed.

The actual experiments were invariably preceded by a preliminary training with all the doors unlocked and the animals running along any path they liked to reach the cage with the food. The preliminary training lasted 6 to 8 days. Over the first few days of the preliminary training the animals were fed normally, and only when they became familiar with the general layout of the maze they were kept fasting 20 hours a day. The rats received food after each run through the maze. When the preliminary training was concluded, and the actual experiments were begun, only one of each four doors in the partitions was left unlocked whereby the path was payed out a rat was to learn. The experiments were begun when the animals were two and a half or three months old, and they were making 6 runs daily. The consecutive tasks the rats were expected to master in the course of the experiments, consisted in learning new paths as determined by the doors left unlocked in the partitions. Each time an animal learned a certain path, the arrangement of unlocked doors was changed and training was resumed without delay. There were 256 different paths through the maze.

Progress of learning was evaluated according to three factors: time, errors, and hesitations. The touching of locked doors was counted as an error. Running up to a door, locked or unlocked without attempting to open it counted as hesitation. Time was counted from the animal passed through the first (single) door till it passed through the last door. A task was considered as mastered when the animal made 6 consecutive runs, — not necessarily in one day — without errors or hesitations. The over-all number of experimental animals was 55, divided into five groups.

EXPERIMENTS WITH THE FIRST FOUR GROUPS OF ANIMALS

In the experiments with the first four groups the animals were made to run through the maze with the additional one-door partitions. The number of tasks varied between the groups. The number of runs the animals of specific groups needed to master the consecutive tasks are shown in Table I.

The numbers of run shown in the table were calculated as an average from all animals in a given group. Individual differences were often very substantial, but usually between 6 and 10 runs in a given group. The most conspicuous differences as between groups concerned the first task. Also individual differences were most pronouced with regard to the first task.

The experiments which involved the first four groups of animals supply evidence of distinct transfer of learning. The transfer was positive since the number of runs the animals needed to master a new task diminished as the consecutive tasks were maste-

red. From an analysis of the data relating to group 3 it will be seen that the changes in the number of runs needed to master the consecutive tasks corresponded to an ordinary curve of learning. After the initial fall (tasks 1—5) the number of runs needed for the mastery of subsequent tasks (5—17) remained on a more or less even level, the slight differences between particular tasks being also characteristic for an ordinary curve of learning. From the 18th task onward there was again a change — a mild decrease

Table I

The number of runs needed to master the consecutive tasks for the four animals groups

Group 1										
Task	1	2	3	4						
No of runs	46	42	34	26						
Group 2										
Task	1	2	3	4	5	6	7			
No of runs	70	30	27	24	18	18	12			
Group 3										
Task	1	2	3	4	5	6	7	8	9	10
No of runs	44	36	24	23	16	19	16	16	15	16
Task	11	12	13	14	15	16	17	18	19	20
No of runs	17	14	15	15	11	14	11	12	9	9
Group 4										
Task	1	2	3	4	5	6				
No of runs	54	42	24	23	21	18				

to 9 runs. It appeares therefore that the changes could be attributed to a gradual improvement of learning. Thus the animals learned to master individual tasks on the one hand, while on the

other they learned also the general features common to all these tasks. The individual tasks appeared as if certain units integrated in the entire process of learning.

From an analysis of the number of errors in the particular tasks we obtain a pattern analogical to that referred to above (Figs. 1 and 2). As the consecutive tasks were mastered, from the 15th

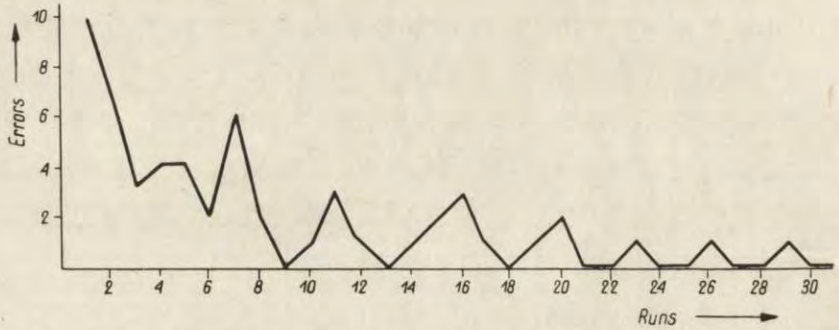

Fig. 1. Number of errors in the learning of the first task

task onward, the learning become more rapid and differences in the number of errors in subsequent runs diminished. In the last tasks learning became very rapid. There was a high number of errors in the first run, which rapidly fell to zero in subsequent

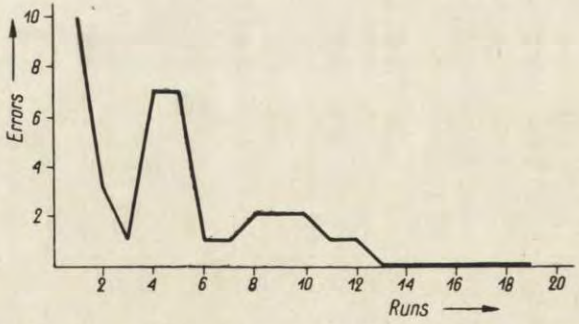

Fig. 2. Number of errors in the learning of the tenth task

runs (Figs. 3 and 4). Here the character of the relevant curves differed considerably from that of a normal learning curve.

From an analysis of the tasks which were mastered by the animals of all groups, it will be seen that the rate of learning increased with the number of tasks mastered. The learning of each

consecutive task involved progressively smaller numbers of runs and errors. The average number of errors per run invariably diminished over the first 6 tasks; subsequently there were considerable differences in the number of errors as between particular tasks, and this remained so till the end of the experiments. (Tab. II).

The initial fall of the average number of errors made in one run and the subsequent inhibition of this fall may be attributed to the factor that over the first few tasks (and first several dozens of

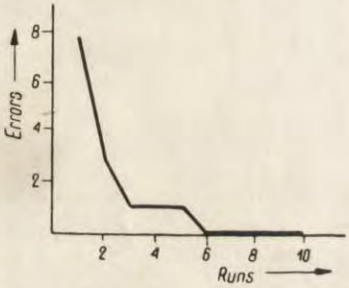

Fig. 3. Number of errors in the learning of the nineteenth task

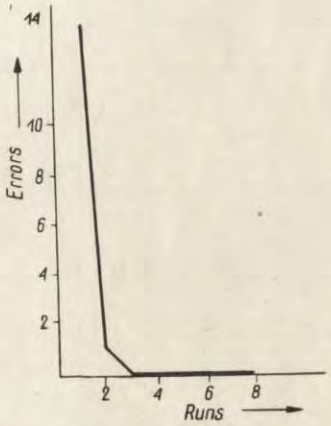

Fig. 4. Number of errors in the learning of the twentieth task

runs) the rats acquired general orientation in the maze and learned the principle that only one of the four doors in a partition is left unlocked. The assumption was also confirmed in unpublished investigations on rats by E. Szulc. The above concerns the entire complex of tasks but contributes only slightly to a more rapid mastery of individual tasks, and therefore the number of errors per run diminished less rapidly than the number of runs required for learning. But once general orientation has been acquired and the principle of one unlocked door per partition learned, and the point became merely to solve individual tasks, all of which were essentially similar, the fall of the number of errors was matched by a fall in the number of runs. A rise or fall in the number of errors was reflected in similar changes in the number of runs, the correlation being very intimate in that period.

The errors made by a rat during consecutive relearning to another task (of the same type) were in close connection with the preceding task, with the new task was to be mastered, and with the preliminary training. The latter obviously affected first and foremost the mastery of the first task. Further learning was increasingly affected by experiences made while mastering the preceding tasks, and by characteristic features of the current task.

Table II

Average number of errors in one run per animal in group 3

Task	1	2	3	4	5	6	7	8	9	10
Av. No of errors	2.1	1.7	1.5	1.5	1.3	0.9	1.1	1.3	1.4	0.9
Task	11	12	13	14	15	16	17	18	19	20
Av. No of errors	1.5	1.5	1.3	1.5	1	1.8	1.5	1.3	1	0.9

An animal made to master a new task remembered over a certain interval the preceding one and made errors at such doors as were unlocked in the preceding task. Inhibition of an established habit required always several consecutive runs, the number of which depended on the degree of difficulty, the characteristic features of the preceding task, and the number of tasks the animal had mastered before. In order to learn how errors due to habits formed by preceding tasks are eliminated, paths were modified only partly in every new experiment, and the number of errors made at such doors as were unlocked in the preceding task was compared with the number of errors made at the remaining doors. These comparisons failed to give an answer to the question, but they showed that in the maze used there were a number of factors affecting the type of errors: for instance, partitions with one centrally located door promoted a preference for the central doors of the next (foor-door) partition because the path between central doors in shorter than that between a central and en extreme door, while the animals, having a natural tendency to follow the shortest path on their way to food, were therefore inclined to try central doors. Here, two antagonistic tendencies became manifest: on the one hand the memory of the preceding task, and on the other,

the natural preference for central doors. Consequently, when the central doors were unlocked in the preceding task, the two tendencies overlapped in the subsequent one and there was a higher ratio of errors at central doors. On the other hand, when in the preceding task the extreme doors were left unlocked, the two tendencies compensated each other and the distribution of errors between the particular doors appeared fortuitous. When individual tasks were divided into two parts, and the errors counted accordingly, there was over first half of each task an increased number of errors at doors that had been unlocked during the preceding task, whereas in the other half there appeared errors at central doors. In addition to the factors referred to, undoubtedly greatly important in learning, there are also others which affect the type of the errors, and therefore the distribution of errors is not altogether accidental, not even in the first task, or during preliminary training. Some such factors are: anticipation repetition of a turn, straight-line running, and others. Consequently, the behaviour of a rat in a maze is a resultant of all these factors.

EXPERIMENTS WITH THE FIFTH GROUP OF ANIMALS

The above experiments failed to give a clue to the question as to how errors due to habits formed by learning of the preceding task, are eliminated. It appears, however, that this elimination determines to a large extent the rate of learning. In order to explore the question of elimination the maze was modified by removing the one-door partitions with a view offsetting to the natural preference for central doors. But for this change, the experiments were run analogically to the previous ones, and the results were calculated in a like manner. The over-all number of doors in the maze was thus 16, the errors being obviously distributed over the 12 locked doors. In the second task, the percentage of errors made at doors had been unlocked in the preceding task was 13.4, the remaining doors accounting for 5.8 per cent. In the twentieth task the respective figures were 9 and 8 per cent. If the distribution of errors had been purely accidental, there would have been 8.3 per cent. of errors for each door. It follows from the caluculations that as the number of mastered tasks rose, the percentage of errors made at the doors which had been left unlocked during the prece-

ding experiment tended to fall to the level of accidental distribution of errors, without however attaining this level owing to the obvious fact that before a new habit could supplant the previous one a certain minimum number of errors had to be made at such doors as had been left unlocked in the preceding task. The number of errors made at other doors might have been fortuitous. They could be made, but were not bound to. Therefore, after between ten and twenty tasks, when new tasks were learned after a fairly small number of errors, some of which had inescapably to be made at the doors that had been left unlocked during the preceding experiment, the percentage of the latter was bound to be larger than would follow from an accidental distribution.

The results of these experiments were evidence of an increasing rate of inhibition of the habit which proved inadequate in a changed situation.

The experiments, in which the method of complete relearning was used, demonstrated that prior training does indeed contribute towards more rapid learning of a new task, but that it may also — if too thorough — make relearning difficult over the first few runs, causing a concentration of errors at such doors as had been left unlocked during the preceding task.

THE QUESTION OF HESITATIONS DURING RELEARNING

As was mentioned before, it counted as a hesitation when the animal made for any door but turned away without touching it.

The term has been defined by E. S z u l c (in an unpublished study). Unlike errors, hesitations may concern also unlocked doors. During the first relearning the curve of hesitations ran as shown in Fig. 5.

During the first few runs, when errors were most frequent, hesitations were nil. As the number of errors began to diminish, hesitations appeared and grew in inverse relation to the errors. A transient increase of errors was usually attended by a similar fall of hesitations. This phenomenon occurred fairly regular until, eventually, both errors and hesitations began to fall to zero. However, errors usually disappeared more rapidly than hesitations. In each consecutive complete relearning (criterion: 6 runs without errors or hesitations) the error-to-hesitation ratio was as described before till the stage was reached at which a rat was learning new tasks

very rapidly (after not more than 15 runs). At this stage, hesitations were rare and the relationship referred to before was not observed (Fig. 6).

Hesitations were an important moment in maze learning. They appeared only at a certain stage and subsequently disappeared

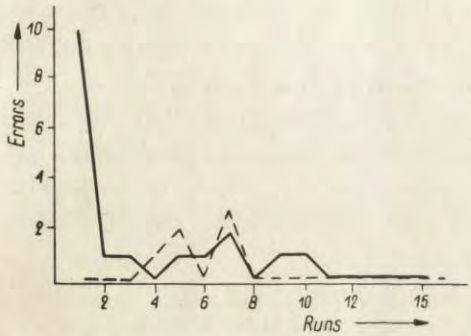

Fig. 5. Error-to-hesitation ratio in the learning of the second task

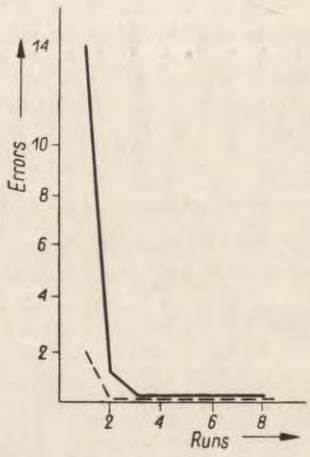

Fig. 6. Error-to-hesitation ratio in the learning of the tenth task

completely. When an animal became familiar with the general features of the tasks, such factors as anticipation, straightline running, and others, began to interfere with correct running. The ani-

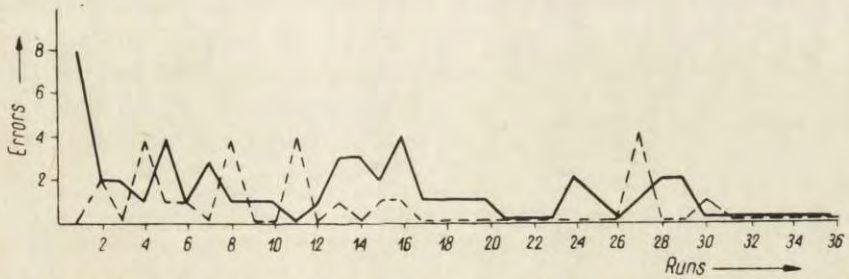

Fig. 7. Error-to-hesitation ratio in the learning of the twentieth task

mals frequently approached a certain door, stopped short at some distance, sniffed, turned back and ran up to the others. This was so over up to ten runs, or more, depending on how many tasks the animal had mastered before, but eventually the hesitations ceased and the task was mastered successfully. Roughly after the

eight consecutive tasks the hesitations were diminishing to disappear almost completely after the seventeenth or eighteenth (Fig. 7). From that moment onward a slight change became noticeable in the behaviour of the animal, which began to run according to the principle of consecutive turns. The rat simply memorized a definite pattern of several turns and followed it through subsequent runs. In the first few runs of a given task the turn was not yet accurately memorized, but errors were made not at doors far from the correct ones but at those next to them. It frequently happened that the rat touched the partition with the nose between the correct door and the one next to it, and only then corrected its course. This was due to the turn being too sharp, or not enough so.

It follows from an analysis of the hesitations that the rats chose, from the many modes of solving the task correctly, that mode which was quickest. It was easier for them to memorize a sequence of four definite turns than to remember out of sixteen doors the four correct ones.

THE TIME FACTOR IN COMPLETE RELEARNING

The time, counted from the beginning of the run, which the animal needed to get to the food was a important factor in relearning. By counting it over a number of successive runs, an animal's stage of learning could be determined with reasonable accuracy. In preliminary training one run lasted more than ten minutes, occasionally even more than a score or two. Actual training started in fact only when the time was cut to 10 seconds. In the first run of the first task, i.e. when the doors were locked for the first time, the time went up to even several minutes. It depended on the number of errors made at the beginning of learning. After several runs, the time of running was between 7 and 15 sec. However, when in the course of mastering a specific task the stage of few errors and numerous hesitations was reached, the time grew again. A rat's behaviour was here characteristic. A given animal was sitting for a few moments at the starting point moving its head in a horizontal plane, and then made a rapid dash slowing down as it was approaching any door; when it opened the door it stopped again, moved its head as before, started rapidly for the next door and slowed down approaching it. This behaviour continued roughly to the eighth run, and from then on the running time fell to between

10 and 12 seconds. Stopping in the door was progressively less frequent and head movements disappeared. By about the 15th run time was cut to about 7 seconds. The animal ceased to stop in the open door and gathered speed as it approached the goal. The rapid decrease of the running time at about the 10th task supplied further evidence that the animal was changing the mode of mastering successive tasks. By abouth the 17th task the running time was cut to roughly 5 seconds. When the task was well mastered and the animal hungry, minimum running time was reduced to 3 seconds.

DISCUSSION

Maze learning of white rats depends strictly on the animal's history. When the animal is for the first time in a maze, in a situation completely new to it, its behaviour is different from what it is when the equipment is familiar. In the first case orientation reaction undoubtedly exists, but various stimuli from without as well within the maze, even weak ones, provoke reactions of fear and have a powerful effect on the animal's behaviour. Very frequently fear inhibits orientation causing the animal to stay motionless for a long while, often even till sleep. This first initial period is then supplanted by the second one when the orientation reaction predominates and only very strong stimuli are capable of evoking fear, which is soon over though. This stage lasts till the animal finds the cage with the food for the first time. To cut down this stage it is advisable to put more animals in the maze which helps to reduce in some way the fear and exploration. Once a rat associates maze running with the reaching of the cage in which it normally lives, further learning proceeds fairly rapidly. Then the rat has only to learn which doors are unlocked and that these lead along the quickest path to the goal. When the next task to be mastered concerns the same maze, the entire preliminary stage, in which the animal becomes familiar with the equipment, is eliminated and the only thing to be learned is the new path. Several authors, e.g. Dashiell (1920, 1930), believe that the reduced number of errors and runs during learning of a subsequent task is due merely to an elimination of the initial stage. However, the experiments here described refute this view since the factors of orientation and fear predominant in the first and second stages of learning, were eliminated during the preliminary training, which lasted about 8 days

and during which the animal became used to the stimuli attending the experiments and learned in which direction to run to obtain food. Furthermore, if Dashiell, and some other authors, were right, changes in the rate and mode of learning would necessarily be confined to the first or few first tasks, and could not possibly follow pattern revealed in the experiments covered by this report. However, the results here discussed show beyond all doubt that, in experiments involving complete learning, the number of runs required in learning diminished with each of consecutive twenty tasks. The fact that consecutive tasks are mastered with increasing rapidity is probably attributable to the nature of the mechanism of learning, which is capable of improvement. The latter ability may also be concluded of the work of Hunter (1922), who stated that in general the learning in rats is capable of improvement. This would imply that learning is in a given case the resultant of the difficulties, specific for the current task, and of the amount of experience the animal gained by solving earlier tasks. This point of view is justified, although it is contradicted by Brockbank's conclusions (1919). However, Brockbank disproved extinction of an earlier habit during development of a new one, but it is difficult to agree with him in that maze tasks have no influence on rope ladder climbing. But since the period of orientation reaction was not eliminated in either case, interference due to this factor changed the results.

A number of authors concerned with the problem of transfer of learning distinguish two types of transfers: a positive and a negative one. One of these types is invariably involved when the animal has mastered a preceding task to a high degree, but is always confined to the first half of next-task learning owing to the fact that what was known to the animal before, and led to the goal, became misleading. Consequently, the earlier habit has to be inhibited and another one, adequate for the new situation, developed. However, once the earlier habit has been inhibited, the new task is mastered more rapidly than the preceding one. Eventually, therefore, the character of the transfer of learning will be positive. Hunter (1922) believes that relearning is the easier the simpler the task involved, provided that conditions of relearning are of the same type as in the case of the preceding task, and that some of the elements of the first task may be directly transferred to

the other. Hunter is right as regards elements transferable from one situation to the other, but — as regards simplicity why should a four-fold selection of one of four doors be simpler than a two-fold selection in response to a specific stimulus? It appears that the difficulty involved in the latter task is entailed not by the peculiar complexity of individual tasks but by the complete reversal of an essentially simple situation.

When we analyse an animal's relearning from one situation to another, a close analysis of the errors will prove it wrong to believe transfer to be only negative or only positive. In addition to the positive influence of the preceding task there is invariably also a negative one, the magnitude of which depends on the degree in which this preceding task was mastered.

Bunch and others (as quoted in the introduction) investigated the influence of an earlier task on learning of another task, in modified conditions. However, they limited themselves to alternative relearning, or rather recollecting, of only two tasks and thereby indeed restricted the problem to that of improvement of recollecting but not of learning.

The experiments described in this report warrant the conclusion that in complete relearning (to a degree of six consecutive runs without errors or hesitations) rats master successive tasks of the same type with: increasing rapidity, i.e. decreasing number of runs (for each specific task) and time required for one run, and hesitations disappearing after a number of tasks has been mastered. All these progressive changes noted throughout an entire course of learning of a sequence of tasks bear evidence of gradual improvement of learning.

CONCLUSIONS

1. During complete relearning (criterion: six runs without error or hesitation) the successive tasks are mastered with progressive rapidity.

2. The runs and errors involved in mastering specific tasks diminish as a sequence of 20 tasks is successively mastered.

3. The average time needed to complete one run varies according to the actual stage of learning.

a) Towards the end of preliminary training the time of one run is roughly 10 seconds.

b) With the beginning of the learning of the first specific task, the time of one run rises to between 1 and 4 minutes, and eventually falls to 7—15 seconds.

c) Beginning with about the 17th task average running time is 5 seconds.

4. Beginning with the 9th task the number of hesitations is in inverse relation to that of errors.

5. The over-all number of hesitations diminishes from the 9th task onward.

6. By about the 17th task hesitations become almost nil.

7. Successive learning of twenty tasks of the same type improves learning in rats.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

AN ANALYSIS OF THE BEHAVIOUR OF A WHITE RAT DURING INCOMPLETE RELEARNING

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(Received 5 July 1958)

The effect of prior training on the mastering of a maze task has been studied in detail by numerous authors. However, the influence of prior partial training evoked little interest, although it is very important for explaining an animal's adaptation to ever changing external conditions, and its increasing ability to profit from earlier experiences in solving new difficulties. As regards influence on current learning, a distinction may be made for two points: 1) influence of prior overlearning, and 2) influence of only a partial mastery of a preceding task.

The influence of overlearning was studied by Jackson (1932), who claimed that the rate of second-task learning is inversely related with the degree to which the preceding task has been mastered. When the first task is mastered to a very high degree of perfection, second-task learning becomes more difficult. Different conclusions are drawn by Reid (1953) and Pubolis (1956). In their experiments, which involved discrimination of a white positive card and a black negative one, overlearning appeared to have a positive effect on a reversion of the problem that is on distinguishing a black positive card from a white negative one.

The problem was approached from a different angle by Wiltbank (1919), Bunch and Rogers (1936), and Bunch and Lange (1939). They varied the amount of training in one or two different mazes, investigated the influence of that amount on second-task learning, and found the latter to be promoted by prior partial training.

Dąbrowska (1959) employed the method of complete relearn-

ing and demonstrated that prior training does indeed facilitate mastery of a new task, but also that overlearning causes such changes in the distribution of errors in second-task learning as would indicate difficulties in relearning. As relearning continues the difficulties do gradually disappear, nevertheless negative transfer is conspicuous at the beginning of second-task learning. In a specific task (a peculiar arrangement of doors in the experiments referred to) memory facilitates the mastery of this task and is therefore a positive factor in learning. However, with regard to the entire sequence of twenty tasks the very same memory has an adverse effect on the rate of learning when the task is changed to another one of the same type. These are mutually interfering influences. In the experiments now to be described it was intended to minimize memory of a specific task and to concentrate on training of the ability to switch over to ever new tasks, which may prove helpful in explaining improvement of relearning.

METHODS

The methods and equipment are described in detail in the paper by Dąbrowska (1959) — Kinaesthetic tasks in relearning albino rats. The only modification was that tasks were changed daily, that is every six runs, over several weeks. There were two equal groups of experimental animals which made a total of fourty. After a complete series of experiments with incomplete relearning, the animals were used in experiments with complete relearning.

EXPERIMENTS WITH INCOMPLETE RELEARNING

Relearning with regard to each consecutive specific task, was regarded as complete when after any amount of training, the animal eventually made six consecutive runs without error or hesitation. As incomplete relearning was regarded the amount of training the animal received over the arbitrary number of six daily runs made after consecutive, i.e. daily change of task, irrespective of the degree to which the task was mastered.

After a preliminary training, during which the animals became familiar with the general features of the maze, direction to be followed, and general experimental conditions, actual experiments were started. They lasted 54 or 84 days (group 1 or 2 respectively) and involved 54 or 84 different tasks; doors left unlocked in one task were invariably locked in the succeding one.

Over several days the rats appeared to make no progress in relearning. In the first ten tasks (60 runs) the number of erros in each failed to diminish noticeably, but then there appeared a mild but steady fall, shown in fig. 1. The graph shows results calculated as average from the first group of 20 animals. The results from the other group, which was trained to only 54 tasks, were essentially similar. For the sake of clarity the errors were not plotted for each task separately but as averages from 5 consecutive tasks (30 runs) and prorated on the basis of 1 run. The axis of ordinates indicates the average number of errors in one run, and the axis of abscissae each successive five tasks (30 runs). It will be seen from the diagram in fig. 1 that over the first ten tasks (60 runs) the average number of errors in one run decreased only by half an error. After 35 tasks (210 runs) however, the average number of errors in one

Fig. 1. Curve of incomplete relearning. Every point in the curve represents an average from five consecutive tasks.

run was halved. Thus, the number of errors in incomplete relearning diminished very slowly and the curve obtained from these experiments differed very essentially from an ordinary curve of learning; in the latter the initial decrease of errors is very rapid and then diminishes, and the curve begins to show oscillations. In

incomplete relearning the errors initially decreased at a mild rate, then rose briefly, and eventually fell to the level of roughly four in one run, but never to zero. This high final level of errors was rooted in the nature of incomplete relearning. The animals were learning every day a different path, and learning entails as a matter of course a certain number of errors. And it was precisely at that minimum level of errors, that the curve in fig. 1 become eventually stabilized. The above considerations refer to the curve of incomplete relearning, where each specific task was treated merely as one of the numerous units in an entire cycle of relearning. In due course it became necessary to consider the units as such, since their individual patterns showed fundamental differences which developped as relearning continued. For the sake of detail each six daily runs were analyzed separately in threes, and the average number of

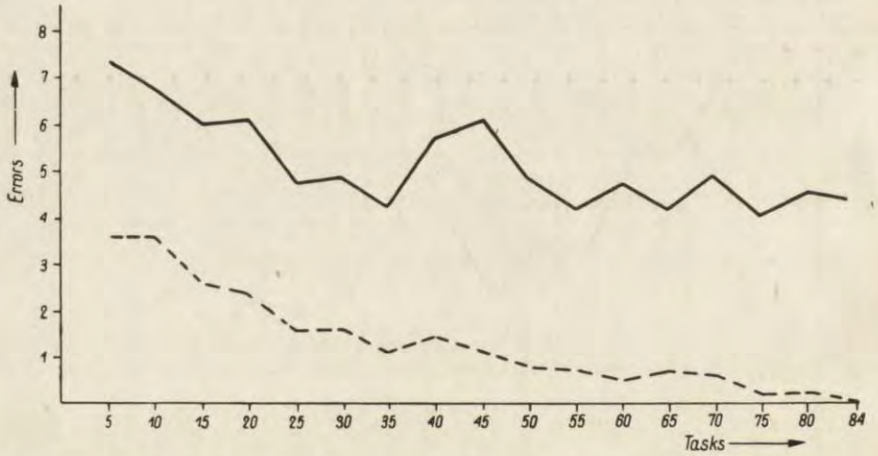

Fig. 2. Curves of incomplete relearning for first and second three of six daily runs (1 day = 1 task). Every point of the curves represents an average number of errors in the first or second three runs computed from five consecutive tasks and prorated on the basis of one run and one animal of group 2.

—— average number of errors in one of the first three runs; - - - - average number of errors in one of the second three runs

errors per run was calculated from all animals separately for the first three runs, and separately for the other three ones, and curves were plotted accordingly (Fig. 2). The first (solid) curve represents the average number of errors in one run calculated per animal from the first three runs in each five of consecutive 84 tasks. The

broken curve has been plotted analogically from the other three daily runs.

By analyzing in this manner the progress of learning it was found that over the first five tasks the difference in errors per run as between the first and the second three daily runs averaged roughly four, the actual figures being 7.5 and 3.5 errors in one of the first and second three runs respectively. When over the next five tasks the average of errors per run went down by half an error in the three first daily runs, there was no change in the relevant figure for the second three runs. Subsequent more or less conspicuous changes in the shapes of the two curves were not correlated. Eventually, the broken curve mildly sloped to zero, whereas the continuous one became more or less stabilized at the level of 4.5 errors per run, which was thus shown to be the minimum number of errors necessary for mastering the task. In this case we may refer to mastering the task because errors in the last three runs became here very rare and there were even unbroken successions of tasks where no errors were made in the last three runs, which became almost a rule by about the 80th task.

It followed from these experiments that although the animals were originally unable to master a specific task in the course of the six daily runs, continued training gave rise to some such changes in the process of learning as eventually made it possible for the animals to master a specific task after as little as three consecutive runs. In order to explore these changes it became necessary to review the errors in all the tasks in succession.

In the process of learning there could be distiguished two essential stages: one involved learning of the arrangement of the maze and of the general pattern of all tasks, and the other involved learning of the specific task. The first principle the animals learned was to try each door only once. They soon learned not to try another time a door they found locked in a given run. The other important principle, which the animals learned fairly late, was that the tasks changed daily: doors unlocked on one day, were invariably locked on the next. In this respect the pattern of errors inthe first run of each day was the following. Over the first ten to twenty days there was an increasing concentration of errors at such doors as very unlocked in the preceding task. When a certain maximum was reached errors became progressively fewer and eventually nil. In result of such training the animals ceased to

try in the first run a door which was unlocked on the preceding day. The question arose, did the animals passively avoid doors which were unlocked on the preceding day — did they not remember the preceding situation? Or was this avoidance active in nature — inhibition of a familiar reaction which was known to be inadequate on the succeeding day? These questions will be answered when further experiments are described.

The principale of avoiding doors that were unlocked on the preceding day was intimately associated with the second stage of learning, that is to say with the learning of specific tasks. If familiarity with this principle was to become manifest, the specific task had to be known to some extent, but even then it did become manifest only when the animal become familiar with the general pattern of the experiments. The learning of this general pattern i.e. treatment of the entire sequence of experiments as a single composite unit, could indeed be referred to as „complex" learning.

EXPERIMENTS WITH COMPLETE RELEARNING AFTER PRIOR INCOMPLETE RELEARNING

Towards the end of the preceding chapter two fundamental questions have been raised which it will be possible to answer by considering the results of the experiments described below.

When the sequence of experiments with incomplete relearning was concluded, both groups of animals were used in experiments involving complete relearning, wherein the animals were required to master successive tasks to such a degree as to be able to make six successive runs without error or hesitation. This means that over several days in succession (six runs daily) the animals were given the same task.

It followed from the results that when switched over to complete relearning the animals continued to behave as if they were given every day a different task irrespective of the fact that the sequence of unlocked doors remained in every detail the same over a number of successive days. For a number of successive days, while the rats were running along the same path, errors averaged in the first run roughly six, diminished by about the fourth run to zero, to rise on the next day again to three in the first run (Fig. 3.). As will be seen from the graph, the curve consisted of several peaks rising abruptly every day to several errors or so and mildy sloping to zero. As the peaks became gradually lower, errors

began to appear in the last three runs, and then the errors disappeared entirely so that the task became completely mastered (six successive runs without error or hesitation). When one task was mastered the animals received subsequent ones in due succession. The number of runs needed to master a task completely was gradually decreasing, except in the second task. Also the number of

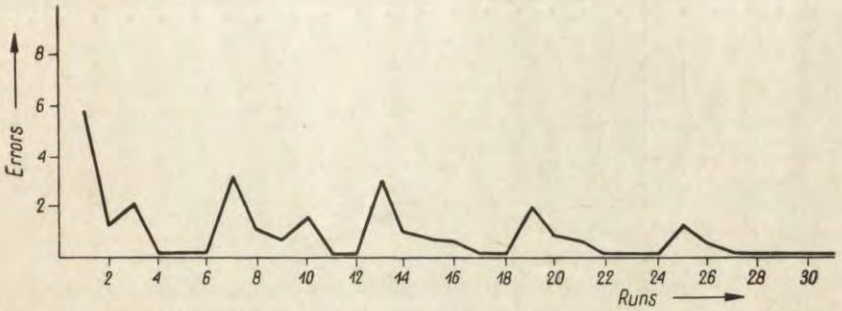

Fig. 3. Curve of complete relearning after prior incomplete relearning. First task; animals of group 2.

peaks diminished with successive tasks (Fig. 4, 5 and 6). It appears that now the questions referred to before can be answered. The questions implied that the animals either (a) avoided passively the doors that were unlocked in the preceding task, having forgotten the situation of the day before, or (b) avoided the doors actively, in-

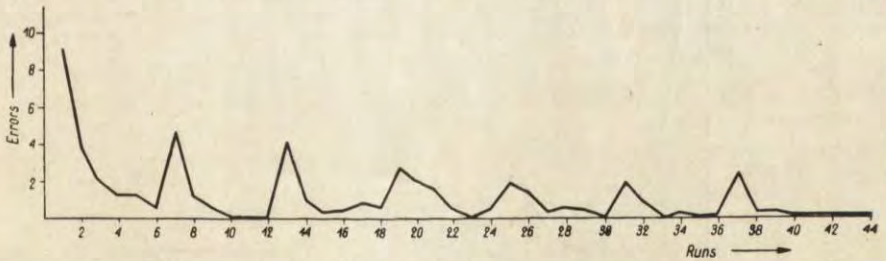

Fig. 4. Curve of complete relearning after prior incomplete relearning. Task 2; animals of group 2.

hibiting a familiar reaction which was known by the animals to be inadequate in the current situation.

The first conclusion would be in obvious disagreement with the experiments covered by the preceding report, concerned with complete relearning (D ą b r o w s k a 1959), and with the experi-

ments of other authors who demonstrated in rats a notable ability to memorize experimental patterns. Furthermore, it would leave completely unexplained the characteristic peaks in the curves covering the first succession of tasks in such complete relearning experiments as were preceded by incomplete relearning. These characteristic peaks, which disappear but gradually, have absolute-

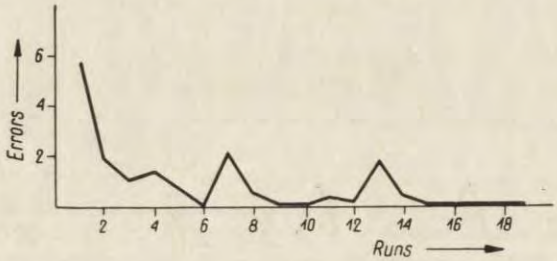

Fig. 5. Curve of complete relearning after prior incomplete relearning. Task 4; animals of group 2

ly no counterpart in, and are in striking contrast to analogical curves obtained from experiments involving complete relearning without a prior course of incomplete retraining, and are essentially the result of significantly persistant avoidance of such doors as were open on the preceding day. Consequently it appears justified

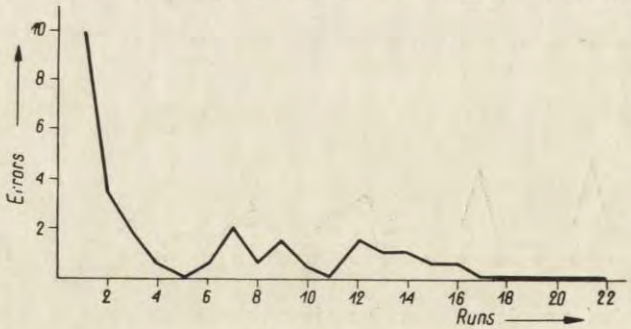

Fig. 6. Curve of complete relearning after prior incomplete relearning. Task 1; animals of group 1

to accept the other conclusion, namely that the rats did remember the pattern of the day before, but avoided repeating it knowing from experience that doors unlocked on one day were invariably locked on the next, and this in fact is the very essence of the

phenomenon reflected in the peculiar pattern of errors — made in the course of incomplete retraining — at such doors as were unlocked on the preceding day. Otherwise this peculiar pattern, that is the initial rise of such errors, subsequent decrease, and eventual complete elimination, would be difficult to explain. Hence it follows that in the course of incomplete training the animals learned the characteristic features of each specific task, but also the features common to all tasks jointly and peculiar to the entire sequence of tasks considered as one composite entity, and these two processes were in a way complementary to each other, and jointly they enabled the animals to master consecutive tasks more or less adequately after as little as three runs, provided that the entire sequence of experiments was continued for a sufficient length of time.

The experiments also revealed that incomplete relearning seriously interfered with subsequent complete relearning. Consequently, it has been attempted to explore the relationship between that interference and the length of the course of incomplete retraining. With this in view, the animals were divided into two groups, of which group 1 was switched over to complete relearning after 54 tasks (324 runs), and group 2 after 84 tasks (504 runs). A comparison of the behaviour of the two groups showed that group 1 (54 tasks), which had a shorter period of incomplete retraining, adapted itself more rapidly than group 2 (84 tasks) to the changed conditions involved in complete relearning. The peaks in the curve of complete relearning plotted for group 1 rose less abruptly, errors in the last three runs were more frequent, and the animals needed fewer days master the task completely (Fig. 6). To master the first task completely, group 1 needed only 22 runs, and group 2 as much as 31 runs, which makes a difference of almost 10. However, this advantage of group 1 over group 2 was restricted merely to the first two tasks in complete relearning. The second task was mastered by group 2 after 38 runs, and by group 2 after 44. In the third task the situation was spectacularly reversed: group 2 needed as little as 18 runs for complete mastery of the task, whereas group 1 toiled for the same purpose through a full 25 runs. Thus it became obvious that the advantage of group 1 was essentially spurious since the third and subsequent tasks revealed group 2 (which had been incompletely trained to a greater number of tasks) as more

receptive and capable of mastering a task in complete relearning more rapidly, except that the process of switching over from one principle to another — effected during the learning of the first and second tasks — was slightly more difficult and required a certain interval. But once this new principle was grasped, the rate of learning promptly increased, unlike in group 1 where it rose more slowly and by degrees. This is evidence of a general improvement of learning in group 2, which went through a longer course of incomplete retraining.

Finally, when the two groups were compared with regard to the ultimate results attained by the end of incomplete relearning, here also group 2 had a certain advantage over group 1. Towards the end of incomplete relearning experiments, animals of group 1 began to master specific tasks after three runs, which appeared to be the limit of their abilities, considering the relative difficulty of the tasks. However, in group 2, by about the eightieth task, some animals managed to master a task even after as little as two runs.

All these experiments show that learning is amenable to training, whereby the rate at which various tasks are mastered increases, as does also the capacity for switching over from one task to another.

HESITATIONS IN INCOMPLETE RELEARNING

The total number of hesitations was in specific tasks very slight, the average being little more than a bare one hesitation per animal per run. Thus it was is even difficult to speak of a numerical diminution in the course of learning, as the ultimate proportion of 0.4 per run differed little from the original 1. However, when prorated on the basis of one run in the first or second three runs of each specific task (similarly as was done with errors), the hesitations showed but insignificant changes, if any, in the first three runs as between the beginning and the end of the sequence of experiments, (the ratios being 0.8 and 0.6 in one of each first three runs at the beginning and end of experiments respectively), whereas differences in their proportion to one run of each second three were more significant, and could be attributed to continued training. The relevant proportions were: beginning of experiments — 1.4 hesitations in one of the second three runs per animal; end of

experiments — 0.2 in one run; furthermore, it must be added that the decrease of hesitations, slight as it was though, was very consistent throughout.

DISCUSSION

It will be seen from this report, as well as from those of other authors, that it is impossible to consider an animal's behaviour as an isolated phenomenon and without taking into account that animal's history. A specific experimental situation will be totally different for each of two animals, depending on prior experience of either. Two evidence supplied by the two groups of animals referred to before is here ample enough. It appears therefore that also disagreements in literature concerned with the behaviour of white rats stem from an inadequate analysis of earlier experimental situations. Different animals used in different experiments had different histories and showed therefore different reactions to specific and exactly similar stimuli, or combinations thereof.

This view is strongly supported by the experiments with incomplete relearning. The curves obtained in the experiments covered by the second chapter of this report, and concerned with complete relearning after a course of incomplete relearning, are essentially different from the curves obtained in the earlier experiments on complete relearning (D ą b r o w s k a, 1959). Repeated and abrupt peaks followed by mild falls to zero or near-zero values, are a new element and would be incomprehensible if the animals histories were unknown. In normal learning curves errors do also increase and diminish alternately, but these changes are relatively mild and have to them none of that consistent regularity which is so conspicuous in the curves obtained from complete relearning with prior incomplete relearning. Moreover, in plain complete relearning (without prior incomplete relearning) none of the rats was ever able to make in the first day of a new task three runs without error, and in one succession at that. Furthermore, in complete relearning, the number of runs needed to master the second consecutive diminished by one half in comparison to the first task, whereas it rose in experiments on complete relearning with prior incomplete relearning. The influence of the duration of prior training on subsequent learning is still more conspicuous if we consider

such factors as the number of runs made in the course of that prior learning. Differences in that number of runs, as between groups 1 and 2, modify in a certain degree the character of complete relearning. The number of runs needed for complete mastery of a task is on the whole smaller in group 2 than in group 1. During learning of the second task, in group 1, the peaks in the curve are not so regular and the last three runs are not entirely without errors. As may be seen, even such relatively slight differences in relearning as 180 runs influence the results of complete relearning. In group 1 complete relearning with prior incomplete relearning is more like the plain relearning without prior training (Dąbrowska 1959), than it is in group 2.

The differences in the number of runs as between groups 1 and 2 may possibly be explained by the fact that group 2, which had a longer course of incomplete relearning, may have learned more thoroughly the principle of constant switching over to new tasks. Since the change from incomplete relearning to complete relearning entailed a change of principle, it is evident that this change would be easier for that group which had been less well trained to the principle to be abandoned. However, the advantage of group 1 over group 2 continued merely over the first two tasks, whereafter the situation was reversed and group 2 began to master successive tasks more rapidly than group 1. This was so because group 2 was trained longer and had greater experience in mastering tasks. This group, therefore, should and did learn successive tasks in complete relearning more rapidly, except for the first two tasks, where it was handicapped by the too firmly established habit of switching over to new tasks daily. However, once this habit was inhibited, the learning of subsequent tasks became abruptly much more rapid.

All these changes which attend learning depend on the scope of the animal's past experiences and show that a rat's behaviour does not consist of strictly isolated reactions to current specific stimuli from without, but is determined by a aggregate of factors such as: a complex of innate reactions, habits acquired in the course of life, and a system of current stimuli which originate within or without the animal. Such of the animal's reactions as become inadequate in a changed situation do not disappear altogether but are transformed in a manner which makes possible better and quicker adaptation to new conditions.

CONCLUSIONS

1. During incomplete relearning the animal learns the features common to all tasks in a given sequence.

2. Incomplete relearning essentially modifies the curve of learning in subsequent complete relearning.

3. During incomplete relearning the animal acquires the ability of actively avoiding such doors as were unlocked on the preceding day.

4. Incomplete relearning facilitates the animal's switching over to a new task.

5. The total number of hesitations is in incomplete relearning smaller than in complete relearning without prior incomplete relearning.

6. In the course of incomplete relearning, consecutive specific tasks are mastered during the last three of the six runs on each experimental day.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

PROLONGED ACTION OF CALCIUM CHLORIDE ON *PARAMECIUM CAUDATUM*

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(Received 3 December 1958)

Many protistologists of this century have studied the adaptation of ciliates to a number of injurious environmental factors. Papers published up till now have shown that the adaptability of these organisms is due mainly to internal changes within the cells. Sonneborn (1943, 1948), Kimball (1947), Beale (1948, 1951, 1954) and Margolin (1956) have recently established that changes in the environment can produce cell changes which are inherited for many generations. *Paramecium* has been the main object of these studies. A series of different chemical agents in sublethal doses were used which caused acclimatization of paramecia consisting in the prolongation of their survival period in lethal solutions (Davenport and Neal 1896, Daniel 1909, Neuhaus 1910, Neuschloss 1919, 1920). The findings of Jollos (1913, 1921) are worthy of particular mention. They concern the inheritance of changes in the cell which have arisen as a result of the prolonged action of different factors (temperature changes, sublethal doses of arsenic or calcium salts ets.). In 1921 there appeared an extensive monograph by this author containing numerous experimental data and hypotheses which aroused a great deal of interest on account of the novel approach to the problem of adaptation in ciliates. Many of Jollos' conceptions concerning the so-called long-lasting changes (Dauermodifikationen) remain pertinent to this day. Many of his experiments have been repeated and further developed by other protistologists and physiologists. The findings of Orlova (1941) are of special interest. The author carried out

a long-lasting adaptation of *Paramecium caudatum* and *Paramecium multimicronucleatum* to calcium salts and obtained a number of individual lines resistant to concentrations which before adaptation would have been lethal for all the cultures. The course of acclimatization was generally in agreement with the observations of Jollos. It is worth mentioning, however, that the Orlova failed to obtain long-lasting resistance in some individual lines.

The results obtained by Orlova induced us to investigate whether *Paramecium caudatum* cultivated on our laboratory would react to some osmotically active agents in a similar fashion.

MATERIAL AND METHODS

In the present paper an attempt was made to analyse the fission-rate and resistance in *Paramecium caudatum* after introducing small amounts of osmotically active substances into the medium.

The experimental method consisted, on the one hand, in cultivating experimental and control lines under uniform conditions, on the other hand, in a uniform method of testing resistance and fission-rate.

The method used was very similar to the method of Orlova. All experiments were carried out on clones M and S cultivated in our laboratory in lettuce infusion. Some individual lines of paramecia received measured doses of *Bacterium coli*. The majority of the experiments was performed on single ciliates of our individual lines. These lines were grown in a medium which was changed daily in some experiments and every few days in others.

Sublethal M/100 — M/20 solutions of calcium chloride were mainly used. In some experiments a sublethal M/500 solution of glucose was used.

Individual lines were derived from a single specimen isolated from mass cultures (clones M and S) which were maintained in a mixture containing nine parts of bidistilled water and one part of stock lettuce infusion.

All experiments were always carried out in a culture medium with the same concentration of food as in the original culture. During the preparation of an appropriate concentration of the osmotically active substances special attention was given to maintaining a constant concentration of food in the medium.

Each culture was treated in the following way:

The experimental lines, i. e. those which were subjected to the prolonged action of sublethal doses of calcium chloride (Ca-lines) or glucose (G-lines), differed from the control lines only in the concentration of these agents in the medium.

From time to time the mass cultures, cultivated parallel to the individual lines, were tested for their calcium content. The calcium was precipitated with ammonium tartrate and titrated against $KMnO_4$. No dimi-

nution in the calcium content of the mass cultures was observed. The calcium content of the mass cultures was constant for several months and as a result of evaporation was even slightly higher than at the beginning. It does not seem probable, therefore, that a diminution of the calcium content would occur in the individual lines where paramecia were repeatedly transferred to a new medium.

The survival period of the paremecia in lethal solutions of calcium chloride, i. e. the resistance of paramecia, was the main test of our experiments. It was determined in small amounts (1ml) of culture medium containing a constant lethal concentration of calcium chloride (M/13.3). The lethal concentration of glucose was M/3.3 which gave the same survival period as the M/13.3 solution of calcium chloride.

Observations on resistance were made on single individuals. They were examined under a low-power lens to the moment when locomotory movement of the paramecia ceased and afterwards the cessation of cilliary movement was observed under the microscope. As the volume of the culture medium usually was small two individuals were observed simultaneously. The cessation of the ciliary movement was observed by means of a stop--watch starting from the introduction of the lethal solution into the medium with paramecium. Death was taken as the moment when the complete cessation of the ciliary movement occurred.

The paramecia of the individual lines were always examined after a definite period following fission. Close attention was also paid to ensuring that the resistance of the control paramecia was measured the same day as that of the experimental paramecia.

All the above measures were undertaken with the aim of creating constant experimental conditions which eliminate the differences in the resistance often occurring in mass cultures.

RESULTS

Fission-rate

As seen in Fig. 1 the rate of fissions decreases immediately after the introduction of calcium chloride into the medium with paramecia. In spite of the considerable variability in the fission-rate of the controls, the fission-rate curve of Ca-lines at all points lies below the curve for the fissions of the controls. The greater is the concentration of the calcium salt in which the paramecia are cultivated, the more marked is the inhibition of fassion-rate. A series of additional observations showed that if strong concentrations are used the fissions may be inhibited for a long period. Such concentrations are, however, harmful for the organisms and sooner or later cause the death of all individuals. It is interesting that even

such weak concentrations as those used in our experiments are disadvantageous for the paramecia since after about two and a half

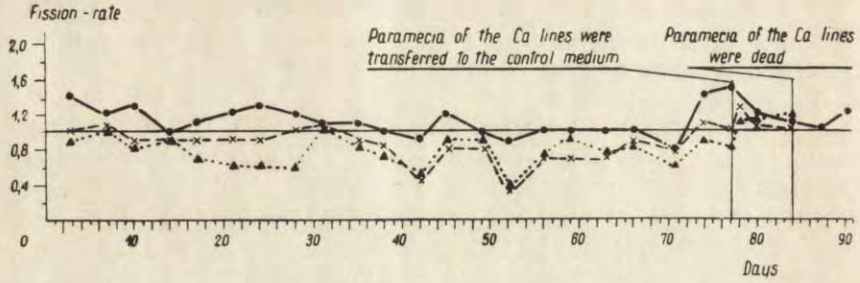

Fig. 1. Fission-rate of individual lines of *Paramecium caudatum*
Abscissae: consecutive days from the introduction of sublethal concentrations of calcium chloride into the medium with paramecia. Ordinates: the average fission-rate of 10 individuals. The dots indicate the fission-rate of the control line. The crosses indicate the fission-rate of the Ca-line cultivated in a M/100 solution of calcium chloride. The triangles indicate the fission-rate of the Ca-line cultivated in a M/50 solution of calcium chloride

months the ability to divide does not return to the level normally observed in the controls. Similar results were obtained from observations on the mass cultures.

Resistance

The effect of sublethal solutions of calcium chloride on the prolongation of the survival time of *Paramecium* in lethal solutions appears relatively quickly and depends on the concentration of this compound in the medium. Individual paramecia cultivated in M/100 $CaCl_2$ solutions exhibit an increase in resistance in about 7—10 days, whereas parmecia cultivated in M/50 solutions exhibit an increased resistance in about 4 days (Fig. 2). When the M/20 solution of calcium chloride was introduced into the medium with individual paramecia, the latters died. However, medium in which there were several paramecia (about 10 per 1 ml) survived in this concentration and some paramecia showed an increased resistance already 24 hours.

It follows from the data presented in Fig. 2 that the increased resistance of paramecia cultivated in sublethal solutions of calcium chloride was gradually lost and after some time returned to normal. In several Ca-lines it was found that after about 30 or 20 days the paramecia cultivated in M/100 or M/50 solutions respectively

did not differ from the control lines. The decrease in resistance of paramecia cultivated in M/20 solution took place abruptly in 4—5 days.

In order to determine whether the decrease in resistance also occurs when the concentration of calcium chloride is gradually increased, a special series of experiments was undertaken.

The paramecia were gradually transferred from a M/50 solution through solutions of increasing concentrations, namely M/40, M/33.3, M/28.6 and finally M/25 (Fig. 3). The mortality was very high in these experiments and sometimes there was a danger that the whole line would perish. However, it proved possible to obtain paramecia living and reproducing in M/25 solution. All attempts to transfer individuals to concentrations greater than M/25 ended in failure. In M/50 — M/25 solutions the paramecia retained the resistance

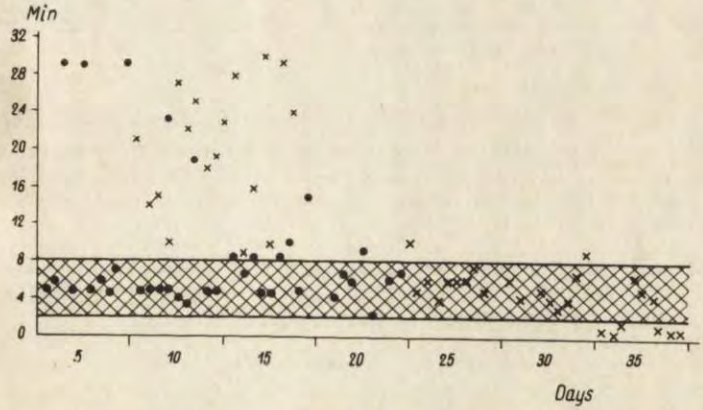

Fig. 2. The course of resistance of individual lines of *Paramecium caudatum* cultivated in constant sublethal concentrations of calcium chloride

Abscissae: consecutive days from the introduction of sublethal concentrations of calcium chloride into the medium with paramecia. Ordinates: survival time (resistance) of the paramecia in lethal solution of calcium chloride. The chequered area represents the resistance of the individuals of the control line. Crosses — the resistance of the individuals of Ca-line cultivated in a/ M/100 solution of calcium chloride. Dots — the resistance of the individuals of Ca-line cultivated in M/50 solution of calcium chloride

for about three months. In contrast to paramecia cultivated constantly in M/100 or M/50 solutions the decline in resistance in those paramecia was small.

In order to analyse the resistance in paramecia more precisely experiments were performed with glucose using M/500 solution. Each experiment lasted about two weeks. The determination of

resistance was carried out regularly every few days. It was established that paremecia exhibit an increase in resistance already after 4 days from the moment of introduction of a M/500 solution of glucose. The increased resistance lasted about 7 days and then it was abruptly lost.

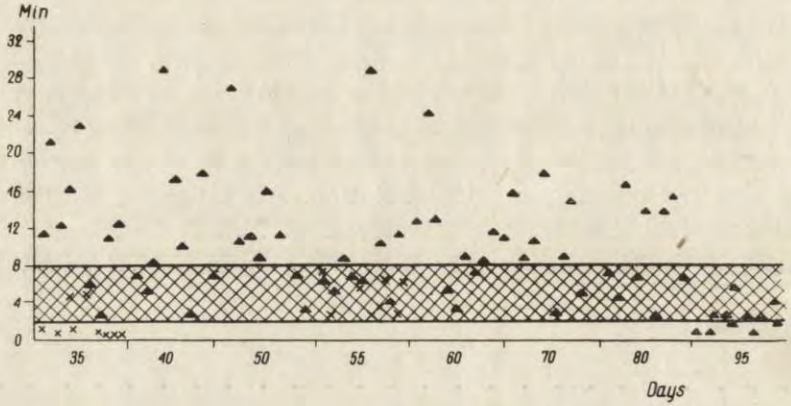

Fig. 3. The course of resistance of an individual line of *Paramecium caudatum* cultivated in grandually increasing concentrations of calcium chloride
Abscissae: consecutive days from the introduction of sublethal concentrations of calcium chloride into the medium with paramecia. Ordinates: survival time (resistance) of the paramecia in lethal solution of calcium chloride. The chequered area represents the resistance of the individuals of the control line. Crosses — the resistance of the individuals of Ca-line cultivated in a M/100 solution of calcium chloride. Triangles — the resistance of the individuals of Ca-line cultivated in gradually increasing concentration of calcium chloride

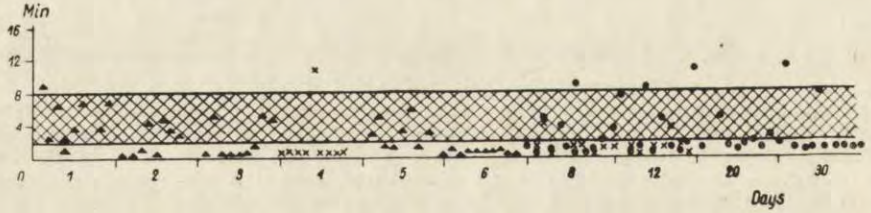

Fig. 4. The course of resistance of individual reverse lines of *Paramecium caudatum*
Abscissae: consecutive days from the transferring paramecia from the sublethal solutions of calcium chloride to the control medium. Ordinates: survival time (resistance) of the paramecia in lethal solution of calcium chloride. The chequered area represents the resistance of the individuals of the control line. Dots — the resistance of the individuals of the reverse line cultivated in a sublethal solution during 60 days. Crosses — the resistance of the individuals of the reverse line cultivated in a sublethal solution during about 75 days. Triangles — the resistance of the individuals of the reverse line cultivated in a sublethal solution during 90 days

The retention of resistance after transferring paramecia from the media with the sublethal concentrations of calcium chloride to the control media is of special interest and it was investigated in a new series of experiments.

In these experiments the so-called reverse lines of paramecia were used. The reverse lines were derived from the Ca-lines and put back into the control medium at various periods after transferring the paramecia into the calcium chloride solution. Five reverse lines were studied carefully of which three are presented in Fig. 4.

It follows from this figure that the increase in resistance to calcium chloride is a temporary phenomenon and it disappears as soon as the paramecia are returned to the control medium. Similarly, the fission-rate immediately returns to normal and often after 24 hours the number of paramecia of the reverse lines equals and even exceeds the number of control individuals. Since further experiments supported the findings concerning the decrease in resistance in spite of the continuous action of calcium chloride, it can be understood that the same phenomenon occurs immediately after transferring the paramecia to a medium free of calcium chloride.

DISCUSSION

In contrast to O r l o v a who obtained a long-lasting adaptation to calcium chloride in many lines of paramecia, in the present experiments such adaptive changes were not observed. Our findings show that calcium chloride has an harmful effect on *Paramecium caudatum*. It follows from the fact that in paramecia, cultivated in sublethal solutions, there is a constant decrease in fission-rate as well as a decrease in resistance, only temporarily preceded by an increased resistance. The disturbances caused by calcium chloride depend upon its concentration: 1) the decrease in fission-rate is greater in stranger than in weaker solutions; 2) the greater the concentration of sublethal solutions, the more rapidly decreases the resistance of paramecia, preceded by a transient increase. The initial increase in resistance to calcium chloride can be maintained for a longer period, if paramecia are transferred to gradually increasing concentrations. However, even in this case the resistance disapppears after about three months, and after a few weeks a lower resistance in paramecia living in stronger solutions and a higher resistance

in paramecia living in weaker solutions is observed. Sometimes, a better effect is obtained by a so-called rapid adaptation, when after being put in a most strong sublethal solution, i.e. approaching the letal one, paramecia exhibit the highest increase in resistance already after about 24 hours.

As far as the action of glucose is concerned, even very small amounts of it cause the paramecia to exceed the resistance limits of the controls. In this case the increased resistance may be seen in 4—5 days. Then, however, it disappears very rapidly.

It is obvious, therefore, that after transferring paramecia to a medium free of calcium chloride or glucose it is impossible to obtain an increased resistance in spite of the previous prolonged action of these chemical compounds. The reactions of these paramecia very quickly return to the norm and sometimes their resistance is smaller than the resistance of the controls.

The changes in resistance in *Paramecium caudatum* exposed to calcium chloride seem to indicate that this animal is able to regulate some of its functions. Owing to this ability it may prevent injurious agents from penetrating into the cell. Shortly after introducing paramecia into a medium containing concentrations of mineral salts stronger than normal, it is observed an acceleration of locomotory movement, a decrease in the body dimensions and a brown colouration of the whole body. Some of these effects were observed by other authors, too, who investigated also the rate of formation of food and contractile vacuoles and found both to be decreased (Chejfec 1939; Frisch 1939). However, the activity of the vacuoles was restored to its normal level after or even without transferring paramecia into a medium free of osmotically active substances. Recently, Kuźnicki noted that after transferring paramecia into the weak solutions of a number of inorganic salts, the rate of pulsation of the contractile vacuoles which had been initially decreased returned to the normal state. In the present experiments it was established that the return to the norm concerns not only the physiological but also the morphological changes.

It seems that all the morphological and physiological changes which are produced in *Paramecium* by the short action of weak sublethal solutions of calcium chloride have a typical regulatory character and fulfil the criterion used for adaptive changes. On

the other hand, the prolonged action of calcium chloride or application of its strong sublethal solutions cause a gradual disintegration of the organism leading eventually to its death. The best evidence for the harmful effects of calcium chloride is the death of the individual Ca-lines maintained for a long time in even weak concentrations (M/100 or M/50). Paramecia perish not only in the presence of calcium chloride but also after being transferred to a normal medium although there is the same fission-rate in them as in the controls.

The theory of Jollos of long-lasting modifications concerns only the adaptive changes, i. e: those changes which enable the organism to survive in disadvantageous conditions. The changes described in this paper and observed during the period when paramecia were subjected to prolonged action of strong concentrations of calcium chloride cannot, therefore, be called a long-lasting modification.

However, many workers have been able to obtain such changes by introduction of various chemical substances to the medium containing paramecia. The results of Jollos and Orlova have been already mentioned. Besides these authors the long-lasting modifications in protozoa obtained: Harnisch (1926), Moevus (1934) and Schuckmann and Piekarski (1939). All these investigators started from the theory of Jollos of long-lasting adaptations. But the obtained results were rather contradictory.

The Jollos' concepts were criticized by Raffel (1932) of the Jennings laboratory. This author considered the long-lasting modifications of Jollos as a so-called reverse mutations. Schuckmann and Piekarski considered that it was unnecessary to introduce the term "long-lasting modifications" since according to them Jollos obtained simple modifications only. However, in order to discriminate between changes which disappear abruptly from those which persist through several generations these workers propose the terms labile and stabile modifications.

Hernisch criticized the Jollos' methods presented in his papers concerning the effect of arsenious acid on paramecia. He considered that Jollos gave too little attention to the bacteriological factor which is impossible to eliminate from any medium containing ciliates. A similar point of view was taken by Neuschloss (1919, 1920) who was of the opinion that the main role

in the process of neutralising the harmful effects of some chemical compounds (quinine, arsenic etc.) must be attributed to the active influence of *Paramecium* on the environment. Harnisch concluded that bacteria cause fundamentally changes in the environment which loses its previously toxic properties. Grębecki and Kuźnicki (1955) came to the similar conclusions. Their experiments showed that in the cultures of paramecia, various toxic chemical compounds can be adsorbed by organic detritus. In this case no changes in the ciliates themselves may be observed but it is possible to determine that the toxic substances previously introduced into the medium are either partly or completely absent.

Even some of the followers of Jollos were unable to obtain his results. For example, Schuckmann and Piekarski failed to obtain clones of *Paramecium caudatum* resistent to arsenic; they observed the long-lasting modifications only in *Colpoda steinii.*

Very extensive work was done by Orlova. Her results were somewhat different from those of Jollos. Nevertheless, in some experiments she made very similar observations. In Jollos' experiments the attention was chiefly concentrated on fission-rate of adapted ciliates. Orlova stated only a temporary decrease in fission-rate and for that reason the main criterion of her experiments was the increase in resistance of paramecia to lethal solutions of calcium chloride. Similarly to the long-lasting modifications of Jollos in some Ca-lines, she obtained a prolonged and very marked increase in resistance of paramecia which very slowly and gradually disappeared after transfering the animals to the control medium.

The results presented in this paper support only partly those of Orlova: an increase in resistance is observed, but it is transient and disappears very quickly. Similar results were obtained by Chejfec (1939). During the prolonged adaptation to calcium chloride an increase in resistance appeared which, however, abruptly disappeared after transferring paramecia to a medium free of this substance. In an unpublished paper Chejfec also obtained an adaptation to the chlorides of magnesium, barium and strontium. Magnesium chloride gave similar results as calcium chloride producing some resistance which disappeared in about 48 hours after transferring paramecia to a normal medium. It is necessary to add

that Chejfec experimented on adaptation of mass cultures in which selection might appear. Besides, he adapted the ciliates to the gradually increasing concentrations in which resistance lasted much longer than the resistance of paramecia kept continuously in the same concentration. Consequently, Chejfec considered that he did not obtain long-lasting modifications. Dembowski (1942) concluded that in *Paramecium caudatum* there was no adaptation to potassium chloride. Recently, Beale (1953) failed to obtain longlasting modifications to sodium chloride. The aquired resistance disappeared shortly after transferring paramecia to a medium containing only traces of this salt. Seravin (1958) achieved a very marked increase in the resistance of paramecia in the early period of adaptation to the salts of calcium, sodium and potassium. After several days a decline in resistance was, however, observed, followed sometimes by a complete return to the norm. In consequence, the Seravin's findings seem to support the results of the present paper.

It would appear that in our experiments a certain permanent change was obtained. This was the decrease in fission-rate which was maintained for over two months. However, the fission-rate returned to the norm immediately after transferring paramecia to a medium free of calcium chloride.

Our results seem to indicate that calcium chloride has in fact an harmful effect on the ciliate organism and the changes produced by it are sometimes irreversible and leading to the death of paramecia. It is true that small doses of calcium chloride applied for a short time can produce adaptation. On the other hand, after calcium chloride has been applied for a longer period its effects appear to be harmful.

It is possible to suppose that there are some strains of *Paramecium* which are particularly resistant to osmotically active agents and they were probably those that Orlova was dealing with. It is also possible that the discrepancies between our results and those of Orlova depend on the different nutritive media used in both studies.

SUMMARY

1. The prolonged action of various sublethal concentrations (M/100 — M/20) of calcium chloride on the fission-rate and resi-

stance in individual lines of *Paramecium caudatum* was investigated. In several experiments paramecia were exposed to glucose.

2. It was found that calcium chloride causes a permanent decrease in fission-rate of *P. caudatum;* the greater the concentration of calcium chloride, the more changed is the fission-rate. The decrease of the fission-rate is lost, however, when paramecia are put back into normal medium.

3. In the first period after exposure of paramecia to calcium chloride a marked increase in resistance is observed. The increased resistance is transient and it disappears in about 1 month in spite of a constant maintainance of paramecia in the sublethal concentrations of the salt. The greater the sublethal concentrations of calcium chloride, the more rapidly appears the increase in resistance, and the more abrupt decrease in it is observed.

4. The increased resistance may be prolonged for about 3 months if paramecia are transferred to gradually increasing concentrations of calcium chloride. But, even in this case the increased resistance disappears finally irreversibly.

5. When paramecia are put back into normal medium the increased resistance disappears abruptly in about 24 hours.

6. In contrast to the control, very high mortality in the individual lines of paramecia exposed to prolonged action of sublethal concentrations of calcium chloride is observed.

7. Similar results were obtained in paramecia exposed to glucose.

I am grateful to Professor J. Dembowski for valuable advice and encouragement throughout this work. My sincere thanks are due to Miss K. Golińska for her technical assistance.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

THE TRANSFORMATION OF DIFFERENTIATED INHIBITORY STIMULI INTO POSITIVE CONDITIONED STIMULI

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(Received 1 October 1958)

In our previous papers (K o n o r s k i and S z w e j k o w s k a 1950, K o n o r s k i and S z w e j k o w s k a 1952) the following facts concerning the transformations of excitatory into inhibitory conditioned reflexes and vice versa were established:

1. If a firmly established conditioned stimulus is chronically extinguished and then restored, the process of extinction is very slow, whereas the process of restoration is rapid, and after a few reinforcements the reflex reverts to its preextinctional level.

2. If a stimulus, not similar to any of the positive conditioned stimuli, is applied without reinforcement from the very beginning of its application, and is transformed later on into positive conditioned stimulus, then the excitatory reflex to it develops very slowly and does not attain the strength of other conditioned reflexes established in the usual way; chronic extinction of such a reflex occurs more rapidly than that of „normal" conditioned reflexes.

Accordingly, we have divided all stimuli applied in conditioned-reflex experiments into two groups: 1) primary excitatory stimuli, i.e. those stimuli, which from the very outset are reinforced by the unconditioned stimulus; 2) primary inhibitory stimuli, i.e. those stimuli, which are not reinforced at all at the beginning of training. The properties of these two groups of stimuli differ considerably even if, in result of appropriate transformations, they are both positive or both negative (cf. K o n o r s k i and S z w e j k o w s k a 1952).

It seemed interesting therefore to investigate to which of these two groups belong the differentiated inhibitory stimuli, i.e. those stimuli which, owing to generalisation, evoke initially a positive conditioned reaction, but are never reinforced from the very beginning of training. The primary positive character of these stimuli makes them analogous to the stimuli of the first group, whereas application without reinforcement brings them near to the second group.

MATERIAL AND METHODS

Experiments were performed on 5 dogs. They were conducted in a typical sound-proof conditioned reflex chamber. Alimentary salivary conditioned reflexes were used. The food was presented by moving into position a bowl in a foodtray. Salivation was recorded by the method described elsewhere (Konorski and Szwejkowska 1950) from the parotid gland fistula. Each experimental session consisted of 5—6 trials, the inter-trial intervals being 4—5 minutes. The isolated period of the conditioned stimuli was 20 sec.

RESULTS

The course of experiments in all our dogs was approximately the same. First, conditioned alimentary reflexes were established to two stimuli, a metronome (M) and bell of a definite sound (B_1). Then, two new stimuli, similar to the bell were introduced, namely: a bell of another sound (B_2) and a buzzer (Bz). The difference between the sound of B_1 and B_2 was much smaller than the difference between B_1 and Bz. Both new stimuli, B_1 and Bz, were applied among positive conditioned stimuli and were not reinforced by food. In three dogs they were applied once daily each, every day in reverse sequence. It was found, however, that more reliable results are obtained if only one differentiated stimulus is given in each experimental session. Therefore, in two other dogs B_2 ad Bz were applied every second day in alternate sequence. The experiments with these two dogs are presented in fig. 2 and 3. The numbers of applications of B_2 and Bz were in each dog strictly the same, however they were different in different dogs depending on the course of inhibitory training. When the level of the conditioned reactions to the differentiated stimuli fell to a more or less stable level, a new series of experiments began, in which B_2 and Bz were applied in the same way as before (i.e. once daily in 3 dogs and every second day in 2 dogs) but they were reinforced by food.

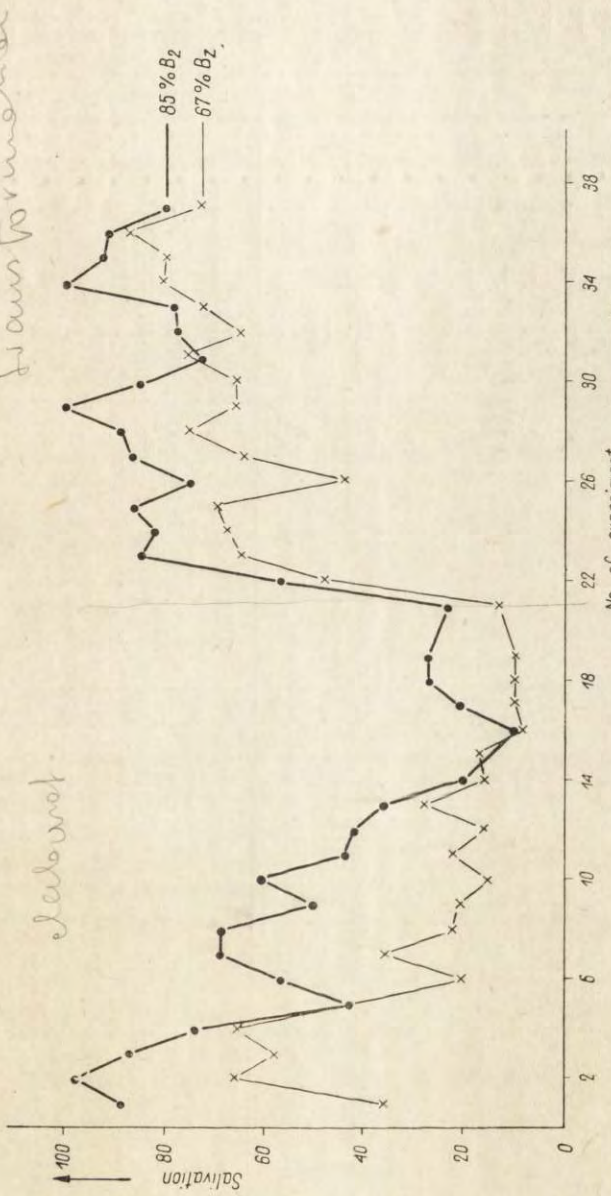

Fig. 1. The elaboration of inhibitory conditioned reflex and its transformation into an excitatory conditioned reflex to a more (B_2) and less (Bz) similar stimulus i respect to the primarily conditioned stimulus (B_1) in dog 1.

Abscissae: successive trials for each of the differentiated stimuli. Ordinates: magnitude of the reflexes to B_2 (dots) and to Bz (crosses) in percentage of the reflexes to positive stimulus, B_1. At arrow the beginning of a series in which B_2 and Bz are reinforced. On the right the mean magnitude of reflexes to B_2 and Bz from the 4 th reinforced trial

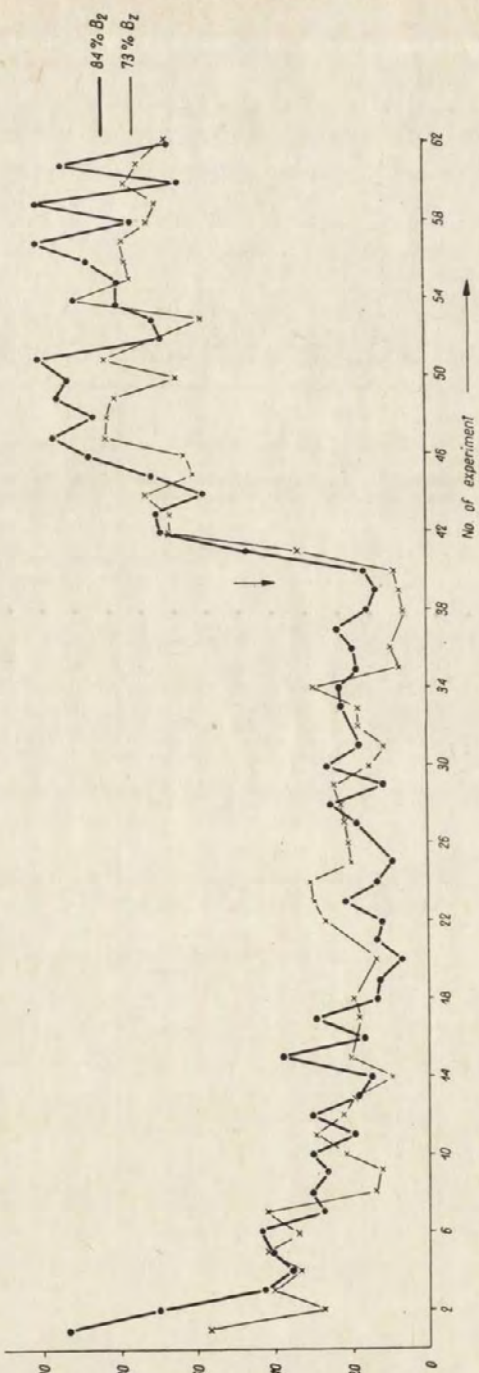

Fig. 2. The elaboration of an inhibitory conditioned reflex and its transformation into an excitatory conditioned reflex to a more (B_2) and less (Bz) similar stimulus in respect to the primarily conditioned stimulus (B_1) in dog 2.

Explanations as in fig. 1

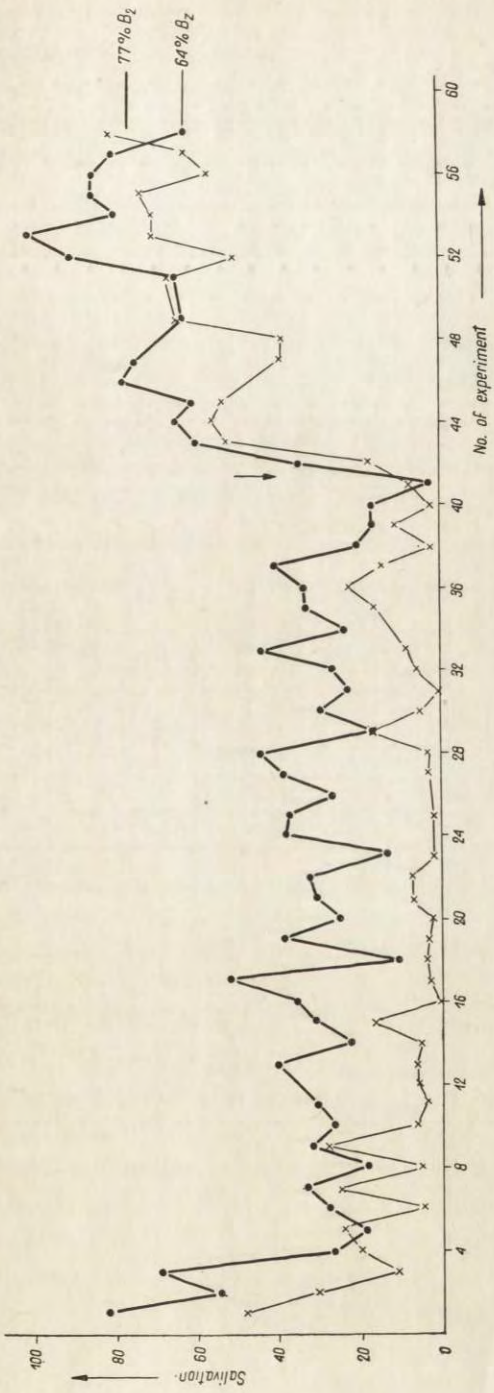

Fig. 3 .The elaboration of an inhibitory conditioned reflex and its transformation into an excitatory conditioned reflex to a more (B_2) and less (Bz) similar stimulus in respect to the primarily conditioned stimulus (B_1) in dog 3.

Explanations as fig. 1

The results obtained in all our dogs are similar. But since in two dogs conditioned reflexes, both positive and negative, were very irregular, and one of the dogs developed experimental neurosis in the course of transformation of differentiated stimuli, the corresponding data are not reported here. We describe in detail the results obtained on three dogs; the material concerning one of them (No 1) was briefly reported in a previous paper (Konorski and Szwejkowska 1952).

The whole course of experiments is presented for each dog separately in Figs. 1, 2 and 3.

Table I

Total salivation in divisions of scale during differentiation of stimuli B_2 and Bz and their transformation into positive conditioned stimuli

Dog	Differentiation			Transformation		
	B_2	Bz	Bz in % of B_2	B_2	Bz	Bz in % of B_2
1	461	239	52	792	688	86
2	305	247	81	632	566	89
3	554	177	30	600	400	66

The following conclusions can be drawn from these figures:

1. In all our dogs the first application of B_2 produced a larger salivary effect than the first Bz. The course of differentiation of Bz was more rapid than of B_2, and in general the ultimate level of reflex to Bz was lower than the level of reflex to B_2. Accordingly, as seen in Table I, the total amount of salivation during the whole period of differentiation was much smaller to Bz than to B_2.

2. The transformation of B_2 and Bz into positive conditioned stimuli appeared to be relatively rapid to both stimuli, since after a few reinforcements only they acquired a more or less stable value. But the growth of the reflex to Bz was somewhat slower than that o B_2, and the level it achieved was lower (see Table II). Accord-

ingly, to the total amount of salivation throughout the period of transformation was smaller to Bz than to B_2, as seen in Table I.

Table II

Average values of transformed conditioned reflexes to B_2 and Bz in percentage of control reflex

Dog 1				Dog 2				Dog 3			
A		A_1		A		A_1		A		A_1	
B_2	Bz	B_2	Bz	B_2	Bz	B_2	Bz	B_2	Bz	B_2	Bz
85	70	85	67	68	67	84	73	65	55	77	64

A — mean values of conditioned reflexes of 3—4 first experiments.

A_1 — mean values of conditioned reflexes of total series (without 3—4 first experiments).

3. As seen in Figs. 1—3 and in Table II, neither Bz nor B_2 attained the value of the reflex to B_1. Only in a few cases did the reflex to B_2 reach 100 per cent of the control reflex, and in no case did this happen as regards Bz.

Table III

Degree of irregularity of conditioned reflexes to the positive stimulus and to the transformed stimuli

Stimulus	Average value of reflex in divisions of scale during 20 sec. isolated period	Mean deviations from average value of reflex in divisions of scale	Mean deviations in percentage of positive reflex
	Dog 2		
B_1	37	3.5	9
B_2	31	4.4	14
Bz	27	2.9	11
	Dog 3		
B_1	50	6.7	13
B_2	42	9.0	21
Bz	28	7.0	25

4. The transformed reflexes to Bz and B_2 were more irregular than the control reflex as shown in Table III.

DISCUSSION

As seen from our results, a stimulus very much similar to the primary conditioned stimulus, when not reinforced, behaves in much the same way as a chronically extinguished stimulus. Its original effect is almost the same as that of its positive counterpart, and its resistance to extinction is considerable. Just as with chronically extinguished stimuli, transformation into the positive stimulus is rapid, and the effect achieves a value not much lower than that of the positive stimulus. On the other hand, a stimulus more remote from the primary conditioned stimulus behaves differently, when not reinforced: the original effect is low, the "extinction" more rapid and complete, and the transformation into the positive stimulus less perfect. Thus, the properties of this stimulus are very similar to those of the primary inhibitory stimulus.

It seems that the difference between the properties of fine and crude differentiation can be understood by reference to our previous concepts of the elaboration of excitatory and inhibitory conditioned reflexes (K o n o r s k i and S z w e j k o w s k a 1952). A stimulus very similar to a primary conditioned stimulus possesses a strong excitatory character, due to a high level of generalization. According to the conception of K o n o r s k i (1948), the central representation of this stimulus largely overlaps with the representation of the primary conditioned stimulus, and, in consequence, it has many connections with the unconditioned centre. This is the reason why a differentiation of such a stimulus resembles the chronic extinction of a primary conditioned stimulus, and its transformation into the positive stimulus resembles the restoration of the extinguished stimuli. By contrast, the central representation of a stimulus which is more remote from the primary conditioned stimulus possesses fewer elements in common with the central representation of the latter, and therefore its connections with the unconditioned centre are less abundant. If this stimulus is applied without reinforcement, a strong inhibitory reflex is formed to it (as is the case with a quite dissimilar stimulus) (cf. K o n o r s k i and S z w e j k o w s k a), and it is this which makes so difficult the subsequent transformation of this stimulus into the positive stimulus.

SUMMARY

1. The properties of the differentiated inhibitory stimuli were investigated in respect to their rate of transformation into the positive conditioned stimuli.

2. It was found that in the case of fine differentiation the inhibitory stimulus is easily transformed into the excitatory stimulus, while in the case of crude differentiation this transformation is more difficult and imperfect.

3. It can be concluded that in the case of a fine differentiation the stimulus behaves like a primary excitatory conditioned stimulus, whereas in the case of a crude differentiation it resembles a primary inhibitory stimulus.

4. The properties of differentiated stimuli are discussed.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

THE INFLUENCE OF THE PRIMARY INHIBITORY STIMULUS UPON THE SALIVARY EFFECT OF EXCITATORY CONDITIONED STIMULUS

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In one of our previous papers (Konorski and Szwejkowska 1952) it was established that if in experiments with alimentary conditioned reflexes a definite stimulus is applied over a long period without reinforcement, and then a positive conditioned reflex is elaborated to it, this reflex develops with great difficulty and does not attain the level of the "normal" conditioned reflexes. From this fact, the conclusion was drawn, that a stimulus applied without reinforcement does not become „neutral", as was supposed earlier, but becomes inhibitory. We called such stimuli „primary inhibitory" in contradistinction to those inhibitory stimuli which have been transformed from excitatory stimuli.

According to Konorski (1948), the mechanism of inhibitory conditioned reflexes consists in formation of inhibitory conditioned connections, alongside the excitatory ones, between the centre of an inhibitory stimulus and the centre of the unconditioned stimulus. Therefore, the normally used inhibitory stimuli (established by extinction or differentiation) were thought to be, in fact, "mixed" stimuli, having both excitatory and inhibitory character. On the other hand, since the primary inhibitory stimulus was never reinforced by the unconditioned stimulus, the connections between its centre and the unconditioned centre would have to be purely inhibitory. In consequence, if such a stimulus is applied simultaneously with an excitatory conditioned stimulus, algebraic summation be-

tween the two effects should occur, and, as a result, the positive conditioned reflex should be diminished. The present paper is concerned with testing this conclusion.

MATERIAL AND METHODS

The experiments were performed on two dogs. The usual salivary conditioned-reflex method was used. The salivation of the parotid glands by chronic fistula was recorded by method described in an earlier paper (Konorski and Szwejkowska 1950). Experiments were performed in a typical sound-proof chamber with the experimenter outside. The food was given from the foodtray by moving into position a bowl filled with a bread-powder moistened with broth. Each experimental session lasted about half an hour and consisted of 4—5 reinforced stimuli, and 1—3 unreinforced stimuli applied in varying order. The isolated period of conditioned stimuli lasted 20 seconds, intertrial intervals being 4—5 minutes.

RESULTS

The whole series of experiments carried out on both dogs covered about $1^1/_2$ years. First, positive conditioned reflexes to two stimuli (bell and bubbling of water) were established. Then, two new stimuli, metronome and whistle, were introduced, which from the very beginning were applied without reinforcement. We shall denote the two positive stimuli as S_1, S_2 respectively, and the two negative stimuli, as S_3, S_4 respectively. At the beginning, the unreinforced stimuli elicited slight salivation due to generalisation, but very soon it diminished considerably, oscillating between 0 and 6 per cent of the value of positive conditioned reflexes. The total number of experiments with each dog was about 300. There were about 1500 positive trials and about 425 negative trials.

After about $2^1/_2$ months of the preliminary conditioned-reflex training (in which 200 positive and 52 negative trials were made) the experiments proper began. They took the following course. About one in twelve of the experimental sessions was a special experiment in which, on one occasion, positive and negative conditioned stimuli were applied jointly. Since there were 4 possible combinations of such stimuli (S_1S_3, S_2S_3, S_1S_4, S_2S_4); each such combination occurred approximately once in about 50 sessions, or once in about 250 trials. Of course, in normal experiments, constituting the background for the crucial onces, both positive and negative conditioned stimuli continued to be applied. From time to time, the

combinations of two positive stimuli (S_1S_2) and two negative (S_3S_4) were also applied by way of control.

In the course of these experiments, various combinations of two stimuli were used.

Application of the combined stimuli in overlapping sequence, the negative stimulus being the leading one

In the first part of our experiments, we applied the positive and negative stimuli in the combined trials in the following manner. First, a negative stimulus (S_3 or S_4) was applied, and after 10 sec. a positive stimulus (S_1 or S_2) was added for further 20 sec. Both stimuli were discontinued simultaneously. Reinforcement was not given after such trials in order to avoid contamination of unreinforced stimuli by a positive element.

Table I

Protocol of a typical experiment with the application of combined stimuli. Dog No 1, No of exper. 110, 18th May 1956

No of trial	Time in min.	Stimulus	Salivary reaction			Reinforcement
			First 10 sec.	Second. 10 sec.	Total	
1	1	Bell	14	20	34	+
2	5	Bell	12	21	33	+
3	9	Bell	14	26	40	+
4	13'50''	Metronome	0			
	14	Metronome +Bell	7	9	16	—
5	18	Bell	9	25	34	+
6	22	Bell	13	22	35	+

Altogether in both dogs 25 such combined trials were made. Table I shows a protocol of such crucial experiment and in Figs. 1 and 2 the entire course of this series is represented for each dog separately.

As is seen from the Figs. the results of these experiments are unequivocal. In all our combined trials, the effect of the positive conditioned stimuli was, on average, diminished to about 50 per

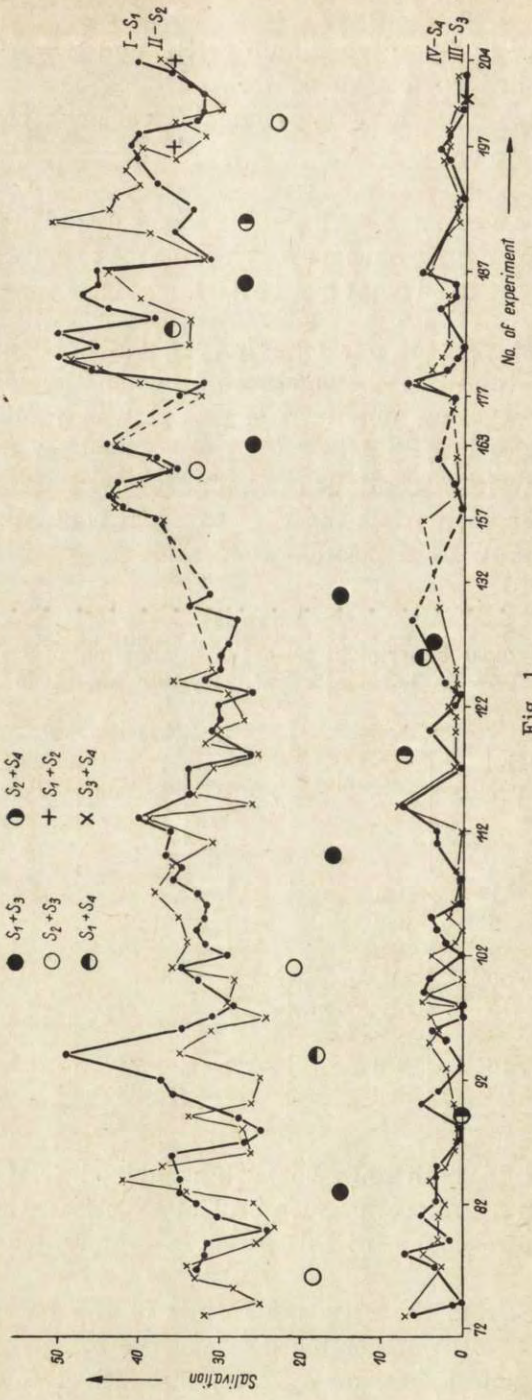

Fig. 1.

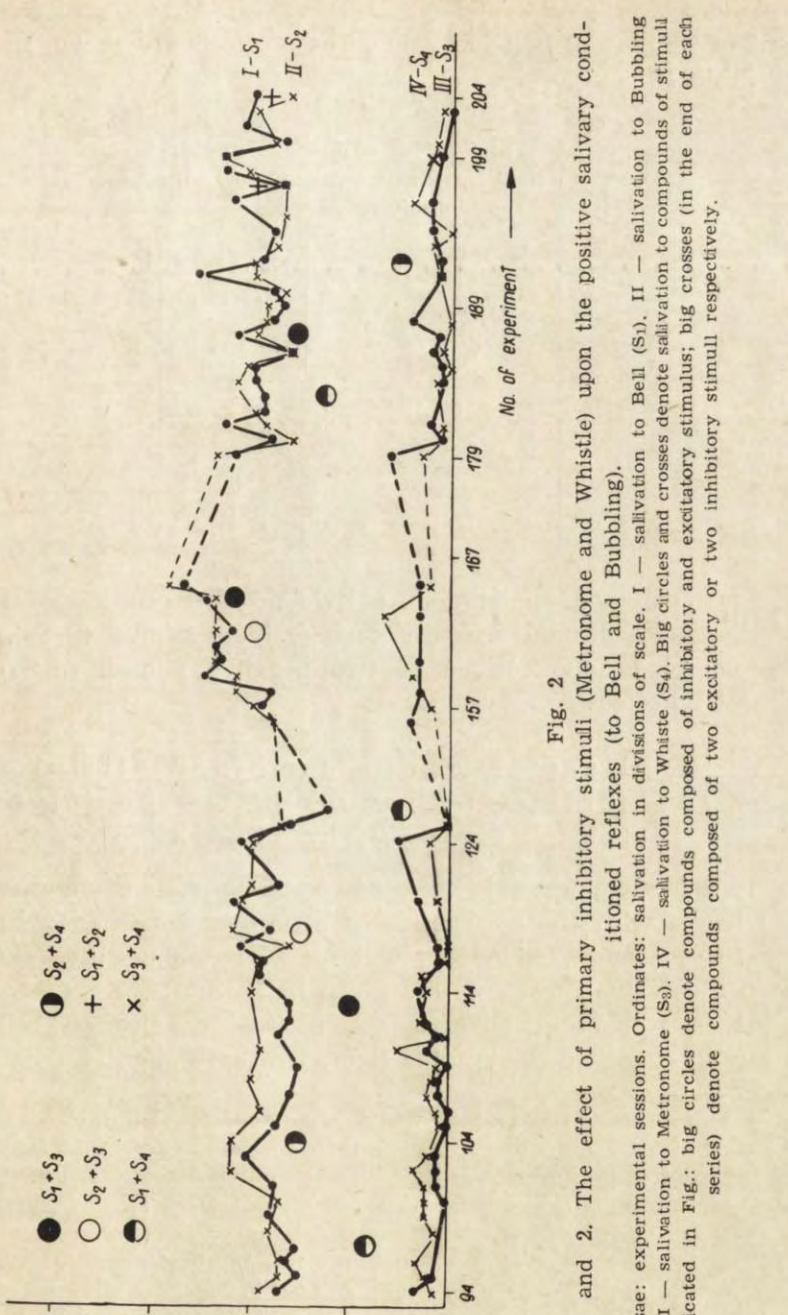

Fig. 2

Fig. 1 and 2. The effect of primary inhibitory stimuli (Metronome and Whistle) upon the positive salivary conditioned reflexes (to Bell and Bubbling).

Abscissae: experimental sessions. Ordinates: salivation in divisions of scale. I — salivation to Bell (S_1). II — salivation to Bubbling (S_2). III — salivation to Metronome (S_3). IV — salivation to Whiste (S_4). Big circles and crosses denote salivation to compounds of stimuli as indicated in Fig.: big circles denote compounds composed of inhibitory and excitatory stimulus; big crosses (in the end of each series) denote compounds composed of two excitatory or two inhibitory stimuli respectively.

cent of its normal positive value. These effects are shown in detail in Table II.

Table II

The diminution of excitatory conditioned reflexes when combined with inhibitory reflexes

Conditioned reflex reduced to in %	Number of cases	
	Dog 1	Dog 2
0—19	3	0
20—39	2	2
40—59	7	1
60—79	2	5
80—99	1	2

The combined applications of two positive conditioned stimuli produced the normal effect of each of these stimuli (S_1S_2), while the combined applications of two negative stimuli (S_3S_4) gave almost no effect*.

Application of the combined stimuli in overlapping sequence, the positive stimulus being the leading one

In a few experiments we used combined trials of another kind. We began with the application of a positive conditioned stimulus for 10 sec., and then added to it for 20 sec. a negative stimulus. Two illustrative protocols are given in Table III.

It is seen, that whereas the positive conditioned reflex to a strong conditioned stimulus (bell) was only slightly reduced by

* The question arose as to whether the repeated non-reinforcement of the combined trials might not lead to the elaboration of the inhibitory reflex to any compound stimulus applied in our experiments. A priori, this was highly improbable, since such compound stimuli were applied very rarely (once in about 50 normal trials), and a determined compound stimulus was applied with each dog only a few times. But the best proof that this elaboration did not take place was that, as seen in Figs. 1 and 2, the reactions to the compound stimuli did not diminish at all with the repetition of these stimuli, and the application of the compound consisting of two positive stimuli produced a full effect.

adjoining the negative stimulus, the conditioned reflex to a weaker stimulus (bubbling) was reduced quite considerably.

Table III

Diminution of the effect of the conditioned stimulus caused by adjoining of the inhibitory stimulus

No of trial	Time in min.	Stimulus	Salivary action				
			First 10 sec.	Second 10 sec.	Third 10 sec.	Total	Reinforcement
Dog No 1, No of exper. 208, 14th February 1957							
1	1	Bell	17	22		39	+
2	5	Bell	14	21		35	+
3	9	Bell	16	19		35	+
4	14	Bell	16				
	14'10"	Bell+Whistle		16	20		—
5	18	Bell	13	17		30	+
6	22	Bell	11	17		28	+
Dog No 1, No of exper. 233, 17th April 1957							
1	1	Bubbling	19	21		40	+
2	9	Bubbling	14	19		33	+
3	13	Bubbling	17	20		37	+
4	17	Bubbling	13		after		
	17'10"	Bubbling+Whistle		7	6		—
5	22	Bubbling	14	15		29	+

Application of the negative stimulus immediately after the positive

In several experiments, the application of a conditioned positive stimulus was discontinued after 20 sec. (normal isolated period), and then, immediately, a negative stimulus was applied for 10 sec. Two types of control were used for these experiments. In one of them, the positive conditioned stimulus lasted 30 seconds, instead of the usual 20 sec.; in another, the positive stimulus was discontinued after 20 sec., and for further 10 sec. nothing occurred.

The results of these experiments are shown in Fig. 3. It is seen that when the action of a positive conditioned stimulus is prolon-

ged to 30 sec., the salivation during the last 10 seconds is, by comparison with the second 10 sec., usually increased. When the conditioned stimulus is interrupted and nothing happens, the sali-

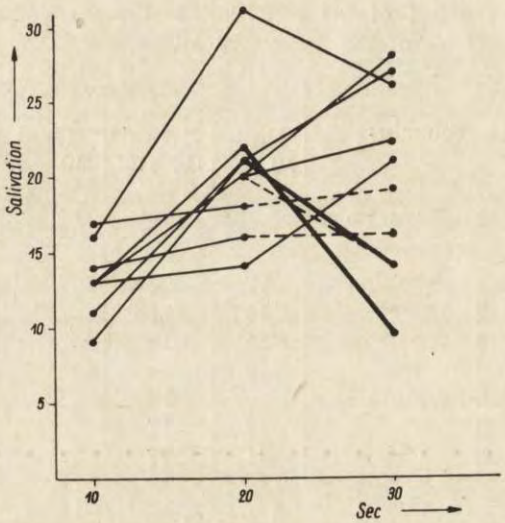

Fig. 3. Influence of inhibitory stimulus on the salivary after-effect of positive conditioned stimulus

Abscissae: time in seconds; ordinates: salivation. The points in the first two rows represent salivation to the positive conditioned stimull in the first and second 10 sec. respectively. The third 10 sec., when either positive conditioned stimulus continues to act (continuous lines ——), or no stimulus acts (broken lines - - -), or else inhibitory stimulus is applied (heavy lines —)

vation is the same as in the second 10 sec. On the other hand, if the positive conditioned stimulus is replaced by the negative one, the salivation drops dramatically.

The inhibitory after-effect of the negative stimuli

In these experiments, a positive conditioned stimulus was applied immediately after the 20 sec. duration of a negative stimulus. The results of these experiments are given in Table IV. It is seen that in almost all cases the conditioned stimulus applied just after the negative stimulus gives a slightly diminished effect, average up to 75 per cent of the normal value.

DISCUSSION

It is seen from these experiments that in every combination of positive and negative stimuli, used in our crucial trials, there is a more or less marked diminution of the positive conditioned reflex. These results confirm our initial hypothesis. This means that the joint application of excitatory and inhibitory conditioned stimuli leads to the same sort of algebraic summation, as in spinal reflexes (Sherrington 1947, Creed and al. 1932), i.e. that the inhibitory conditioned stimulus in fact sends to the unconditioned centre inhibitory impulses, which oppose the excitatory impulses sent to it by the excitatory conditioned stimulus.

Table IV

The inhibitory after-effect of negative stimuli

No of dog	No of exper.	Salivation in divisions of scale			
		Excitatory conditioned stimulus	Inhibitory stimulus	Excitatory conditioned stimulus following inhibitory stimulus	The effect diminished to in %
1	218	28	0	15	54
	221	40	2	35	88
	323	31	4	31	100
	327	38	0	34	90
2	216	18	3	8	44
	218	20	2	16	80
	222	23	2	15	65

It is worth mentioning that combinations of stimuli, similar to those used in our experiments, had also been applied in many earlier papers of the Pavlovian school. In most of them various differentiated stimuli were used in the role of inhibitory stimuli. It has been shown in these papers that the combined applications of negative and positive stimuli result either in insignificant diminution of the positive conditioned reflex (Ivanov-Smolensky 1924, Kałmykov 1926, Podkopayev 1924), or else in its augmentation (Fursikov 1923, Kałmykov 1926, Kogan 1914, Kreps 1924, Podkopayev 1924) (positive induction). In general, the more fresh or fine was the given differen-

tiation, the stronger was the tendency to the enhancement of the positive conditioned reflex. If, on the other hand, differentiation was more crude or well established, the combined effect of the two stimuli was to diminish the conditioned reflex.

As shown by Konorski (1948) these results can be easily explained by reference to the different composition of particular inhibitory reflexes. All differentiated inhibitory reflexes are, in fact, "mixed" excitatory-inhibitory reflexes. The more recent or fine is the given differentiation, the stronger is its excitatory element, whereas the more crude and fixed it is, the stronger is the inhibitory element. In consequence, combining such stimuli with positive conditioned stimuli leads either to the supremacy of excitatory effects, or to a slight predominance of the inhibitory effect. On the other hand, in our experiments, the inhibitory stimuli, not being similar to any of the positive stimuli, were more or less „purely" inhibitory and, therefore, their combination with the positive stimuli always led to the diminution of the positive conditioned reflexes.

It is worth noticing that the diminution of the positive conditioned effect caused by the inhibitory stimuli was not identical in all our experimental settings. When the excitatory stimulus was applied immediately after the inhibitory stimulus, its effect was far less reduced than when it was applied during the action of the inhibitory stimulus (compare Tables II and IV). That was so because the inhibitory after-effect of the stimulus is weaker than its actual effect. On the contrary, if the inhibitory stimulus is applied immediately after the excitatory stimulus, the effect is much greater than the normal effect when it is applied alone (see Fig. 3). Again, this is because the excitatory after-effect of the positive conditioned stimulus (well seen when this stimulus is simply discontinued — Fig. 3) weakens the inhibitory effect of the negative stimulus.

One more point should be commented upon. As shown in experiments by Hernandez-Peon et al. (1955) and Galambos et al. (1956), the repetitive application of an acoustic stimulus without any reinforcement leads to the complete abolition of action potentials produced by such stimulus both in the cochlear nucleus and in the acoustic area of the cortex. This means that the stimulus in such a case is not heard at all by the animal. If such

a mechanism of inhibition were working in the case of the application of our inhibitory stimuli, this would not lead, of course, to any diminution of the positive conditioned reflex, for it would be unthinkable that a stimulus which does not reach the animal's central nervous system could produce any effect on the other stimulus operating at the same time. We think that the difference between the properties of our inhibitory stimuli and those used in Hernandez-Peon and Galambos's experiments is that whereas in the experiments of those authors the acoustic stimulus (click) was applied thousands of times, in very rapid succession, in our experiments it was applied much less frequently. In experiments by Sharpless and Jasper (1956) in which the unreinforced stimulus was applied in a somewhat similar manner to that in our experiments, the Hernandez-Peon and Galambos effect was also absent.

There remains a final problem to be considered in this paper — that of the organization of the conditioned inhibitory reflex-arc. According to our previous hypothesis (Konorski 1948) the mechanism of the inhibitory conditioned reflexes was conceived in the following way: we supposed that when the conditioned stimulus is not reinforced by the unconditioned stimulus, then the inhibitory conditioned connections are formed between the conditioned centre and the unconditioned centre. According to this hypothesis the chronically extinguished or differentiated conditioned stimuli were of mixed character, i.e. their centres were connected with the unconditioned centre both by excitatory and inhibitory connections. The primary inhibitory stimulus would on the contrary be joined with the unconditioned centre by inhibitory connections only.

We think now that, according to the new evidence concerning the organization of certain unconditioned centres, the mechanism of the inhibitory conditioned reflexes can be depicted in another way (cf. Konorski 1958). The experiments of Anand and Brobeck 1951, Hetherington and Ranson 1941, and others have shown that the hypothalamic alimentary centre is composed of two subcentres, one positive (P) and one negative (N). After destruction of the centre P the animal does not take food, nor manifest any hunger drive, while the destruction of centre N leads to the impairment of inhibitory alimentary me-

chanism and to hyperphagia. Therefore, we may suppose that the positive centre is responsible for the excitatory alimentary reflexes and is activated in the course of the unconditioned, as well as conditioned, alimentary reflexes, while the negative centre is responsible for the inhibitory alimentary reflexes and is activated when the animal stops eating, or when the conditioned alimentary stimulus is not reinforced. Accordingly, we may suppose that in positive conditioned reflexes the connections are formed between the conditioned centre and the hypothalamic positive alimentary centre (or rather with the representation of the latter in the cerebral cortex), whereas the inhibitory conditioned reflexes are formed by establishment of connections between the stimulus and the negative alimentary centre.

In this way the conditioned „cessation reflex" found by A. Zbrożyna (1957) can be explained. The abrupt withdrawal of food evokes the excitation of the negative alimentary centre, and the stimulus signalizing this event becomes an inhibitory conditioned stimulus. Similarly, when a well-established conditioned stimulus is not reinforced, the excitation of the positive alimentary centre is interrupted and the negative centre is excited; in consequence, conditioned connections are formed between the centre of the stimulus and the negative centre.

Now, the problem arises why is it that the stimulus simply not reinforced by food acquires strong inhibitory properties, as shown in our previous and present experiments. We think that this fact can be explained in the following way. In a routine conditioned reflex experiment, the dog is taught in hundreds of trials that he should obtain food only to conditioned stimuli and never in the intervals. Therefore, at the end of each food consumption, and during the greater part of the interval, the negative alimentary centre is active, and in consequence any stimulus which acts then becomes connected with this centre. And so, the physiological structure of a primary inhibitory reflex is simply that conditioned connections are formed between the centre of the stimulus and the negative alimentary centre. This explains why the joint application of the primary inhibitory stimulus and the excitatory conditioned stimulus diminishes the reaction of the latter, and why it is so difficult to transform a primary inhibitory stimulus into a positive conditioned stimulus.

The present interpretation makes also intelligible the fact that similar relations exist between the excitatory and inhibitory conditioned reflexes and between excitatory alimentary and defensive conditioned reflexes (Konorski and Szwejkowska 1956). This is because between excitatory and inhibitory subcentres of the alimentary centre there exists the same sort of antagonism as between positive alimentary subcentre and the defensive centre. Since it seems that in the latter case the antagonism is stronger, the conflict between conditioned alimentary and defensive reflexes is even more pronounced.

SUMMARY

1. The combined application of an excitatory conditioned stimulus and a primary inhibitory stimulus leads to the diminution of the effect of the former stimulus.

2. This diminishing effect is stronger during the action of the inhibitory stimulus than after its cessation.

3. The mechanism of the inhibitory conditioned reflexes is discussed.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

PHYSIOLOGICAL MECHANISM OF DELAYED REACTIONS
I. THE ANALYSIS AND CLASSIFICATION OF DELAYED REACTIONS

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The chief, and, perhaps, the only subject of study in the physiology of higher nervous activity has so far been the conditioned reflex (in the broadest sense of the word), i.e. the more or less fixed form of the animal's behaviour conditioned by its previous experience. Both the formation and the stability of these reflexes is guaranteed by the fact, that higher nervous centres of the animal, and especially the cerebral cortex, are endowed with some peculiar functional property called in psychology „permanent memory", for which in physiology the term „plasticity" has been proposed (Konorski 1948).

However, if we take into account the behaviour of animals and man under normal conditions, we can easily observe that it is not only determined by the unconditioned and conditioned reflexes established in the course of phylogenesis and individual life respectively, but it is also based on some transient traces of recent events called "recent memory". These traces persist generally for minutes or hours and then are, in contrast to the lasting character of conditioned reflexes, completely obliterated. Perhaps the best manifestation of such traces in experimental conditions lies in the so called delayed responses.

The method of delayed responses introduced first into psychological research by Hunter (1913) and applied with various modifications by a number of other authors (Walton 1915, Yarbrough 1917, Yerkes a. Yerkes 1928, Tinklepaugh 1932, McAllister 1932, Nissen, Riesen a. Nowlis 1938,

Wojtonis 1951 and many others), is based on the following principles:

The animal is taught to receive food in two or more different places (or to run to food by several paths, as was the case in Hunter's original experiments) in such a way, that feeding in each place is preceded by different signals, with regard either to location or quality. The animal is allowed to perform the movement which secures the food only some time (of the order of seconds or minutes) after the cessation of the signal. The correct choice of the route shows that the animal has temporarily kept in mind whence the signal has come or which signal has been given.

The method of delayed reactions gained greatly in importance in the thirties of this century when Jacobsen (1936) showed that after prefrontal ablations in monkeys and apes the ability to form and retain conditioned reflexes (habits) is fully preserved, while the delayed reactions are greatly impaired. In other words it has been proved that the prefrontal areas are indispensable for the preservation of recent memory traces of direction. This fact makes it even more desirable to shift the whole problem of delayed reactions onto a firm physiological basis.

In the present series of papers we intend to analyze both the physiological mechanisms underlying the delayed responses in the normal animal and the impairment they undergo after prefrontal ablations.

The first paper of this series is concerned with an analysis of the functional structure of delayed responses and their classification according to various settings of the experimental conditions.

It is easy to see that the method of delayed responses generally applied in both psychology and physiology is by no means simple. It contains as an essential element the reaction of choice. The animal has to choose in which of several feeding places the food is to be obtained in response to a given stimulus, quite independently of whether this reaction will be delayed or not. However, one can devise experiments in which there is a delayed reaction without any element of choice, when the animal gets food only in one place some time after the cessation of the stimulus. The combination of these two methods represents the method normally used in the majority of experimental studies.

And so, we ought to consider and analyse separately the following three methods:

1. The method of choice without delay.
2. The method of simple delay without choice.
3. The method of delay with choice.

THE METHOD OF CHOICE

By the method of choice we denote an experimental setting in which: a) the animal is fed in at least two places, b) the place in which the animal is to be fed in a given trial depends on the stimulus applied in this trial, and c) if the animal turns to an „incorrect" feeding place he does not get food.

The various types of experiment based on the method of choice may differ from one another in the following respects:

A. The relative localisation of various feeding places (or paths leading to food). They may be situated either close to one another, or at more or less widely divergent angles (taking the starting point as apex).

B. The nature of the instrumental reactions securing food. These are either manipulatory reactions (in experiments with monkeys) consisting in removing the cup under which the food has been hidden, or locomotor reactions consisting in the animal running from the starting point toward the feeding place.

C. Stimuli determining in which of the several feeding places the food is to be found. These stimuli may be classified in two categories:

We shall call d i r e c t i o n a l stimuli those stimuli which determine the direction of the animal's response according to its experience acquired in normal life. Here will belong such stimuli as the placing of food in the foodtray in front of the animal's eyes, application of auditory or visual stimuli acting from the place where the animal gets food, etc. These stimuli elicit a natural orienting reaction toward the source of the stimulus (and concequently, toward a given foodtray), which reaction is allied with, and soon becomes preliminary to, the reactions of running in the same direction. Consequently, the animal does not have to learn which foodtray to run to in response to any given stimulus.

In the second category of stimuli, which we shall call s i g n stimuli, we classify those stimuli, which are not spatially connected

with the respective foodplaces, and which, therefore, evoke an orienting response not allied with the respective reaction toward food.

Since in our further considerations we shall base ourselves chiefly on the experimental setting used in our own experiments, it seems appropriate, in order to be quite specific, to give here a brief outline of this setting.

Our experiments were conducted on dogs and cats in a rectangular room (Fig 1). Three foodtrays F_1, F_2, F_3 were situated at the same distance from the starting place (which is also where the experimenter sat) at angles of almost 90° from one another. The sound of three buzzers B_1, B_2, and B_3, or light from the three lamps L_1, L_2, L_3, located on the foodtrays, supplied directional stimuli signalling food in the respective foodtrays. For experiments with sign stimuli we used the bell and the metronome placed at the start and signalling food in the foodtrays F_1 and F_3 respec-

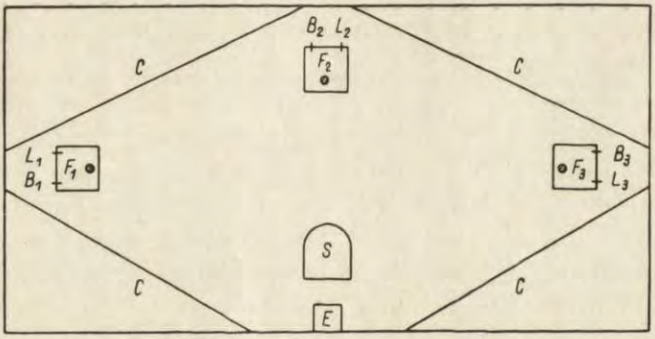

Fig. 1. Experimental setting of the experiments
F_1, F_2, F_3 — left, middle and right foodtray. The bowls are automatically moved into position by the experimenter using an electromagnetic device; E — table and seat for experimenter; S — starting platform; B_1, B_2, B_3 — buzzers; L_1, L_2, L_3 — lamps situated on the respective foodtrays

tively. When the animal at the given signal ran to the correct foodtray the bowl containing food was automatically presented.

The course of elaboration of choice reactions to directional and sign stimuli is quite different. The application of the directional stimuli evokes an orienting reaction towards the given foodtray which very soon, after only a few reinforcements, is succeeded by the proper instrumental reaction, viz. the run towards the respective foodtray. Therefore, under these conditions each stimulus

elicits a correct reaction to the respective foodtray even if the task of multiple choice is presented.

By contrast, if sign stimuli are applied the animal must learn by trial and error which stimulus signals food in which foodtray, in other words, he must learn to inhibit the incorrect run to a given stimulus in order that the correct run may be established.

To pass to the explanation of the physiological mechanism of the choice reactions it must be first stated that they represent a form of instrumental (type II) conditioned reflexes, i.e. of those reflexes in which the performance of a certain motor act in the presence of a certain stimulus leads to a positive unconditioned stimulus (or to the avoidance of a negative one). According to a scheme presented by Wyrwicka (1952), the structure of these reflexes is based on the establishment of both "direct" and "indirect" connec-

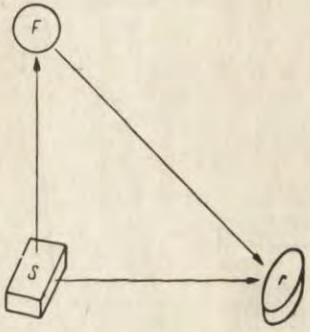

Fig. 2. The diagram of the conditioned-reflex arc in type II (instrumental) conditioning

S — central representation of the conditioned stimulus; F — central representation of the unconditioned stimulus (alimentary centre); r — centres involved in the instrumental motor reaction (Wyrwicka 1952)

tions between the "centre" of a conditioned stimulus and the "centre" of the motor reaction, the indirect connections passing via the unconditioned centre (Fig. 2)* (see appendix 1).

Let us take into consideration, first, the elaboration of a choice reaction to a directional stimulus. Each such stimulus evokes an orienting reaction towards its source, which is readily transformed

* In operating with the terms "the centre of the stimulus", "the centre of movement", "connections between centres" we by no means have in mind any anatomically defined structures; we apply them in a strictly physiological sense.

into a strong instrumental alimentary reflex consisting in the run to the respective foodtray. According to what has been just said, conditioned connections are set up on the one hand between the centres of the conditioned stimuli and the centres of the respective motor reactions, and on the other hand between the centres of all the stimuli and the alimentary centre, as well as between the alimentary centre and the centres of all the motor reactions concerned (Fig. 3a). The existence of these latter connections explains the fact that if the dog after running to the foodtray signalled by the respective stimulus will not get food, he may go to the other foodtrays where he would otherwise never go after this stimulus. Thus, inhibition of the motor reaction to the first foodtray may reveal the existence of the connections between the given stimulus and other motor reactions, the connections presumably running via the alimentary centre. But it must be noticed that in the case

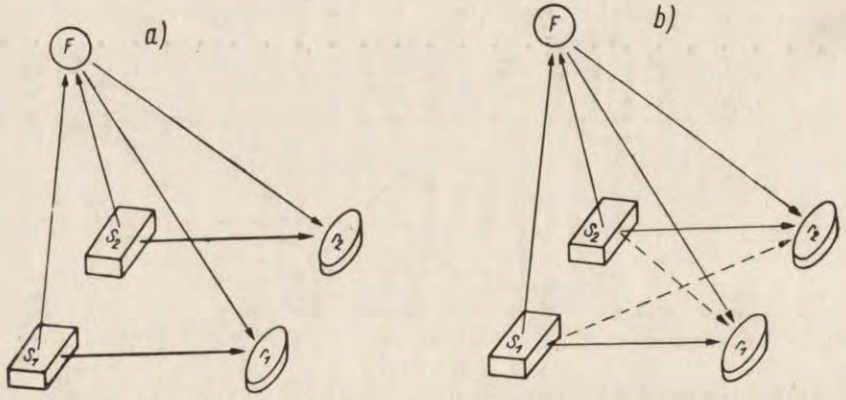

Fig. 3. The diagram representing the conditioned-reflex arc in the reaction of choice to directional (a) and sign (b) stimuli

For simplicity only the choice of two reactions is represented. S_1, S_2 — central representation of conditioned stimuli; r_1, r_2 — central representation of two respective reactions; F — alimentary centre. Solid lines, excitatory connections, broken lines, inhibitory connections

The differences between fig. 3a and 3b are: i — the connections $S_1 \rightarrow r_1$, and $S_2 \rightarrow r_2$ are stronger in a than in b; ii — in b there are inhibitory connections between S_1 and r_2, and between S_2 and r_1 which do not exist in a. Further explanations in text

of choice reactions determined by directional stimuli the indirect cross-connections running through the alimentary centre are far less significant than the half-inborn direct connections between the centres of stimuli and the respective motor reactions (see appendix 1).

Quite different is the situation in the case of choice reactions to the sign stimuli (Fig. 3b). Here „natural" direct connections between the stimulus and its respective reaction do not exist, therefore each of the sign stimuli forms connections first and foremost with the alimentary centre and thence with the centres of e a c h instrumental reaction. Consequently, in the first stage of training the animal will run to either foodtray in response to either stimulus. The elaboration of the correct motor reaction to each stimulus must be accomplished by differentiation: to the given stimulus the dog must learn not to go to the incorrect foodtray in order to be able to go to the correct one. In other words, the incorrect run must be inhibited for the correct one to be established.

For these reasons the establishment of the reactions of choice to the sign stimuli encounters some difficulties even with only two foodtrays. Probably with more foodtrays these difficulties would increase very rapidly. The existence of strong connections between e a c h stimulus and e a c h reaction (via the alimentary centre) explains, why even after a prolonged training the animal is prone to make errors and to go to the wrong foodtray. This happens either when the inhibitory processes are for some reasons temporarily impaired, or when one of the reactions is temporarily strengthened in preceding trials.

One may depict the situation arising in both the aforesaid experimental settings in the following way: the directional stimuli "attract" the animal toward a definite foodtray, hence even in the case of a m u l t i p l e choice situation the task of running correctly will not produce any difficulties, provided that the source of the stimulus is quite distinct; on the other hand the sign stimuli "push" the animal toward the foodtrays in general, hence the correct run is determined by inhibition of all those runs which have proved in the past to be ineffective with this stimulus.

SIMPLE DELAYED REACTION

As has already been mentioned, in the simple delayed reaction the animal does not have to choose between various motor reactions, since only one reaction leads to food in this particular experimental situation. This reaction however is effective only when it is performed to a definite stimulus preceded by another stimulus.

The stimulus upon which the animal must perform a given movement in order to obtain food we shall call the releasing stimulus (S_{rl}). The stimulus preceding the releasing stimulus and which is a necessary condition for the latter to be reinforced (after the instrumental reactions is performed) will be called the preparatory stimulus (S_p).

Accordingly the experimental setting of simple delay is as follows. We establish an instrumental conditioned reflex (with the motor effect, r) to the compound of stimuli S_pS_{rl} applied consecutively. In some trials we apply stimulus S_{rl} alone and we do not reinforce the reaction r which, owing to generalization, appears to this stimulus. So the stimulus S_{rl}, when acting alone, is differentiated from the compound S_pS_{rl} and transformed into an inhibitory stimulus. This achieved, we prolong the interval between S_p and S_{rl}, continuing repeatedly to test the inhibitory character of stimulus S_{rl} on its own. The interval between S_p and S_{rl} will be called the delay period.

To put it briefly we have:

$$[S_p]\, S_{rl} \rightarrow r - F$$
$$S_{rl} \rightarrow \sim r$$

where $[S_p]$ represents the trace of the stimulus S_p, F represents food, $\rightarrow$ means "elicits", — means "leading to", $\sim$ r means "not r".

Just as with choice reactions, so with simple delayed reactions we can produce different varieties of experimental settings according to the character of stimuli S_p and S_{rl}, and of reaction r.

A. The preparatory stimulus may be (just as the conditioned stimulus in choise reactions) either a directional (the sight of baiting, or a signal acting from the feeding place) or a sign stimulus (any signal acting at some site unrelated to the feeding place).

B. The releasing stimulus may be also either a "natural", or a sign stimulus. If during the whole delay interval the animal is either fastened or enclosed at the starting point and the stimulus is simply a physical release enabling him to perform the instrumental reaction, then we have to do with a natural releasing stimulus. On the other hand, if the animal has a physical possibility to perform the instrumental movement during the delay period, but it is reinforced by food only at a given signal, then this signal represents a sign releasing stimulus.

C. Finally, the conditioned instrumental reaction may be either any manipulatory act (opening of a lid, lever pressing, etc.) or locomotor act (running to the foodtray). Since in the case of simple delay we have to do only with one conditioned reaction and not with several, it would be, as a matter of fact, quite possible to conduct the experiments by the method of classical conditioned reflexes, without any instrumental reaction. In this case the stimulus S_{rl} when preceded by the stimulus S_p would be reinforced by food, whereas S_{rl} alone would not be reinforced. Salivation to the stimulus S_{rl} preceded by S_p would be the measure of the delay capacity of the animal.

The interesting question arises, what is the relation between: a) simple delayed reaction, b) conditioned inhibition when the conditioned inhibitor precedes the conditioned stimulus, and c) trace conditioned reflex. The comparison between these three forms of reactions is discussed in appendix 2.

We pass now to the explanation of the physiological mechanism of simple delayed reactions. This is the more important because in this situation the process of delay is present in a simplest form and is not complicated by the process of choice, as in the more complex situation of delay with choice. We shall begin with the examination of the case when the releasing stimulus is a sign stimulus, i.e. the animal is not constrained and hence is physically able to perform a given instrumental reaction, but this reaction is reinforced only when S_{rl} is preceded by S_p.

To begin with, suppose that the releasing stimulus is applied not on the traces of the preparatory stimulus but during its action (Fig. 4a). To stimulus S_p (before S_{rl} has been added), and to stimulus S_{rl} applied separately, the animal, even if it performs the reaction r, does not obtain food, getting it only when S_p and S_{rl} act jointly. So gradually, differentiation develops between the compound S_pS_{rl} and its components acting separately, these latter becoming inhibitory. As said previously when dealing with the structure of instrumental reflexes, the centre of the compound stimulus S_pS_{rl}* becomes connected with the motor centre both directly and indirectly through the alimentary centre. The connections of the centres of the single stimuli S_p and S_{rl}, however are more complicated.

* The problem of the central representation of compound stimuli is briefly discussed in appendix 3.

In the first stage of training these stimuli, owing to generalization with the positive compound stimulus, also become positive, i.e. they develop connections both with the alimentary centre F and with the motor centre, r. Subsequently, as these stimuli are not reinforced, additional connections responsible for their transformation into the inhibitory stimuli are formed*.

We have to do exactly with the same processes when the releasing stimulus acts, not during, but some time after the preparatory stimulus (Fig. 4b). There is a great body of evidence, both phy-

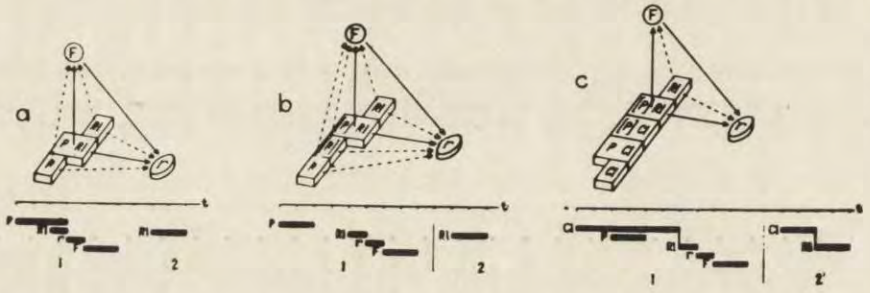

Fig. 4. The diagram representing the conditioned reflex, type II, to the compound stimulus (a), the simple delayed reaction to the sign releasing stimulus (b), and to the „natural" releasing stimulus (c)

P — central representation of the preparatory stimulus; [P] — of its trace; Rl — of the releasing stimulus alone: P Rl and [P] Rl — of the respective compounds; Cl — of the stimulus „of being restrained"; P Cl and [P] Cl — of respective compounds; F — alimentary centre; r — central representation of instrumentall reaction. Solid lines —— represent excitatory connections, broken lines - - - - represent inhibitory connections. Below each figure the actual sequence of events (each sort of event drawn on separate line) is represented for an excitatory trial (1) and inhibitory trial (2); t — time

siological and psychological, to show that any stimulus, after its cessation, leaves in the nervous system a trace which may be revealed by many methods. In other words the trace of a stimulus, just like its actual operation, gives rise to central processes, to

* These connections can be either thought of as inhibitory connections between the respective centres as was proposed by Konorski (1948), or else in a somewhat modified form, as excitatory connections formed between the conditioned centre and the negative counterpart of the unconditioned centre (Konorski 1958). Although the new hypothesis seems to us more justified than the previous one, for the sake of simplicity we shall draw in our schemes inhibitory reflexes as based on the inhibitory connections established between the respective centres.

which, as experiments of Pavlov's school have shown (Pavlov 1940), excitatory as well as inhibitory conditioned reflexes may be formed. In delayed reactions the trace left after the cessation of the stimulus S_p ($[S_p]$) plays the same role as does the preparatory stimulus itself in the reflex just described: both stimulus S_p and its traces $[S_p]$ have an inhibitory character, while the compound composed of this trace „stimulus" and the releasing stimulus, $[S_p]$ S_{rl}, is an excitatory conditioned stimulus.

A slightly different situation is encountered when a natural agent is used in the role of the releasing stimulus, i.e. the animal, being attached or enclosed, is not able to perform the response as long as it is not released. Here the stimulus S_p and its trace act jointly with the stimulus of „being attached" (the pressure of the collar) or enclosed (the sight of the enclosure) S_{cl}. These stimuli, according to the animal's previous experience elicit a strong inhibitory reflex in respect to any attempt to move away, and, among other things, to go to the foodtray. As a result, the normal animal, if not in a state of some unusual excitement, does not try to break loose from the confinement, but behaves more or less calmly, waiting for the release.

In the preliminary training the stimulus S_p was applied when the animal was free and could immediately run to the foodtray and obtain food. If this stimulus S_p is first applied when the animal is attached, he tends to get loose, but as this is unsuccessful the attempts to do so are usually abandoned. Thus the compound $S_{cl}S_p$ and also S_{cl} $[S_p]$ may become inhibitory in respect to the reaction r*, whereas the stimulus S_p acting alone (i.e. when the animal is not attached) preserves its excitatory character (cf. experiments by Chorążyna reported in appendix 3). As to the stimulus S_{rl} acting alone it is bound to become inhibitory as in previous cases, i.e. the animal has to restrain from going to the foodtray when released not after S_p, otherwise our experiments with simple delay would be meaningless.

The reflex structure of the simple delayed response with natural releasing stimuli is presented in Fig. 4c.

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* It should be noticed that in this case it is not relevant for the course of experiments whether or not $S_{cl}S_p$ becomes inhibitory, as the animal is physically prevented from performing the complete reaction r.

How may we imagine the physiological mechanism of the trace "stimulus"? According to our present knowledge of the functioning of the nervous system, we may suppose (as indeed do most authors), that any stimulus impinging on a particular cortical centre throws into activity closed, reverberating chains of neurons, whose activity outlives the operation of the actual stimulus, causing a continuous and more or less long-lasting activation of the centre.

Is there any difference between the pool of neurons excited by the stimulus itself and that excited by the "trace" stimulus, and, if so, what is its nature? It seems clear that the actual stimulus and the trace stimulus are not identical for the animal: the ease of differentiation between the two appears to supply objective proof on this question. It may be supposed that with the cessation of

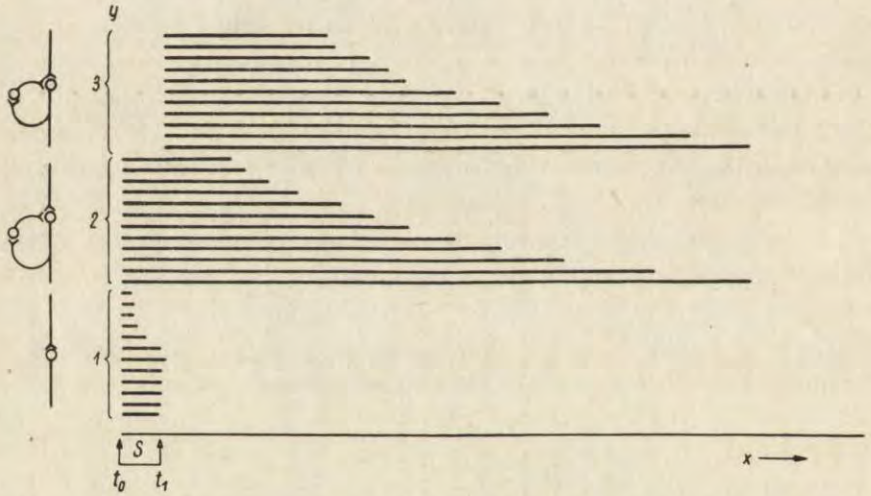

Fig. 5. Diagrammatic representation of the physiological structure of a trace of stimulus

The x axis represents time; t_0 is the beginning of the operation of the particular stimulus, t_1 its termination. Along the y axis are represented three groups of neurons. 1 — a group of neurons activated during, and only during, the operation of the stimulus; 2 — a group of neurons activated both during the operation of the stimulus and owing to the reverberating circuits of neurons, for some time after its termination; 3 — a group of neurons activated after the cessation of the stimulus (off-neurons). All off-neurons are represented as acting for some time after the cessation of the stimulus by way of reverberating circuits. On the left the respective type of neurons of each groups indicated. The horizontal lines represent the periods of excitation of each particular neuron. The whole group 1 is activated only during the operation of the stimulus, some quickly adapting on-elements being also shown. Group 2 is activated during the operation of the stimulus and after its cessation, gradually becoming less active in the course of time. The whole group 3 is activated by the cessation of stimulus asd then becomes gradually less active as in the case of group 2. Further explanation in text

the stimulus some of the neurons activated during its operation quickly become inactive, while others continue to be excited by closed chains of neurons superimposed on them; there is also the possibility that the cessation of the stimulus has a positive off--effect, throwing into activity a new group of neurons. Thus, while some of the neurons excited during the operation of the stimulus may be active also after its cessation, (and may therefore be responsible for the generalization between the actual stimulus and its trace), other groups of neurons are either withdrawn from, or on the contrary, may be put into activity when the stimulus ceases to act. As time passes, the assembly of neurons activated by the trace of the stimulus gradually diminishes and finally drops to zero, as more and more neurons fall out of action. At this time the trace of the stimulus has faded out and, consequently, the delayed reaction is no longer possible. These hypotheses are shown diagrammatically in Fig. 5.

To end these considerations it may be supposed that the higher the phylogentic development of the brain, the more numerous are the systems of superimposed closed chains of neurons in the cerebral cortex, and this could account for the more longlasting and perfect recent memory of higher animals. It may also be supposed that recent memory is not necessarily equal to all kinds of stimuli in any particular animal, but that it is highly selective, being more or less pronounced according to the analyser concerned. Further consideration of this problem goes beyond the scope of the present study.

DELAYED REACTION WITH CHOICE

After elucidation of the conditioned-reflex pattern of the choice reaction and the simple delayed reaction, there is no difficulty in understanding the nature of the delayed reaction with choice. This method is the one generally employed (in contrast to the method of simple delay) because it avoids some experimental difficulties encountered with the latter. As mentioned before, a necessary condition for the correct application of the simple delay method is the perfect differentiation of the compound stimulus consisting of the preparatory and releasing stimuli, from the releasing stimulus alone. To the extent that this differentiation is not attained (and its attainment is not always an easy matter) we can draw no con-

clusion about the real delay capacity of the animal. On the other hand, in the method of delay with choice, this differentiation need not be established at all; for in this case the animal must remember t o w h i c h foodtray he must run after a given preparatory stimulus rather than simply whether he should go or not go. Therefore the run in the correct direction is a sufficient criterion of the delay capacity of the animal, and the application of the single releasing stimulus without any reinforcement is thus superfluous*.

The varieties of experiments possible with the method of delay with choice are the same as in both previous methods. And so we shall have to deal here with varieties depending on the different localisation of the goals, on the character of preparatory stimuli (sign or directional), of releasing stimuli (sign or natural) and on the character of the conditioned reaction (locomotor or manipulatory).

The setting of the experiments with delayed reactions reported in this series was similar to that employed with the choice reactions. Buzzers and lamps on the foodtrays were used as the directional preparatory stimuli; and the metronome and bell placed at the starting point acted as sign preparatory stimuli, signalling food in two opposite foodtrays (F_1 and F_3) respectively. During the operation of such a preparatory stimulus, and in the delay period, the dogs were on leash, and the cats enclosed in a cage. As a releasing stimulus the natural stimulus, consisting simply in releasing the animal, was the one regularly employed.

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* It is important to realize that this difference between the two methods discussed has only practical and not theoretical significance. For, in both methods the given instrumental reaction r_m (say, going to a particular foodtray F_m) elicited by stimulus S_{rl} leads to food only when this stimulus is preceded by a d e f i n i t e stimulus S_{pm}. Therefore, the performance of this very reaction either to stimulus S_{rl} alone (in the simple delay method) or to stimulus S_{rl} preceded by another preparatory stimulus S_{pn} (in the delay method with choice) does not lead to food. In other words in the simple delay method the conditioned reflex is established according to the formula:

$$[S_p]\ S_{rl} \rightarrow r - F$$
$$S_{rl} \rightarrow r - \sim F$$

while in the delay method with choice the same effect is obtained according to the formula:

$$[S_{pm}]\ S_{rl} \rightarrow r_m - F$$
$$[S_{pn}]\ S_{rl} \rightarrow r_m - \sim F$$

Regarding the conditioned-reflex structure of the delayed response with choice, it is clearly a superposition of the reflex-arc of the simple delayed response, represented in Fig. 4b or 4c, upon either of the reflex-arcs of the choice reactions, represented in Fig. 3a and 3b. As a result we obtain the reflex-arcs represented in Fig. 6a and 6c for directional preparatory stimuli and in Fig. 6b and 6d for sign preparatory stimuli. We shall now study in more detail the schemes represented in these figures and compare them with the previous schemes.

As seen in Fig. 6, instead of a single preparatory stimulus S_p, as in Fig. 4, we now have a number of such stimuli; each of them not only p r e p a r e s the animal for the performance of the instrumental reaction, but also d e t e r m i n e s which of these reactions should be performed. On the other hand, when comparing the schemes of Fig. 3 and 6 we notice that the single conditioned

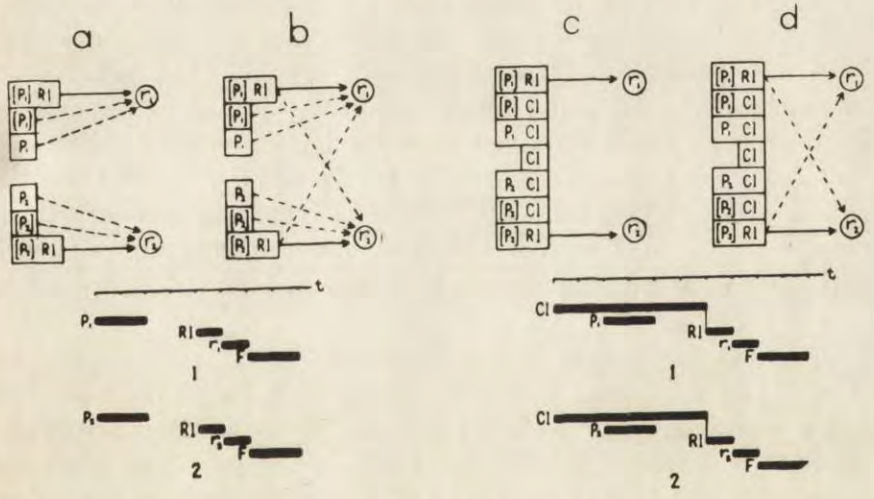

Fig. 6. Diagrammatic representation of delayed reactions with choice. For the sake of simplicity only connections of the conditioned stimuli with motor reactions are shown, while the connections leading to, and from, the alimentary center, are omitted. The same denotations as in fig. 4

a — preparatory stimuli are directional stimuli, releasing stimulus is a sign stimulus; b — preparatory stimuli are sign stimuli, releasing stimulus is a sign stimulus; c — preparatory stimuli are directional stimuli, releasing stimulus is a natural stimulus; d — preparatory stimuli are sign stimuli, releasing stimulus is a natural stimulus. Fig. 6a is the combination of fig. 3a and fig. 4b, fig. 6b, of fig. 3b and 4b, fig. 6c, of fig. 3a and 4c, fig. 6d, of fig. 3b and fig. 4c. Below fig. 6a and 6b, and fig. 6c and 6d the respective course of events is represented for trial with each preparatory stimulus (S_{p_1} and S_{p_2})

stimuli, S_1 and S_2, eliciting the respective reactions r_1 and r_2 are now split into compound stimuli $[S_{p1}]\ S_{rl}$ and $[S_{p2}]\ S_{rl}$: the preparatory stimuli elicit only the orienting reactions toward the particular foodtrays while the releasing stimulus evokes the instrumental reaction toward the foodtray designated by the preparatory stimulus.

On comparison of Fig. 6a and 6b, as well as Fig. 6c and 6d, it is seen that there is a considerable difference between the structure of the delayed reactions with choice according to whether directional or sign preparatory stimuli are used. In the first case each of the orienting reactions to stimuli S_{p1} and S_{p2} is directly determined by the stimulus itself, whereas in the second case each orienting reaction is established by way of differentiation, involving an inhibition of other possible orienting reactions (cf. the same discussion concerning the choice reactions).

It should be also remembered that alongside the direct connections between every compound stimulus $[S_{pm}]\ S_{rl}$ and the respective motor reaction r_m there exist connections linking these compounds with the alimentary centre, and thence with a l l the motor reactions concerned. Speaking freely, we may say that in response to stimulus S_{rl} following a given stimulus S_{pm} the animal either may remember t o w h i c h foodtray it has to run (i.e. it may use the reflex pathway $[S_{pm}]\ S_{rl} \rightarrow r_m$) or it may only remember that it must run for food to some foodtray not remembering to which one (i.e. to have at its disposal only the pathway $[S_{pm}]\ S_{rl} \rightarrow F \rightarrow \rightarrow r_x$).

To end our discussion of delayed reactions with choice, we should like to oppose the view expressed by some authors that these reactions may be considered as „one trial learning with secondary reinforcement" (N i s s e n et al. 1938, M a l m o 1942 and others), baiting in front of the animal playing a role of such a reinforcement. It seems to us that the above analysis helps us to reveal the fallacy of this conception. We can have to do with one trial conditioning when the single reinforcement (primary or secondary) of the motor reaction performed in a given situation causes this reaction to reappear in that situation. E.g. if in a given situation the animal, after having run to a certain place, has obtained food there, and after that runs again to that place, even after several days' interval, we have to do with just this sort of con-

ditioning. If the first run to the given place is „reinforced" by the mere sight of food which is unattainable, then the sight of food as a very strong „natural" conditioned stimulus could play the role of secondary reinforcement and thus provoke the animal to repeat this run after its next release*. But in contrast to reactions of this sort, the essential feature of delayed reactions is that the animal is not allowed to perform the given reaction to the preparatory stimulus at all, and nevertheless, when released, he runs to the proper feeding place. We think that the difference between the one trial learning and the delayed response may be clearly seen in our experimental situation. It happens sometimes that the animal in a given trial runs not to that foodtray which has been signalled by the preparatory stimulus, but to that foodtray in which he obtained food in the preceding trial. This has happened because in this case the trace left by one trial learning was stronger than the trace left by the preparatory stimulus. This sort of errors (which will be discussed in detail in the following paper of this series) shows that there is a competition between one trial learning reaction and delayed reaction, competition in which this or that reaction takes an upper hand according to the strength of respective traces.

CONCLUSIONS

To sum up, we can characterize delayed responses, both with and without choice, in the following way. These reactions take place when a certain stimulus S_{rl}, applied after a stimulus S_{pn} gives rise to the performance of a movement r_n which leads to a positive reinforcement (or to the avoidance of a negative one), while the same movement performed to stimulus S_{rl} not preceded by stimulus S_{pn} does not lead to positive reinforcement (or leads to a negative one). As a result of such an experimental procedure differentiation develops, in which stimulus S_{rl} applied after stimulus S_{pn} elicits the instrumental reaction r_n, whereas this stimulus applied without a preceding stimulus S_{pn} does not elicit this reaction. The very fact that higher animals are capable of performing delayed reactions shows that the stimulus S_p leaves some

* In normal life such one trial learning based on a secondary reinforcement appears for instance when an animal buries food in some place and finds it later, even after a long period of time.

sort of trace in their nervous system which enables them to react properly to stimulus S_{rl} applied some time later.

According to our analysis, in the course of delayed reactions the processes of recent memory, as well as stable memory processes are involved. The stable memory processes are represented in our diagrams of conditioned-reflex structure of various varieties of delayed responses. In order to correctly perform these responses the animal must l e a r n to differentiate between the compound S_pS_{rl} (which is positive) from its elements applied separately (which are inhibitory). He also must learn which preparatory stimulus points to which foodtray. All this learning is based of course on stable memory. On the other hand, the bridging of the delay period is fully based on the recent memory, i.e. the traces of the given preparatory stimulus, preserved for a short period of time in the animal's brain, are responsible for the correct response to the releasing stimuli.

The physiological mechanism of delayed reactions will be elucidated when the three following questions have been answered: a) what nervous mechanisms underlie the trace of a stimulus, b) what are the elements of the stimulus S_p (or its consequences in the nervous system) which activate these mechanisms, and c) what parts of the brain are concerned with the formation of these traces.

The hypothetical answer to the first question is that excitation produced in the nervous system (especially in the cerebral cortex) by a given stimulus throws into activity certain closed chains of neurons and thereby outlives the action of the stimulus. The second question is one of the fundamental ones concerning the delayed reactions; in the following papers we shall partly answer it, but a full answer requires further experimentation. Lastly to the question of the areas concerned in delayed reactions, the first step was made by J a c o b s e n (1936) in the thirties of this century by showing the role played in these reactions by the prefrontal areas. Our own investigations (presented in the third paper of this series) confirm J a c o b s e n's data: they also allow the intimate nature of the disorders of these reactions after prefrontal ablation to be submitted to a detailed analysis which has enabled us to explain some of the controversies between various authors concerned with this question.

APPENDIX 1

On the structure of the reflex arc in type II (instrumental) conditioning

Because of the importance of the scheme shown in Fig. 2 for our further considerations we present here the main experimental evidence on which it is based:

The existence of the indirect pathway $S \rightarrow F \rightarrow r$ is substantiated by the following facts. It is well known that both the elaboration and the preservation of an alimentary instrumental reflex depends on food reinforcement. This reinforcement being withdrawn the reflex becomes extinguished, and, as shown by Wyrwicka (1952), the mere reinforcement of the stimulus leads to the re-establishment of the instrumental reflex without any special training. Similarly, the introduction of a new stimulus and its reinforcement by food very often is sufficient for the establishment of the same instrumental reaction which in the given situation was elaborated to other stimuli (Wyrwicka 1952). When the animal is satiated the instrumental reaction either does not appear at all, or is greatly reduced. If in a given situation alimentary and defensive instrumental conditioned reflexes are established with different motor effects, then all the alimentary conditioned stimuli evoke exclusively the „alimentary" movement, and all defensive conditioned stimuli evoke only the „defensive" movement (Konorski 1939). Finally, the direct electrical stimulation of a particular unconditioned centre in the hypothalamus evokes the instrumental reaction connected with the respective unconditioned reflex (Andersson and Wyrwicka 1957).

On the other hand, if the direct connections between the central representation of S and r did not exist it would be impossible to establish different instrumental reflexes to different stimuli by using one and the same reinforcement. Therefore the connections $S \rightarrow r$ rather determine which instrumental reaction is to be performed, while connections $F \rightarrow r$ are responsible for the elicitation of the reaction designated by connections $S \rightarrow r$.

The experimental evidence gathered in this laboratory shows that, according to the experimental setting, the direct and indirect connections between S and r may play, respectively, a greater or lesser role in the establishment of the given instrumental reflex. According to the unpublished data of Dobrzecka (1957), if the

instrumental reflex of lifting of the leg is established to the tactile stimulus applied to that leg, then such a reflex is very stable and difficult to be extinguished. If in the experimental situation two different instrumental reactions r_1 and r_2 (e.g. lifting of the two legs) are established to two different acoustic stimuli, s_1 and s_2, then it is very difficult to achieve such a state that the animal does not confuse both movements and performs them separately to the respective stimulus. On the other hand, if s_1 and s_2 are tactile stimuli applied to the respective legs, these two movements are never confused. This goes to show that between the central representation of the tactile stimulus to the given leg and lifting of this leg the strong „potential" connections exist (cf. Konorski 1948), which make the conditioned bond $s \rightarrow r$ particularly strong.

We are confronted with the same situation in the experimental setting dealt with in the present paper. The specific bond $S_n \rightarrow \rightarrow r_n$ is much stronger in the case when the stimulus S_n is a directional stimulus, than when it is a sign stimulus. This is because the orienting reaction towards the directional stimulus S_n is allied with the reaction r_n, while the orienting reaction to the sign stimulus is antagonistic to it.

APPENDIX 2

Simple delayed reaction, conditioned inhibition and trace conditioned reflex

As shown in text the experimental setting of the simple delayed response is that the releasing stimulus acting on the traces of the preparatory stimulus is reinforced by food, whereas the releasing stimulus itself is not reinforced. Whether or not we have here to do with classical or instrumental conditioning is not relevant.

Taking into account classical conditioning (as more simple) the formula of simple delay reaction will be:

$$[S_p]\, S_{r1} - F$$
$$S_{r1} - \sim F$$

On the other hand in conditioned inhibition the conditioned stimulus itself is reinforced, while this stimulus acting together with, or preceded by, the „conditioned inhibitor" is not reinforced. For

the case when the conditioned inhibitor precedes the conditioned stimulus by some interval we have the formula:

$$S_{r1} — F$$
$$[S_p]\, S_{r1} — \sim F$$

So we see that the experimental setting of simple delay and conditioned inhibition is reverse. In the first case the preparatory stimulus announces that the releasing stimulus will be reinforced, in the second case, on the contrary, it announces that the positive significance of the next releasing stimulus will be cancelled.

We have as yet no experimental evidence whether or not, all conditions being equal, the maximal possible delay period is in both cases the same, or whether it is different, and if so, which of them is longer. It seems a priori that the conditioned inhibition setting is more difficult than that of the delayed reaction, since the animal has not only to preserve the traces of the conditioned inhibitor, but it must also be able to inhibit the conditioned reaction to the actual positive stimulus acting on its traces.

The simple delayed reaction has also something in common with trace conditioned reflexes (cf. Shustin 1952). The experimental setting of the trace conditioned reflex is that not the actual stimulus is reinforced by food but some particular moment after its cessation. So, in this case a given lapse of time plays a role of the sign releasing stimulus.

APPENDIX 3

On the central representation of compound stimuli

There is much evidence collected both by Pavlov's school and in our laboratory showing that the cortical representation of the compound of conditioned stimuli cannot be simply considered as composed from the particular centres representing each element of the compound. First, the possibility of differentiation in which the compound plays a role of the positive conditioned stimulus while its elements are inhibitory speaks in favour of this conclusion. If the representation of this compound were nothing else than the sum of the centres of its elements such a differentiation would be of course impossible, because the sum of two inhibitory reflexes cannot result in the excitatory reflex. Another piece of experi-

mental evidence showing the relative independence of the central representation of the compound and its elements is given in a paper by Chorążyna (1957). To stimulus S the alimentary conditioned reflex was elaborated whereas the compound consisting of S_0S, applied in succession (and even separated by several seconds' interval) was inhibitory (conditioned inhibition). When the differentiation between S and S_0S was established the stimulus S alone was no more applied, while the compound S_0S was repeatedly applied without reinforcement. When after a long period S was again tested it was found that it did not lose its positive character. And so we see that the repeated application of this stimulus without reinforcement in a compound with S_0, did not lead to its extinction, as would be the case if S were applied alone. This means that the central representations of S_0S and S, and even of $[S_0]$ S and S, are mutually independent.

All these data go to show that the compound stimulus must be considered as a stimulus different from its component stimuli. Of course it may be, and most often it is, similar to its elements, as judged by the high level of generalization between them. But it can be differentiated from its elements in exactly the same way as are differentiated simple stimuli when they are similar to one another.

Passing to the experiments dealt with in the present series of papers we have, on the one hand, single stimulus S_p (or its trace $[S_p]$) and single stimulus S_{rl}, and on the other hand, their compound S_pS_{rl} (or $[S_p]$ S_{rl}). Although the compound stimulus is much similar to each of its elements (as judged by strong generalization), it must be considered as a separate stimulus with its own central representation.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

PHYSIOLOGICAL MECHANISM OF DELAYED REACTIONS II. DELAYED REACTIONS IN DOGS AND CATS TO DIRECTIONAL STIMULI

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(Received 2 October 1958)

In the first paper of this series, we introduced the following terminology of the stimuli involved in delayed reactions. Those stimuli which determine, in any particular trial, the direction in which the animal should run in order to get food were called preparatory stimuli, while a stimulus which evokes the reaction determined by the preparatory stimulus was called the releasing stimulus. Further, we distinguished „directional" and „sign" preparatory stimuli. If the source of the preparatory stimulus is in the same place as the source of food and if, therefore, the orienting reaction evoked by the stimulus is allied with the reaction to food (when the animal is released), we have to do with a „directional" preparatory stimulus. On the other hand, if the orienting reaction to the preparatory stimulus provides no cue for the proper reaction to food, we have to do with a „sign" preparatory stimulus.

The present paper is concerned with the description of delayed responses to directional preparatory stimuli in normal dogs and cats.

MATERIAL AND GENERAL EXPERIMENTAL PROCEDURE

The experiments here described were performed on 8 dogs and 7 cats. They were carried out in a rectangular room 8 m × 4 m shown in Fig. 1. Three foodtrays F_1, F_2 and F_3 were placed near the middle of each of the three walls of the room, whereas the starting platform and the place of the experimenter was located at the fourth wall. On each foodtray was fixed a lamp and a buzzer. Both the moving of the bowl into position in

the foodtrays, and the application of the stimuli were operated automatically by the experimenter by means of an electrical device.

In the initial experiments, the animals were allowed to move freely about the room, and when they happened to come near to one of the foodtrays, the bowl with food was presented. Since moving it into position produced a short click, the animals soon learnt to come to that foodtray from which they heard the noise of the moving bowl. Afterwards, the animals were immobilized at the starting platform (the dogs by leash and the cats by lowering a small round covering cage), and they were released during the operation of one of the buzzers followed immediately by the moving into position of the appropriate bowl. They very soon learnt to run to a given foodtray at the sound of the buzzer, the bowl with food being presented when the animal drew near to the foodtray. All this preliminary training lasted not more than a few days.

Then the experiments with delay began. They ran as follows. The animal being immobilized on the starting platform, one of the buzzers sounded for 3 seconds and then, after some time, the animal was released. If he came to that foodtray which had been signalled by the buzzer, the bowl with food was presented to him. If he went to the wrong foodtray, and then, correcting himself, to the right one, the food was not presented. This

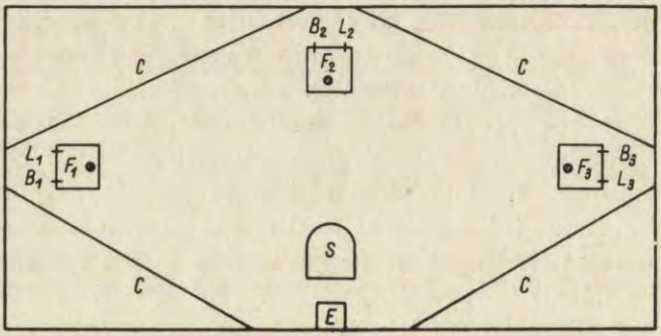

Fig. 1. Experimental setting used in our study

F_1, F_2, F_3 — left, middle and right foodtray. The bowls are automatically moved into position by the experimenter using an electromagnetic device; E — table and seat for experimenter using an electromagnetic device; E — table and seat for experimenter; S — starting platform; B_1, B_2, B_3 —— buzzers, L_1, L_2, L_3 — lamps situated on the respective foodtrays

restriction was adopted to prevent the animal from getting used to wander from one foodtray to another in search of food. As the result, at the termination of each trial (irrespective of whether reinforced or not) the animals got used to return immediately to the starting platform, or to walking around for some time in the room without examining other foodtrays.

When the animal was seen to be able to react correctly after the delay of a few seconds (which in all our cases occurred very soon), the delays were gradually prolonged. In most cases the following delay periods

were used: for dogs — 6 sec., 15 sec., 30 sec., 1 min., 3 min., 6 min., and more; for cats — 6 sec., 15 sec., 30 sec., 1 min., 2 min., 3 min., 6 min.

In each experimental session there were 9, 12 or 15 reinforced trials, the number of reinforcements from each foodtray being the same in each session. The sequence of the applications of signals was not entirely random: in the first period of training, we avoided repetition of the same signal twice in succession, in order to be sure that the correct run was due to the preservation of the traces of the signal itself and not of the traces of the last response. In some experiments, on the other hand, we repeated the same signal a number of times in order to examine the influence of such a „training" on the subsequent delayed reaction. If the animal chose a wrong foodtray and did not get food, the same signal was repeated in subsequent trials until the animal reacted correctly. Usually the delay in the following trial was shortened in order to make the task of choice easier.

The inter-trial intervals were irregular and lasted at least some minutes. We observed that sufficiently long intervals (especially when the delays were long) are very important in preserving the animal in good condition. Although the experimental sessions lasted about from 45 min. to 1½ hours, the animals very rarely reacted less satisfactorily at the end of a session or revealed any tendency to go away.

The application of three foodtrays — one on the left, one in front, and one on the right in relation to the starting place — seems to be very convenient for experiments of this kind. First, since the probability of the chance performance of correct deactions is in n trials $1/3^n$, and not $1/2^n$ as in double choice, far fewer trials are needed to test whether or not the given task (e.g. the particular duration of delay, the use of some sort of disturbance, etc.) can be performed by the animal. Secondly, the existence of three goals made possible an analysis of errors impossible with two goals. Thirdly, the use of three foodtrays made it possible to apply two signals in the trial and to see whether or not the animal is able to perform such a task. This problem will be dealt with in a later paper of this series.

The releasing stimulus in the experiments with dogs consisted in unleashing, and in the experiments on cats in raising the cage. Since the dogs usually lay or stood quietly during the delay period the sound made by unhooking the leash was often the only signal announcing that the animal was free. Hence it happened sometimes, that the dog was not attached at all during the delay period and nevertheless remained quietly on the starting platform until he heard the sound of "unhooking".

RESULTS

1. The length of the delay period

We did not attempt in this paper to investigate in detail the problem of the maximum delay periods which could be achieved by our animals. This task is not very rewarding, for the following

reasons. First, it has been frequently stressed that the maximal delays which can be achieved in a given experimental setting may be quite different even in slightly modified condition, and therefore do not represent any absolute value. Further, with the

Table I

Effect of length of delay on the number of errors in dogs

Dog No	Delay in minutes											
	3		6		9		12		15		18 a. more	
	tr.	er.	tr.	er.	tr.	er.	tr.	er.	tr.	er.	tr.	er.
1	60	0	30	0	6	0	4	0	7	0	7	0
2	60	5	30	2	6	1						
3	30	1	30	0	10	1	12	2				
4	60	0	25	2								
5	50	2	18	2								
6	30	0										

tr. — trials; er. — errors

same experimental condition, the delay ability of different animals of the same species may vary considerably, and even in one and the same animal this ability is far from being constant, depending

Table II

Effect of length of delay on the number of errors in cats

Cat No	Delay in minutes							
	1		2		3		6	
	trials	errors	trials	errors	trials	errors	trials	errors
1	45	0	25	5	20	1		
2	60	4	45	4	40	2	11	0
3	60	3	60	9	13	9		
4	60	4	45	1	20	4	12	3
5	60	2	15	0	13	3		
6	60	2						
7	60	0						

to a marked degree on his general "condition". If, for instance, the animal is presented with an excessively difficult delay task, his ability to delay drops sharply and he is liable to make mistakes even in such tasks that normally are quite easy for him. For this reason our usual procedure was to apply those delays after which

the animal gave 100% correct responses; only occasionally did we test the longer periods.

As far as dogs are concerned, their ability to delay under our experimental condition is really amazing. Since a delay of one minute did not present any difficulty to our dogs, we ordinarily used this delay, and occasionally tested longer delays. The results of these tests are presented in Table I. As this table shows, even in delay periods of a dozen or more minutes, the reactions of our dogs were most often correct.

The errors made by cats were in general more frequent than those made by dogs. The delay periods did not exceed here 6 min. As seen in Table II, one cat was not able to react correctly after a delay of 3 min., whereas for two cats the delay of 6 min. was still available. It must be observed however, that both dogs and cats — particularly cats — did not tolerate well excessively long periods of delay. They became reluctant to return to the starting platform and became angry when enclosed in the release cage.

2. The behaviour of animals to the preparatory stimuli during the delay period and after release

Since the behaviour of normal dogs and cats during the delay period is very characteristic, and differs strikingly from that observed after prefrontal ablations, we shall propose to describe it in greater detail.

Dogs. To the preparatory stimulus, all the animals always manifested a more or less clear orienting reaction directed towards the source of the stimulus. This reaction varied, both in intensity and properties, in different dogs. The animals either started up and oriented their whole body towards the stimulus, or on the contrary, they continued lying on the platform, the only sign of paying attention on the stimulus being a turn of the head (or often of the eyes only) and a pricking of the ears in the appropriate direction. There were even cases in which, though we were not at all sure whether or not the animal "noticed" the signal, he went on release to the proper foodtray without hesitation, even after a long delay period. Some dogs barked vehemently at the stimulus, others remained quite quiet.

After the preparatory stimulus was discontinued again the behaviour of the animals was very diverse and variable. Some of the dogs were almost entirely quiet during the delay; they remained

lying on the platform turned in any direction, sometimes even opposite to that of the source of the stimulus. Often they went to sleep when the delay was very prolonged. Sometimes they played with the leash, scratched and licked themselves, rolled on their backs etc. Usually, the behaviour of these dogs during the delay period did not differ visibly from that in inter-trial intervals; the difference was observed only after the release (section 6). Other dogs were more excited during the delay period; they tried to break loose, turned chaotically in all directions, climbed on the wall of the platform towards the experimenter. Only one dog (spaniel) had a tendency to keep his bodily orientation towards the proper foodtry throughout the delay, but if occasionally he failed to do so, this did not prevent him from making a good response.

When the dogs were unleashed they hurried to the proper foodtray quite irrespective of the position they had taken during the delay. In most cases, they went straight to it, but some of them habitually described a small circle.

C a t s. The behaviour of cats was in general very similar to that of dogs. To the preparatory stimulus, they also exhibited the orienting reaction which consisted in turning the head towards the stimulus. Then they were slightly agitated, turned in all directions, frequently towards the experimenter, climbed the bars of the cage, washed themselves, sometimes scratched, or sat quite motionless for some time. They never maintained the direction towards the source of the stimulus. When the cage was removed, they either ran quickly to the proper foodtray or, when the delay was relatively long, they walked slowly and uncertainly.

In both cats and dogs, the bodily orientation towards the source of the signal during the delay not only did not constitute an essential condition for making a correct post-delay response, but, in fact, was almost never maintained.

3. Comparison of delayed reactions after the acoustic and visual preparatory stimuli

Since in some of our dogs (both normal and lobectomised), we applied as preparatory stimuli both buzzers and lamps, it was possible to observe certain interesting and rather essential differences between these two categories of stimuli.

As regards the orienting reactions to the buzzer, these were always correct and unmistakable, even if the dog was separated from the foodtrays by a screen. Whatever the orientation of the dog before the signal, he was always (except for the rare cases in which he was turned strictly back to the source of the stimulus) able to localise precisely the source of sound.

Quite different were the reactions of the dogs to visual stimuli. It was clearly seen that the dogs, in contradistinction to man, are not able to localise precisely the source of light. When the animals learnt that the lighting of the lamp on the given foodtray meant food in that foodtray, it often happened that the orienting reaction was not directed towards the source of light, but towards that foodtray to which the dog had just been turned. In such cases, the animal when released, either after a short or a long delay period, or even during the operation of the stimulus, ran to that foodtray and stayed there for a long time. In Table III is presented the percentage of wrong runs to the visual stimuli in various dogs. It will be seen that this percentage is the same whether the delay is 6 seconds or 30 seconds, and whether during the delay period the platform is screened or not. This makes it quite clear that the errors are not the result of the difficulty of delay but of the difficulty of perception.

Table III

Effects of various delays on the visual preparatory stimuli in dogs under different conditions

Dog No	6 secs delay						30 secs delay					
	with screen						with screen					
	tr.	er.	% of er.	tr.	er.	% of er.	tr.	er.	% of er.	tr.	er.	% of er.
1	66	6	9	66	6	9	64	6	9	32	3	9
2	66	6	9	63	5	8	60	4	7	30	2	7
3	63	6	9.5	65	5	8	60	6	10	32	4	12.5

Usually, in the course of experiments with visual stimuli, the animals (not only the normal ones but also those subjected to prefrontal ablation) managed to learn to scan the foodtrays after the lamp was lit and to find by comparison that from which the

intensity of light was the strongest. In such cases the reaction, whether immediate or delayed, was always correct. Sometimes, in one and the same experiment, both forms of responses were seen; in some trials, when the lamp was lit, the dog very carefully scanned all the foodtrays and then made a correct orienting reaction, while in other trials he reacted quite fortuitously to the random foodtray, and then, of course, often made mistakes.

4. The effect of distracting stimuli on the delayed responses

The problem of the effect of distracting stimuli on the delayed reactions is of great importance as regards the mechanism of these reactions and of their impairment following prefrontal ablations. We therefore examined this problem, particularly in dogs, in some detail by applying various types of disturbance during the delay period.

The following distracting stimuli were used in dogs:

a) The experimenter rising from his seat, walking around in the room, going out of the room, calling the dog, playing with him, etc. All the movements of the experimenter, and particularly his rising and walking around, evoke in dogs a very strong orienting reaction, the posture of expectation, wagging the tail, barking etc. Therefore it might be expected that these stimuli would, when applied during the delay period, very much disturb the correct postdelay response.

b) The presentation of food on the starting platform at various moments of delay. This was done in order to see whether or not the alimentary unconditioned reflex washes out the traces of the preparatory stimulus.

c) Taking the animal out of the room and keeping him outside throughout the delay. In the other room, the dog was allowed to wander round, play with the experimenter, etc. After being let in again, the dog was immediately released without returning to the platform.

d) Screening the starting platform by drawing curtains around it, or dividing off with wooden partitions the part of the room around the platform. The curtains were drawn either before or after the application of the preparatory stimulus. The wooden partitions were kept during the whole, or part of, an experimental session, the passage opening being situated in various places. In

consequence, during the application of a signal, the animal performed the orienting reaction without seeing the foodtray to which he should go. After being released, he was compelled to go through the only passage left, which often meant that he must go round the screen from outside to reach the proper foodtray.

Table IV

The effect of distractions on delayed reaction in dogs cats

Dog No	Number of trials	Per cent correct	Cat No	Number of trials	Per cent correct
1	50	98	2	85	95
2	35	86	3	85	88
3	45	87	4	60	95
4	50	98	5	30	87
5	25	76	6	20	90
6	30	100	7	20	90

e) Screening of the starting platform by wooden partitions throughout the experiment and taking the dog out of the room in the delay periods. Since the door outside was situated in the shielded part of the room the animal could neither see the foodtray from which the appropriate signal was applied, nor maintain the bodily orientation towards it during the delay.

f) The application of acoustic and visual stimuli from various points of the room.

The general result of such tests was quite unequivocal. In normal dogs in contradistinction to those subjected to prefrontal ablations all these sorts of disturbing agents did not, as a rule, prevent the animals to perform the correct dalayed response (Table IV).

In the experiments with cats only giving food in the releasing cage and drawing the curtains before or after the application of the stimulus were applied. As may be seen from Table IV, the animals were able, generally, to cope with these tasks.

5. Analysis of errors

As already indicated, our triple choice experimental method has the advantage over the double choice method in that the animal, even when making a mistake, has to "choose" between two remaining foodtrays, and therefore, it is possible to examine the question as to why, when making a mistake, he has preferred this and not that foodtray. In particular, it was possible properly

to investigate the problem of the effect of the preceding reaction on the post-delay run.

As stated above, the mistakes made by dogs were so rare* that it was hardly possible to make a more detailed analysis of them. As to the cats, they were more prone to make errors, and since these errors presented some regularity in all our animals, it was possible to draw certain conclusions as to their origin and character.

Table V

The types of errors made by cats

Cat No	Number of trials	Per cent of errors			
		total	persev.	semi-persev.	other
1	386	5,2	3,1	0,8	1,3
2	681	4,5	2,5	1,0	1,0
3	698	6,4	4,3	0,3	1,7
4	571	7,7	5,1	0,3	2,3
5	224	6,2	4,9	0,4	0,9
6	281	7,1	3,9	1,4	1,8
7	226	8,8	7,5	—	1,3

In the preliminary training, when the cats were allowed to react to the actual sound of the buzzer, they made practically no errors at all. But as soon as the releasing cage was introduced, and the animals were set free only some time after the termination of the signal, then from time to time (once in 10— 20 trials) they made errors. These errors consisted for the most part in running to that foodtray which had been reinforced in the previous trial ("perseverative error"**). After arriving at that foodtray, the animal either stood there for some time waiting for food and then returned to the starting platform (error without correction), or even more often, he ran from it straight to the proper foodtray and remained there for a long time (error with correction). Sometimes, when the previously reinforced foodtray was just op-

* Except in the experiments with visual preparatory stimuli, discussed in section 3.

** As a matter of fact, these errors should be termed not "perseverative errors" but "one-trial-learning errors", since the reaction of the animal is based on the fresh habit he acquired in the preceding trial (cf. discussion of this problem in the preceding paper of this series).

Table VI

The most common kinds of mistakes made by cats in delayed responses

	Trials	Time in minutes	Preparatory stimulus	Delay in secs	The direction of run	Reinforcement	Remarks
			1. Mistake at the beginning of training (short intertrial interval)				
Cat No 4	8	16	B_3	3	F_3	+	
Exp. 3	9	19	B_2	3	F_2	+	
	10	20	B_1	3	F_2, F_1	—	From F_2 straight to F_1
	11	21 ½	B_1	3	F_1	+	With hesitation
	12	23	B_1	3	F_1	+	
			2. Mistake in a trial with prolonged delay period. Early stage				
Cat No 1	9	22 ½	B_3	6	F_3	+	
Exp. 13	10	24 ½	B_1	30	F_3, F_1	—	From F_2 straight to F_1
	11	26 ½	B_1	30	F_1	+	
			3. Mistake in a trial with prolonged delay period. Later stage				
Cat No 1	4	9 ½	B_3	6	F_3	+	
Exp. 21	5	11 ½	B_2	120	F_3, F_2	—	From F_3 ran to F_2
	6	15 ½	B_2	6	F_2	+	With hesitation between F_3 and F_2
			4. Mistake in the second trial with screening				
Cat No 2	3	5 ½	B_2	15	F_2	+	
Exp. 56	4	7 ½	Scr. B_3	15	F_3	+	
	5	9 ½	Scr. B_1	15	F_2	—	With strong hesitation between F_3 and F_2
	6	11 ½	Scr. B_1	15	F_1	+	With hesitation between F_2 and F_1
	7	14 ½	Scr. B_1	15	F_1	+	Quite surely to F_1
	8	17	Scr. B_2	15	F_2	+	Surely to F_2
			5. Mistake in a short-delay trial following a long-delay trial				
Cat No 4	4	4	B_1	60	F_1	+	
Exp. 37	5	9	B_2	360	F_2	+	
	6	19	B_3	15	F_2, F_3	—	From F_2 in a great hurry to F_3
	7	21	B_3	15	F_3	+	First step to F_2, afterwards rapidly to F_3
	8	23	B_1	15	F_1	+	

posite to that signalled in the subsequent trial, the cat, after being released, ran to the middle foodtray — a compromise between the conflicting tendencies to run to the two opposite places ("semi-perseverative error"). This explanation seems probable, because such a compromise may take place also when two neighbouring foodtrays are signalled one after another; it then happens that the animal begins his run along the diagonal between the two foodtrays. In Table V, we present the frequencies of various types of errors made by our cats.

The analysis of the protocols of particular experiments in which perseverative or semi-perseverative errors were made, suggests certain conclusions as to their origins. The conditions under which such errors may occur are the following (for examples see table VI):

a) The error could be made in the first trial in which the delay was protracted or some new disturbing factor was introduced (Table VI, 2. 3.).

b) The error could be made not in that trial in which the difficulty was introduced for the first time, but in the next trial in

Table VII

Perseveration in dog

	Trials	Time in min	Preparatory stimulus	Delay in minutes	The direction of run	Reinforcement	Remarks
				Protocol No 1			
Dog No 1	1	1½	B_3	1	F_3	+	
	2	4	B_3	1	F_3	+	
Exp. 38	3	6½	B_3	1	F_3	+	
	4	9½	B_3	1	F_3	+	
	5	12	B_3	1	F_3	+	
	6	15	B_1	8	F_1	+	
				Protocol No 2			
Dog No 1	4	10	B_3	1	F_3	+	In each trial
	5	12	B_3	1	F_3	+	the dog is
Exp. 39	6	15	B_3	1	F_3	+	taken out of
	7	18	B_3	1	F_3	+	the room
	8	21	B_3	1	F_3	+	
	9	24	B_1	4	F_3	—	
	10	31	B_1	4	F_1	+	(Not taken away)

which the same difficulty was repeated (Table VI, 4). Even, in some instances, a perseverative error appeared not in that trial in which the delay was protracted but in the following trial in which it was again short (Table VI, 5). The explanation of these forms of error will be given in the discussion.

c) The tendency to make a perseverative error was increased when the same signal was applied several times in succession, followed by some other signal.

d) The tendency to make a perseverative error was increased when the preceding inter-trial interval was very short (Table VI, 1).

It must, however, be emphasised once more that even in cats, the errors were very rare and that the above protocols do not mean that the same mistake will be repeated when the same experimental condition is reproduced. On the contrary, the animals were able to learn, even very rapidly, not to repeat the same perseverative mistake in a given circumstance. This learning took a particularly clear form in cases when the animal, after having been released, either hesitated where to go or even began his run to the previously reinforced foodtray, but on the way "changed his mind", and turned to the foodtray signalled by the preparatory stimulus.

Table VIII

Results in delayed response tests of dogs in respect to the sequence of visual stimuli

Dog No	Total number of trials	Non repetitive trials			Repetitive trials		
		totals	errors	per cent of errors	totals	errors	per cent of errors
1	490	419	40	9	71	17	24
3	482	398	25	6	84	14	117
5	266	225	23	10	41	5	12

Although, as already mentioned, the errors made by our dogs were less numerous than those made by cats, the general rules of their occurrence were the same. Here, too, perseverative errors were the most frequent and they principally occurred when the delay was prolonged or some disturbance introduced. In Table VII, are presented two protocols of experiments with dog No 1, in which the same signal was repeatedly applied to create favourable

conditions for perseveration. Five applications of B_3 in a normal experiment did not provoke the perseverative error when B_1 was applied and the delay was 8 minutes. On the other hand, when the dog was taken out of the room in each trial, then after a series of applications of the same signal, the perseverative error appeared even after 4 minutes. This was the only error made by this dog after being taken out of the room.

Since the animals exhibited such a strong tendency to be directed in their behaviour by one trial learning (in those cases, of course, in which the preparatory stimulus for one reason or another did not supply a sufficient cue for their run), it was to be expected that if the same signal was applied in the two consecutive trials, the animal would never fail to react correctly to the second application. However, to our great surprise this proved not always to be the case. As mentioned in section 3 concerning experiments with visual stimuli, performed in dogs, the animals made mistakes in approximately 10% of trials because not always were they able to localise the source of light (cf. Table III). Now, analysis of errors made by the dogs indicates that the percentage of errors made to the second application of the given signal is not smaller but even larger than the mean percentage of all errors (Table VIII). This means that the animals display here the tendency to avoid actively the most recently reinforced foodtray rather than to seek it. We have termed such errors "anti-perseverative errors". What is not less interesting is the fact that in the majority of such cases the animal chooses that foodtray which was reinforced in the most recent trial but one. We shall make an attempt to explain this sort of error in the discussion.

With the cats we were not able to observe this type of errors unequivocally. There were cases in which the repetition of the same signal with a very protracted delay period led indeed to an error, but these cases were too scarce to warrant drawing any conclusions from them.

6. The releasing of the animals in intervals without any preparatory stimulus

As explained in the previous paper, in the method of delayed responses with choice, the application of the releasing stimulus alone is not necessary since the delay ability of the animal is

confirmed by the very fact of his choosing the proper foodtray. Nevertheless, with four dogs we performed several series of experiments in which we released them in inter-trial intervals to see whether or not they would go to a foodtray, and if so to which one.

The results of these experiments are given in Fig. 2. It will be seen that the tendency to go to the foodtrays after releasing is relatively poor. Two of the dogs when released in intervals did

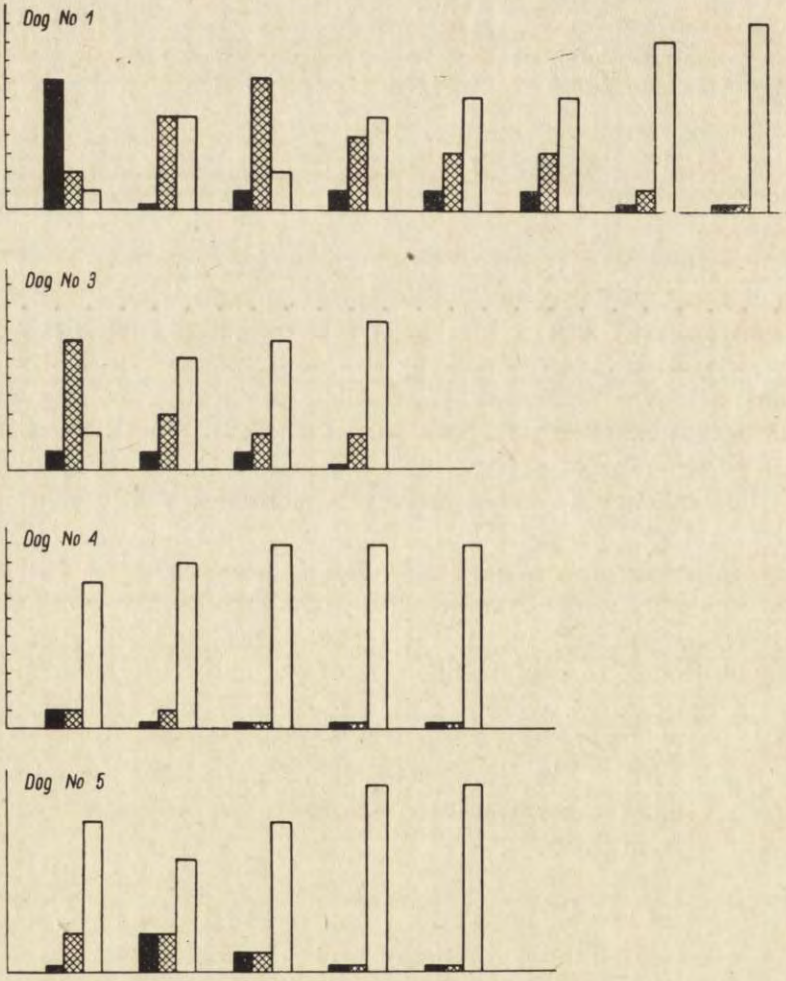

Fig. 2. Releases in intertrial intervals (dogs)

Each block represents 10 successive releases in intervals. Black, the dog goes to the reinforced foodtray; cross-hatched, the dog goes to another foodtray; white, the dog remains on the platform

not manifest any tendency at all to run to the foodtrays, while others did run at first but very soon stopped doing so. But even if the reaction was positive, it differed greatly from the normal run in the delay trial. In the latter case, the animal ran quickly and confidently to the signalled foodtray, and, if the food was not presented at once, he stayed there for a long time obstinately fixing the bowl, while, when released in the interval, he walked slowly and reluctantly to some foodtray and very soon returned to the platform.

As seen in Fig. 2, one dog manifested a tendency to go in the intervals to that foodtray which was previously reinforced, and then, on the contrary, tended to avoid that particular foodtray and to go to another one. The other dog avoided that foodtray from the very beginning. Thus, here, too, we encounter both tendencies indicated in the preceding section, namely the tendency to repeat the last run and also the tendency to avoid repeating it.

7. Experimental neuroses

Several times in the course of the present experiments, disorders of a neurotic character developed in our animals. They were usually caused by too intensive applications of distracting stimuli and by too long delay periods. They were characterised by the following features:

a) The animals became very reluctant to return to the starting platform and manifested a strong tendency to get away from the experimental room.

b) Sometimes they refused to take food in the experimental room.

c) They became "inattentive" and made far more errors (even after short delays) than in the normal state; the majority of these errors were of the perseverative character.

When the neurogenic factor was withdrawn, the animals after some time gradually returned to normal.

DISCUSSION

Our results are not in full accord with previous studies on delayed responses in two respects: first, in both dogs and cats we reached much longer delay periods than those found by Hunter (1913) and Walton (1915) in dogs, and by Yarbrough (1917)

and Cowan (1923) in cats; secondly, our animals (by contrast with those of Hunter and Yarbrough) were able to respond correctly without preserving the bodily orientation towards the signalled foodtray. As to the first discrepancy, it is possible that certain small technical points are partly responsible for the good performance in our animals: each animal was under experimentation for a long time, delay periods were only gradually protracted, and neurotic disorders, which strongly deteriorate the delay performance of animals, were carefully avoided. Nevertheless, we think that the chief factor making possible such long delays was the wide spacing of the foodtrays. In fact, as will be reported in another paper of this series, we found that when the foodtrays are put closer to one another, the delay ability of the animals is drastically reduced. It will also be shown in that paper that with the foodtrays very near one another the preservation of the bodily orientation of the animal plays an important role in the correct solution of the task, which fact accounts for the second discrepancy between our results and those of Yarbrough and Hunter.

If the animal makes a proper choice by virtue of keeping the right bodily (or head) orientation during the delay, his reaction is, as a matter of fact, not a true delayed response, but a pseudo-delayed response, since he reacts to the actual proprioceptive stimulus operating at the moment of release. If that is so, we can say that our experimental arrangement is precisely the best one to enable the animals to manifest their ability — if they possess such ability — for the true delayed reactions. We shall see in another paper of this series that this is indeed true in the case of the normal dogs and cats, and that after prefrontal ablations this ability is lost totally and permanently.

It can be argued, however, that there is another actual factor which might serve as a cue for post-delay response, namely frequent glancing at the foodtray to which the animal should go. But this supposition is ruled out not only by the general observation of the animal's behaviour during the delay — behaviour which does not manifest any such tendency — but also by those experiments in which the foodtrays were screened, and by those in which the animal was taken away from the experimental room for the entire period of delay.

Thus we are bound to admit that there are no actual stimuli during the delay period to determine the animal's reaction, and that this reaction is fully based on the traces of stimuli which were present at the beginning of the delay trial. And so the problem arises what actually does the animal remember during the delay, i.e. what is the immediate preparatory stimulus the traces of which remain in the cortex in the form of reverberating activity and determine the direction of the animal's run after release.

We think that the analysis of the effects of distracting stimuli can provide some evidence as regards this issue. For, if the given factor is supposed to be responsible for the post-delay response, then by applying a factor of the same kind but of antagonistic action during the delay period, we should be able to disturb the proper reaction. On the other hand, if the given extra-agent has no negative effect on the delayed response, this means that it has nothing to do with the trace "stimulus" responsible for the delay. In this way we can eliminate a number of factors which might be suspected of playing a role in determining the post-delay run of the animal, and, perhaps, find those which are relevant to it.

1. It was emphasized in section 2 that the preparatory signal always produces the orienting reaction in the direction of the source of the stimulus; therefore, it might be supposed that the memory of this orienting reaction can provide a cue determining the post-delay reaction. This supposition is, however, ruled out by the fact, that although during the delay period we applied a number of stimuli eliciting a strong orienting reaction in the opposite direction, yet this did not in any way impair the post-delay run.

2. It might also be supposed that what is kept in mind during the delay period is the memory of the direction in which to go — to the right, forward, or to the left. This supposition is again ruled out by the fact that the animal can be taken away from the room and start from the side perpendicular to that where the starting platform is placed. When released and allowed to go to the goal, the animal cannot use the directional cue established at the beginning of the trial.

3. Further, one might assume that the animal can use various cues interchangeably, and that being deprived of one of them he bases himself on the other. For instance, the animal may use, alternatively, either his memory of the glance at the signalled

foodtray, or the memory of his bodily orientation. If so, the animal should fail if he is deprived of these two cues. Such a test arises when the starting platform is separated by partitions from the foodtrays so that the animal cannot see it when the signal is given, and then he is taken out of the room not being able to glance at the foodtray. The fact that he makes a correct run after being released seems to prove that this assumption also is not valid.

4. There remains the last possibility, namely that in the instant of the application of the preparatory stimulus, the animal associates it with the given foodtray (even if it is not seen during the exposition of the stimulus), and then, what he remembers, is only to which of the three foodtrays he should run, irrespective of the point of the room from which he starts.

According to this hypothesis, the disturbing factor capable of diverting the animal's reaction may be the remembrance of the reinforcing value of other foodtrays. Such remembrance is provided by the preceding positive trial and is connected with that foodtray which was then reinforced. Thus, during the delay period the animal has two competing memory traces: the one of the most recently reinforced foodtray and the other of the foodtray signalled by the preparatory stimulus. Although the first stimulus (reinforcement) is more remote than the second (preparatory stimulus), it may be the stronger since it includes the instrumental reaction performed and the reinforced foodtray (the so called one trial learning). Consequently, there may exist experimental situations in which the trace of the previous trial can predominate over the trace of the preparatory stimulus, and then the animal will make a perseverative error.

These errors are made as a rule: i. when the given delay period is very long; ii. when the interval between trials is short; iii. when the preceding delay period was very long; and iv. when in some preceding trial some sort of difficulty was introduced. The first two cases are quite clear: the trace of the stronger — although more remote — stimulus may easily predominate over a more fresh but weaker trace produced by the preparatory stimulus. The third and fourth cases are more difficult to account for. Probably, they should be explained as follows: When the delay is long, or complicated by some disturbing factor, the trace pro-

duced by the preparatory stimulus becomes very faint. If, nevertheless, the animal does find his way to the proper foodtray, then both the run and the place are strongly reinforced, and, consequently, the conditions for one trial learning are particularly favourable. Therefore, the trace left after this trial is strong and enduring, and it may predominate over the fresh trace produced by the preparatory stimulus.

Our experiments give clear evidence that even when the trace of the last reinforcement predominates over the trace of the preparatory stimulus, the latter is by no means lost. This is shown by the fact that in many cases the animal, after visiting the wrong foodtray, immediately passes to the correct one and remains there for a long time waiting for food (errors with correction). In a later paper of this series it will be shown that normal dogs and cats are perfectly able to preserve simultaneously the traces of two foodtrays — namely, when such foodtrays are both signalled in the same trial. While in that case the animal remembers two correct foodtrays, in the case under discussion, he remembers one correct and one wrong foodtray.

The problem arises as to whether or not the animal is able to learn to inhibit the tendency to go to the foodtray reinforced in the preceding trial. The answer is yes, as the following facts show. It was observed in section 6 that at the beginning of training, cats are liable to make perseverative errors even after very short delay periods. These errors are then totally eliminated, only to reappear if the delay periods are protracted or if disturbing factors are introduced, but again they tend, after some time, to be eliminated. In many cases, the animal makes no further error but in his run he displays a considerable hesitation, ostensibly suppressing the tendency to go to the foodtray last reinforced. Hence, the stronger the inhibition of the tendency to be directed by one trial learning, the less the number of mistakes made by the animal. This inhibition is much stronger in dogs than in cats and is one of the reasons of the correctness of their performance. It is worth noticing that this infallibility breaks down if the animal is neurotic; then the perseverative errors appear in abundance.

This brings us to the explanation of the seemingly paradoxical phenomenon of "anti-perseverative" tendency manifested both in " anti-perseverative errors" made by dogs in experiments with

visual stimuli, and also in not repeating the last run when the dog is released in the inter-trial interval. If we admit that the animal learns to inhibit the fresh habit acquired by one trial learning — i.e. to inhibit the tendency to repeat the previous run — then this inhibition may lead to an active avoidance of going to that foodtray where he recently obtained food.

In other words, we are dealing with the following state of affairs. If the task of performing the delayed reaction is not difficult, because the preparatory stimulus is strong and the delay not too long, then the animal reacts correctly. But when the animal is not sure where to go because of the vagueness of the preparatory stimulus and or of the length of delay, he tries to find other, more distinct, cues. The more primitive cue is that provided by one trial learning, the more elaborated one, based partly on the experience of the animal in the experimental situation, is that of suppressing the reaction directed by one trial learning. While the first tendency was rather manifested by cats, the second one was rather observed in the dogs.

SUMMARY

1. The present paper is concerned with the properties of the delayed reactions in normal dogs and cats to acoustic and visual directional preparatory stimuli in a triple choice experimental situation.

2. The delays attained under our experimental condition exceeded twelve minutes for dogs and six minutes for cats, and were probably considerably longer. During the delay period, the animals did not preserve their bodily orientation towards the signalled foodtray; on the contrary, they moved freely on the starting platform, adopting any posture and direction of the body and head.

3. Such distracting factors applied during the delay period as extraneous stimuli evoking an orienting reaction, screening of the starting platform, receiving food on the platform, and (in dogs) taking the animal out of the room, did not significantly disturb the correct post-delay response.

4. When visual preparatory stimuli were used in dogs, it was found that in about 10 percent of trials, the animals did not respond correctly. This was due not to the difficulty of delay but to the difficulty of locating the source of the stimulus.

5. The cats were in general more prone to make mistakes than the dogs. Most of their mistakes had a "perseverative" character, i.e. the animals had a tendency to repeat their last reinforced run.

6. The "anti-perseverative" type of error was found in dogs in experiments with visual preparatory stimuli.

7. The problem of the cues directing the animal in his postdelay run, and the mechanisms of errors are discussed.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

PHYSIOLOGICAL MECHANISM OF DELAYED REACTIONS III. THE EFFECTS OF PREFRONTAL ABLATIONS ON DELAYED REACTIONS IN DOGS

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(Received 3 October 1958)

It has been firmly established that one of the most striking symptoms of prefrontal ablations in monkeys and apes is the disturbance of the delayed responses (Jacobsen 1936, Finan 1940, 1942, Malmo 1942, Wade 1947, Harlow et al. 1952, Pribram et al. 1952, Mishkin and Pribram 1956). Although the fact itself has never been put in doubt, its interpretation has evoked considerable controversy. While the first discoverer of this symptom, Jacobsen, attributed it to the impairment of recent memory (as contrasted with the preservation of permanent memory), other authors connected it with increased distractability of the animals following prefrontal ablation (Malmo 1942, Wade 1947, Harlow et al 1952), to hypermotility (Wade 1947), or to the impairment of associative functions (Nissen et al. 1938, Finan 1942).

In previous papers from this laboratory (Brutkowski et al. 1956, Brutkowski 1957, Ławicka 1957), it has been established that after limited lesions of the prefrontal areas in dogs (involving g. proreus and g. orbitalis), positive alimentary conditioned reflexes are fully preserved while inhibitory reflexes (both classical and instrumental) are greatly disturbed. This disturbance is not permanent, and after some postoperational training the inhibitory ability of the animals is partially, or even completely, restored. It has also been shown that some well known symptoms of prefrontal ablations found by other authors, e.g. the impairment

of difficult discriminations (Harlow and Speat 1943, Settlage, Zable and Harlow 1948) etc. can be easily explained by the lack of inhibition (cf. also the article of Stanley and Jaynes 1949).

The problem arose whether the impairment of delayed responses is also connected with the disturbance of inhibitory processes, or whether it constitutes a quite independent symptom. The aim of this paper is, first, to ascertain whether or not the small lesions of prefrontal areas in dogs will also produce the disturbances of delayed reactions, and, if so, what is the character of these disturbances and what may be their physiological mechanism.

MATERIAL AND METHOD

The experiments were performed on 4 dogs which have earlier (1—2 years ago) been subjected to prefrontal ablations. All these dogs had been used previously in experiments with conditioned reflexes and the symptom of disinhibition after prefrontal ablation was clearly seen in them. The behaviour of these dogs in the delayed response situation was carefully studied and compared with the behaviour of normal dogs described in part II of this series of papers (Ławicka 1959). In two other dogs, the experiments with delayed responses began before the prefrontal ablation, so that their behaviour before and after operation could be compared.

The experimental setting in the present study was exactly the same as that used in the study with normal dogs (Ławicka 1959). Buzzers and lamps fixed on the three foodtrays were used as preparatory stimuli, and unleashing served as the natural releasing stimulus. The length of the delay periods together with the effects of various disturbing factors were studied. After a careful investigation for over 1 year, the dogs were sacrificed. Histological examination of the brains is in progress.

RESULTS

The preliminary training of the prefrontal dogs did not differ from that of normal dogs. They very easily became accustomed to the experimental situation and learned to run to the appropriate foodtrays, first at the click of the moving bowl and then at the preparatory signal. As was observed in part II of this series, the normal animals experienced at the beginning of training some difficulties in locating the source of visual preparatory stimulus (lamp), and gradually learned to scan the foodtrays and see where the visual stimulus came from. The same phenomena were observed in our prefrontal dogs. They also learned to scan the foodtrays and

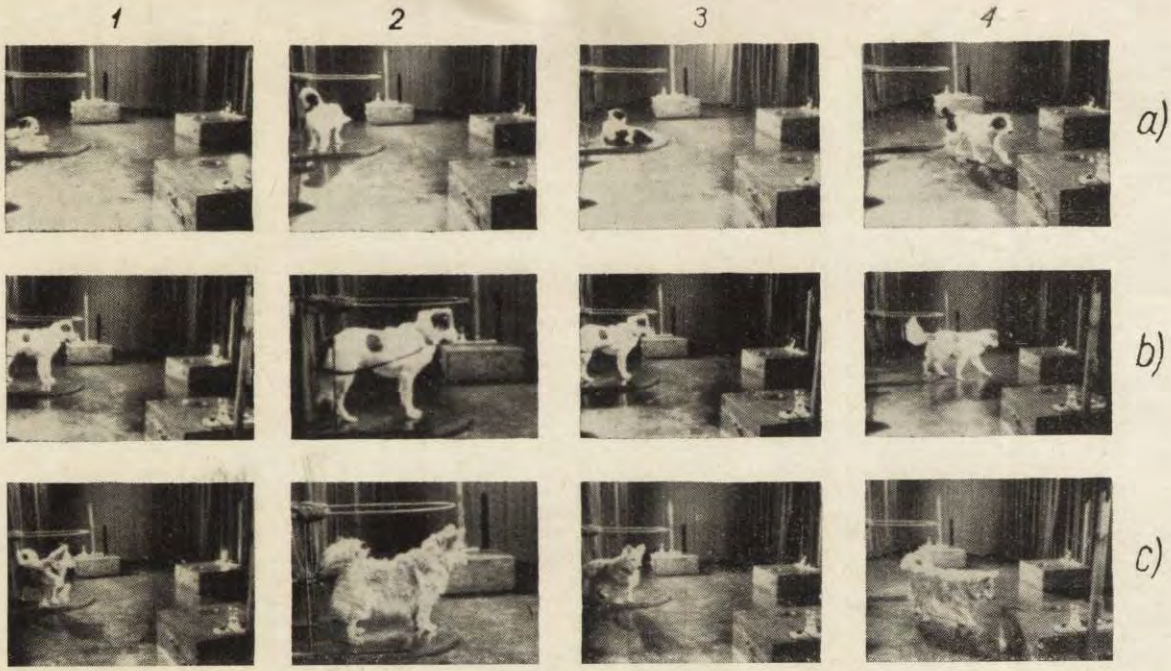

Fig. 1. The behaviour of dogs during the delay period, delay 3 minutes; a — the typical behaviour of normal dog; b and c — the typical behaviour of two prefrontal dogs

1 — the moment of the action of the preparatory stimulus — buzzer; the place of the buzzer is indicated by the lighting bulb: in a the right foodtray is signalled, in b and c, the middle one; 2 and 3 — various moments of the delay periods; in a normal dog behaves quite freely being turned in various directions; in b the dog is turned towards the signalled foodtray throughout the delay period; in c he is first turned to the proper foodtray (2) but then changes his bodily orientation (3); 4 — post-delay reaction: in a and b the dog goes to the proper foodtray, in c he goes to that to which he has been lastly turned

were in this respect not worse than the normal animals. The only difference between the operated and the normal dogs in the preliminary training was that some of the prefrontal dogs manifested an increased tendency to look into other foodtrays in search of food at the end of each trial.

The orienting reaction to the preparatory stimuli was in these dogs also quite normal, and if the delay was short they easily found their way to the proper foodtray. But when the delay periods were prolonged to a minute or more the striking defect characteristic of those animals was manifested. As shown in part II of this series, the normal dog behaves during the delay period quite freely, and finds his way to the proper foodtray independently of his bodily orientation. By contrast with the normal animals, the prefrontal dogs were able to go to the proper foodtray only if at the moment of release they were turned towards it. When during the delay period they had changed their bodily orientation, they went to that foodtray to which they were directed at that moment. In consequence, their performance was much worse than that of the normal dogs, and this was particularly clearly seen under conditions which caused a change in their bodily orientation (Fig. 1).

Length of delay

At the beginning of the experiments with delayed reactions, the prefrontal animals were very poor in their performance. When

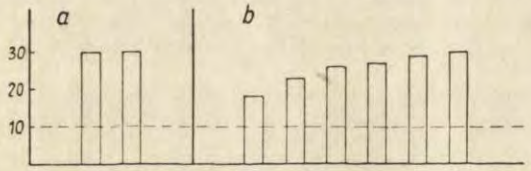

Fig. 2. Gradual improvement of delayed reaction in a prefrontal dog due to learning to keep the bodily orientation during the delay period unchanged. Delay 1 minute; the columns denote the numbers of correct responses in blocs of thirty trials a) before operation, b) after operation. Note the increasing numbers of correct responses in succesive blocks after operation

the animal was released immediately after the application of the preparatory stimulus, or after several seconds, his reactions was correct, but if the delay period was longer, the chance of changing

his posture increased and, therefore, he was more and more prone to make mistakes. It was observed, however, that in the course of experiments the animals gradually learned to keep their bodily orientation unchanged during the delay period and, therefore, they were able to react correctly even after longer delays (Fig. 2).

As has been stated elsewhere (B r u t k o w s k i et al. 1956), the dogs with limited prefrontal lesions (not extending beyond the presylvian gyrus) are never hyperactive. They are able, therefore, quite easily to preserve their bodily orientation even for a number of minutes. And so, those dogs who were generally more quiet, were able to react properly even if the delay period amounted to 6 minutes. On the other hand, those dogs who were generally more excited were less able to maintain their posture unchanged for

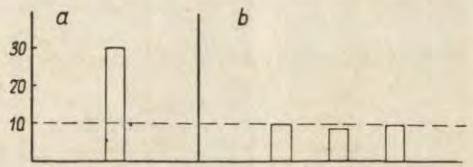

Fig. 3. The effects of disturbances (presentation of food during the delay period) before (a) and after (b) prefrontal ablation. Each column denotes the number of correct responses in blocs of thirty trials with 1 minute delay. Note the lack of any disturbance of the delayed response before operation and the chance level of the response after operation

several minutes and, therefore, they made more mistakes.

In those dogs trained to both visual and acoustic preparatory stimuli the difference between the reactions to these two sorts of stimuli was observed. Since the visual preparatory stimuli elicited in general a weaker orienting reaction than did the acoustic stimuli, the bodily orientation in the correct direction lasted for a shorter time, and in consequence the post-delay reactions to buzzers were better than those to lamps.

Disturbing factors

As indicated in part II of this series, all our normal dogs were exceedingly resistant to various sorts of distracting stimuli applied during the delay — screening, offering food on the starting platform, taking the dog out of the room, etc. The prefrontal dogs were in most cases unable to react properly after these distractions: they went to that foodtray to which they happened to be turned

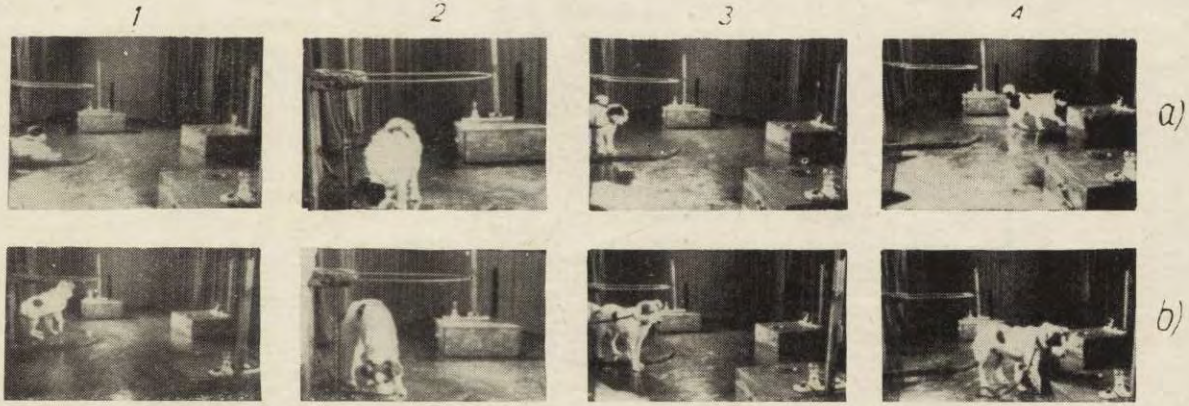

Fig. 4. The disturbance during the delay period: a, normal dog, by prefrontal dog

1 — the moment of the action of the preparatory stimulus; in a the middle foodtray is signalled, in b the left one; 2 — the dog receives food on the starting platform; 3 — the moment after food has been eaten; note that the prefrontal dog changes his position; 4 — the post-delay reaction: the normal dog goes to the proper foodtray, the prefrontal dog goes to the wrong one

at the moment of unleashing, or, sometimes, they remained on the starting platform and went nowhere. Only in those cases in which the distracting stimulus was weak and evoked no more than a turn of the animal's head without change of posture, was the attitude of the dog towards the given foodtray not disturbed, and his post-delay reaction correct (Fig. 3 and 4).

DISCUSSION

Our findings make it quite clear that in the delayed-response test there is a striking difference in the behaviour of normal and prefrontal dogs. This difference is not quantitative, i.e. it does not consist simply in the worse performance of animals after operations, but it is qualitative, since the prefrontal animals behave in a d i f f e r e n t way from the normal ones. As was seen in part II of this series, normal dogs (as well as cats) do not preserve the bodily orientation to the source of the preparatory stimulus during the delay period. It can be positively stated that the choice of the direction of their run does not, whether correct or incorrect, depend at all on the position held at the moment of release. On the other hand, it seems that in prefrontal dogs the only cue which determines the direction of the run is the bodily orientation, and that the correctness of the run depends on whether this orientation was preserved throughout the delay period. In consequence, all those distracting factors which cause a change in this orientation make the correct run of the prefrontal animal impossible. But if the animals are undistracted, they can preserve their bodily orientation unchanged even for many minutes, and hence they can quantitatively approximate the performance of normal animals.

Thus we conclude that prefrontal animals are guided in the delayed-response situation not by the trace of the preparatory stimulus but by the actual orientation of the body. Accordingly, we call the way in which they solve this task a p s e u d o-d e l a y e d reaction, as contrasted with the true delayed reaction shown by normal dogs. As already observed, the difference between the two modes of behaviour is so clear that there is no difficulty at all in distinguishing them.

It was explained in part I of this series that the true delayed response is based on the ability of the animal to preserve the traces of the preparatory stimulus, or rather of some aspect of it.

The physiological mechanism of these traces was thought to be the activity of reverberating chains of neurons, which continues after the cessation of the actual stimulus. The fact that in prefrontal dogs the ability to keep these traces is obliterated suggests that these reverberating chains of neurons are situated in the prefrontal area. Thus, after removal of this area the animal is able to react properly to the stimulus (either external or postural) during its action, but is lost when this stimulus is discontinued.

We think, therefore, that the original Jacobsen's view that prefrontal ablation destroys the recent memory of the animal is, in fact, correct — but subject to one essential reservation. In the delayed-response tests, we have to do only with recent memory traces of the direction determining „where to go" after the release. It does not follow from this that any other sorts of recent memory are also destroyed by prefrontal ablation.

The prefrontal area represents one of the so-called associative areas which is, topographically, situated just in front of the so-called premotor area. According to the data collected in this laboratory (Stępień et al. 1959) the premotor area (probably in connection with caudate nucleus) controls the gross bodily orientation in space, whereas the sensorimotor cortex is concerned with discrete motor reactions of limbs. It may, therefore, be supposed that the prefrontal association area represents an adjunct to the premotor cortex, and that its role is precisely to maintain the traces of its activity by means of reverberating circuits attached to it.

As observed at the beginning of this paper, the impairment of the delayed responses after prefrontal lobectomies has been explained by various authors by reference to certain other mechanisms — increased susceptibility to retroactive inhibition, hyperactivity, or a defect of associative function. It is clear that the defects in delayed responses found in our experiments cannot be attributed to any of these factors. Our dogs did not exhibit any tendency to an increased activity: they were able to stay motionless throughout the period of delay, which lasted several minutes. Neither did they display a very high distractibility: it was often observed that not too strong extra-stimuli failed to divert an animal's orienting reaction. Moreover, we observed no impairment of associative function in our dogs — they were as capable of learning new motor tricks as were normal dogs.

It is obvious that our prefrontal dogs seemed to perform the delay task much better than did the prefrontal monkeys used in experiments by other authors. This difference may be due to the following factors: First, in our method the foodtrays were situated at considerable distances from one another, while in the generally used Wisconsin Test Apparatus the sources of food are situated in close proximity. Secondly, as shown by Jacobsen, screening ordinarily used in such experiments represents an important distracting factor. Thirdly, the prefrontal ablations performed in monkeys and dogs are not quite homologous. In a subsequent paper we shall show that the more extensive prefrontal lesions in dogs, encroaching on the anterior sigmoid gyrus, still further deteriorate the performance of the delayed responses.

The problem arises why the prefrontal dogs stay motionless fixing the signalled foodtray even for several minutes while the normal animals behave quite freely during the delay period without any stable postural attitude. The fact that usually, in the early experiments, the animals make more errors with long delays than in later experiments suggests that they gradually l e a r n to preserve the bodily orientation for progressively longer periods. This learning is closely connected with their essential defect, because every changing of posture leads to a wrong run which is not reinforced by food.

In previous papers from this laboratory concerning the effects of prefrontal ablation it was shown that one of the most striking symptoms of this operation is the disinhibition of the inhibitory alimentary conditioned reflexes. This symptom is usually transient, and with lapse of time the inhibitory ability of the animals is gradually restored. The symptom described in this paper is, on the contrary, permanent: Even after a long period of experimentation, the animals were not able to perform the true delayed reaction. Whether or not these two symptoms — impairment of inhibitory processes and loss of the ability to perform true delayed responses — are functionally or topographically interconnected is a matter for further research.

SUMMARY

1. An investigation of the performance of dogs with limited prefrontal lesions (involving g. proreus and orbitalis) in the triple delayed-response test is presented.

2. It is shown that the prefrontal animals are able to go to the correct foodtray in the post-delay run only if they have, throughout the delay period, preserved their bodily orientation towards that foodtray. If during this period the bodily orientation has been changed, they go, on release, to that foodtray to which they are immediately turned.

3. The animals are able to learn to keep their bodily orientation unchanged during the delay periods, and thus their performance in the course of experimentation gradually improves.

4. All those distracting stimuli which provoke a sufficiently strong orientation reaction to change the animal's posture inevitably disturb the post-delay reaction: after release, the animal runs in the direction which was imposed on him by the distracting stimulus.

5. The mechanism of the impairment of the delayed-response performance in prefrontal dogs is discussed.

We should like to thank Prof. L. Stępień very much for performing operations in dogs used in this study.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

INSTRUMENTAL SCRATCH REFLEX OF THE DEAFFERENTATED LIMB IN CATS AND RATS

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(Received 7 October 1958)

All investigations on the problem whether animals are able to perform instrumental movements with the deafferentated limb have so far dealt only with natural and simple reactions that can be performed without any special training. These consisted of such movements as stretching out the limb for food, grasping the food and conveying it to the mouth (Mott and Sherrington 1895, Kopczyński 1907, Twitchell 1954), removing a source of nociceptive stimuli (Twitchell 1954) etc. It has been established that the deafferentated limb was not used for the performance of such movements. The animals tried to reach their aim by using the head or other limbs, even when they were tied up and the deafferentated limb was the only free one.

Incidental observations have shown, however, that the performance with the deafferentated limb of some even complicated movements, like grasping, is sometimes possible (Lassek 1953, Lassek and Moyer 1953, Mott and Sherington 1895). Such movements were observed when the animal was very excited, e.g. while fighting or running away.

Unlike other authors, we used in our experiments not the "natural" instrumental reflexes, formed in ordinary life of the animal but the "artificial" reflexes obtained by special training. As in cats and rats the deafferentation of the hindlimb is the easiest to perform, we used the movements of this limb. We have chosen the scratch movements which, as our previous experiments have shown,

can be easily instrumentalized, i.e. the animal can be trained to perform the scratch movements in order to get food*.

In the experiments described here we tried to find out: 1° whether it is possible to perform with the deafferentated limb an instrumental scratch reflex established before operation and 2° whether the establishment of such a reflex is possible after deafferentation. Both these questions have been answered affirmatively.

MATERIAL AND METHODS

15 cats and 15 rats were used in the experiments. In 10 cats and 10 rats the instrumental scratch reflex was established before deafferentation. 5 cats and 5 rats were trained after operation. On 11 cats and on all rats unilateral deafferentation of the hindlimb was performed (more or less extensive) and on 4 cats the bilateral one. The kind of training and number of dorsal roots cut in different animals are shown in Table I.

Table I

Extent of deafferentation and time of training

Object	Extent of deafferentation	Number of animals	
		trained before deafferentation	trained after deafferentation
Cats	$L_4 - S_4$	2	
	$L_3 - S_4$	4	1
	$L_2 - S_4$	2*	4**
	$L_1 - S_4$	1	
	$Th_{13} - S_4$	1	
Cats	$Th_{13} - L_6$	1	
	$Th_{12} - L_6$	8	5
	$Th_{11} - L_6$	1	

* Bilateral deafferentation.
** Bilateral deafferentation in 2 cats.

In the experiments on the effects of deafferentation upon a previously established instrumental scratch reflex, the preoperative training lasted till the conditioned reflex was firmly established, usually for 2—5 months (30—50 experimental days). After the operation the reflex was followed up for further 2—6 months, usually once or twice a week.

* The properties of the instrumental scratch reflex will be described in another paper.

In the experiments in which the instrumental scratch reflex was trained after the deafferentation, the experimentally naive animals were first operated on and then, as soon as they were just able to walk (about 3 weeks after the operation in cats, and about 1—2 weeks in rats) the training was begun.

More detailed data concerning the experimental procedure will be given together with the results.

Operative technique

The deafferentation on cats was performed under asceptic conditions in nembutal anaesthesia (40—50 mg/kg of the body weight). The spinal card was exposed from the level of L_7 upwards. The dorsal roots were cut intradurally (between the spinal cord and spinal ganglion). All the sacral roots were cut on the level of L_6—L_7. The edge of the dura matter from the side where the dorsal roots were cut, was sutured to the muscles of the opposite side. Then the muscles, fascia and skin were sutured in layers.

The deafferentation on rats was performed in non aseptic conditions, under uretan anaesthesia (1—1.2 g/kg of the body weight). The spinal cord was exposed from the level of L_4 to Th_{12} or Th_{11} and all the dorsal roots of this area were cut. The dura mater was not sutured. The muscles, fascia and skin were sutured in layers.

The general state of animals after deafferentation

We observed in our animals a number of symptoms identical with those found in earlier works on deafferentation. These symptoms are described here in order to give a fuller characteristic of the animals.

1. The posture of the deafferentated limb after unilateral and bilateral operation

We observed, in accordance with many authors (Bickel 1897, Ranson 1928, Ranson, Hinsey and Taylor 1929, Hnik 1956) the tendency to extension of the deafferentated limbs in both cats and rats. After the unilateral operation the animals, while sitting, usually kept the deafferentated limb stretched forward and in adduction. After the bilateral operation the cats kept both their deafferentated limbs either stretched to one side (they were then sitting on one buttock), or else stretched backwards in the kneeling position (Bickel 1897).

2. Walking

After unilateral deafferentation cats began walking in 1—2 weeks and rats in 2—3 days. In the beginning the cats walked only on three legs, dragging the fourth one behind and only occasionally performing with it

some irregular movements. In 3—5 weeks the movements of the deafferentated limb became more and more coordinated with those of other limbs. The animals were able to support themselves on this limb, stepping on the dorsal side of the toes, but also sometimes normally. The rats used the deafferentated limb from the very beginning. For the first few days they put it in the normal position, later on they dragged it behind them, most frequently on the dorsal side of toes, and flexed it rhythmically. After the bilateral deafferentation cats started to move in the box after 2—3 weeks.

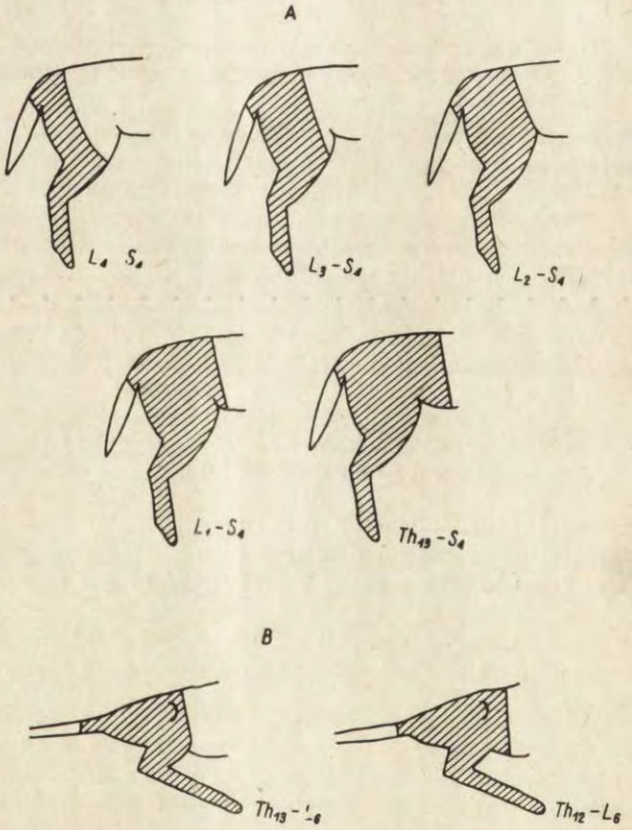

Fig. 1. Area anaesthetized after section of dorsal roots
A — Cats (reconstuction from Klessens 1914); B — (determined by pricking)

In the beginning, using only the forelimbs, they dragged along the whole posterior part of their body which was lying on one side. After 4—5 weeks the manner of walking was changed. Now they could stretch out both their hindlimbs behind them in the kneeling position, support the body weight on them and perform slight flexions.

3. Cutaneous sensation

In accordance with Klessens (1914) we have observed that section of the dorsal roots from S_4 to L_4, L_3, L_2 and L_1 respectively, results in the complete loss of sensation (reaction on pricking) on the surface of foot and leg, and partial or complete on the surface of thigh according to the extent of deafferentation. This is shown on the schematic pictures in Fig. 1A. The loss of the cutaneous sensation in rats is illustrated in Fig. 1B.

4. Trophic changes

Trophic skin changes, ulcers etc. were observed only in some animals. They were chiefly the result of mechanical damage, such as the chafening of leg while walking. They were absent in animals kept in cages with chips on the floor.

5. Some other anomalies

In some cats and in about 30% of rats biting all over the deafferentated limbs took place. In cats it usually happened some months after the operation, and in rats it appeared in the first week or in some weeks or months after deafferentation.

After the more extensive deafferentation (from L_3, L_2, L_1 or Th_{13}) a very distinct curving and twisting of the spine towards the normal limb was observed in cats. In some cases this made even walking on 3 legs impossible because the normal hindleg could not touch the floor.

Another factor which made the experiments with bilaterally deafferentated cats very difficult were disturbances in digestion, in the urination and defecation.

6. The scratch reflex of the deafferentated limb (unconditioned)

In accordance with Sherrington's (1906) observations on the spinal cats and dogs and Lassek's (1953) chronic experiments on apes, we noted the presence of rhythmic scratch movements performed by the deafferentated limb. In practically all our animals they could be very regularly evoked by putting a bit of cotton wool into the ear on the side of the deafferentated limb. They appeared, however, with greater difficulty than similar movements of the normal limb. There were longer latent periods, and animals tried to remove the cotton wool, first performing other movements, such as shaking or rubbing the ear with the forelimb. The scratch movements were also very easily inhibited both by the alimentary reactions (very distinctly in cats) and by the defensive ones (particularly in rats). The scratch movements of the deafferentated limb were performed only in the air without touching the skin, although the animal assumed the appropriate position for reaching the stimulated region. The movements were rhythmic and their amplitude, frequency and strength were not very different from those in the normal scratch reflexes. All joints were involved in these movements.

7. Other movements of the deafferentated limb

A very distinct crossed extensor reflex of the deafferentated limb was present (Bremer 1928, Hnik, 1956, Ranson, Hinsey and Taylor 1929).

No respiratory movements described by Orbeli and Kunstman (1924) were seen, except in one cat, where they appeared only in the first three weeks after bilateral deafferentation.

RESULTS

The effect of deafferentation on the instrumental scratch reflex established in the preoperative period

1. Establishment and fixation of the instrumental scratch reflex in normal animals

Cats. The instrumental scratch reflex was established by reinforcement of the unconditioned scratch movements. These were more frequently evoked by the experimenter, but reinforcement of spontaneous scratch movements was also used. In order to evoke

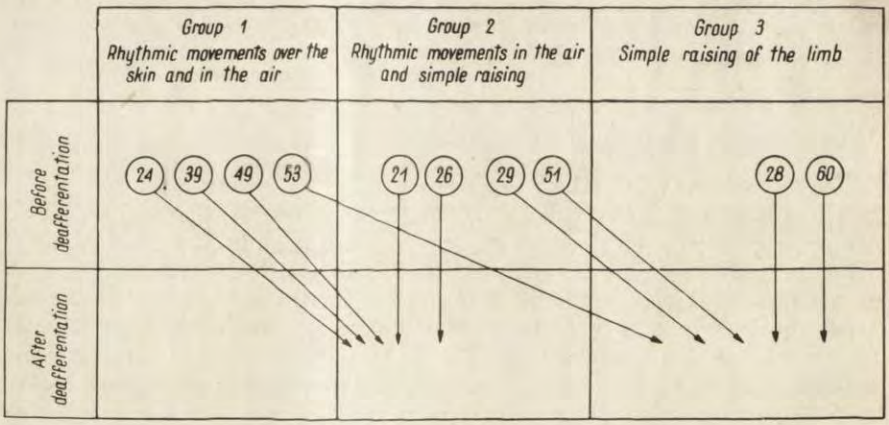

Fig. 2. Changes of character of instrumental movements after deafferentation. In circles there are shown numbers of cats

scratching a bit of cotton wool was put into the right ear of animal, and was left there for a few minutes, unless it was removed earlier by the cat. A piece of boiled horse meat was used as a reinforcement. During the training, from the 2nd day onwards, each

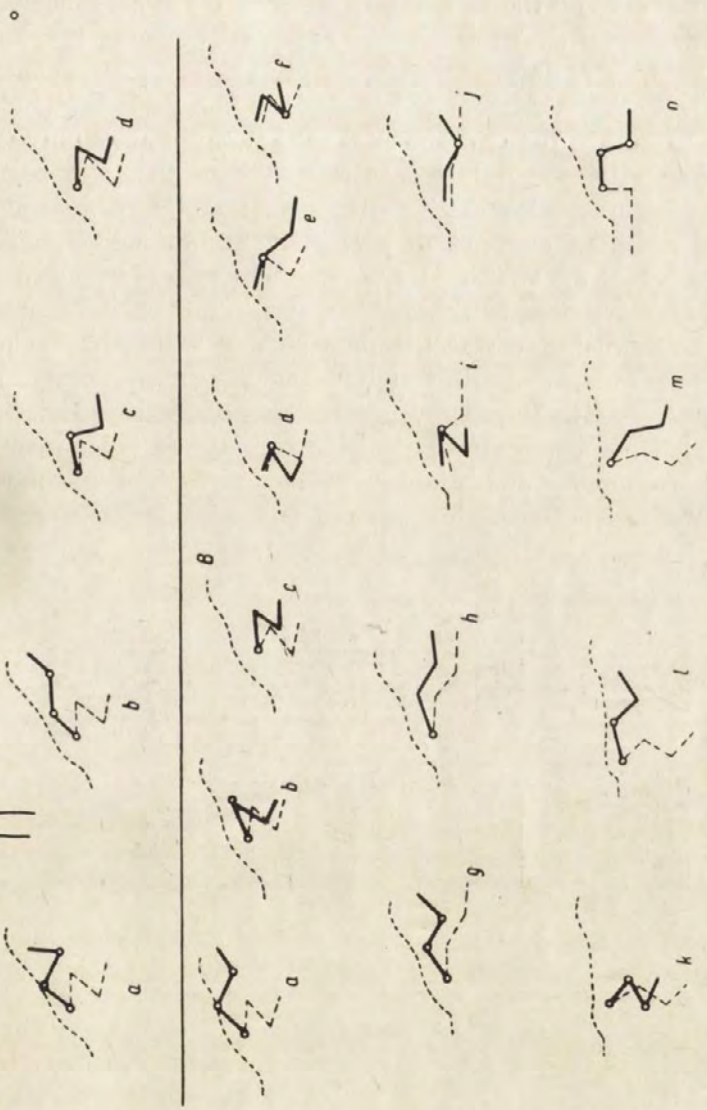

Fig. 3. Conditioned movements before (A) and after (B) deafferentation of the hind limb. Dashed line shows the outline of cats back and the starting position of the limb; continuous — maximal displacement of the limb during movement; circles — joints involved in the movement

experimental session was subdivided into 3 periods, each lasting about 5 minutes. The cotton wool was in the ear during the middle period only. At the beginning and at the end there was no cotton wool in the ear and the animal was watched for appearance of spontaneous movements. They usually appeared between the 2nd and 10th experimental days. During some preliminary experiments these scratch movements without cotton wool in the ear were performed only at the end of the experimental session, and it was only in later experiments that they started to appear from the very beginning. As the instrumental scratch reflex was established to the experimental situation and not to a sporadic conditioned stimulus, so in the course of the training the scratch movements began to appear more and more frequently and regularly immediately after the animal had finished eating the food portion which had reinforced its previous movements. But at the same time they became gradually weaker and weaker. Only in some cats were the normal rhythmic scratch movements over the skin (usually of lesser amplitude, strength frequency and number of beats than in unconditioned scratch reflex) preserved during the whole period of training. In the majority of cats the normal scratch movements were replaced

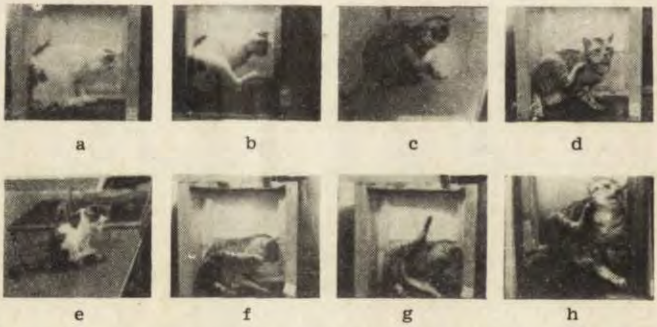

Fig. 4. Examples of conditioned movements performed before (a—d) and after (e—h) deafferentation

by movements of raising the limb (as for scratching) with or without slight rhythmic throbs. The same has been observed by F e d o r o w (1955). Figure 3a — d shows in a schematic manner typical conditioned movements performed by normal cats. Examples of such movements are given on photographs a — d Fig. 4. Figure 2 shows which of these movements were most frequently performed by those cats which were to be deafferentated later on.

Rats. The training of the instrumental scratch reflex in rats was similar to that in cats except that sweetened milk (about 1 ml) was given as reinforcement. The conditioned movements appeared usually at the same time as in cats. The quality of the conditioned scratch movements was however better in rats, if by better movements we mean movements more similar to the unconditioned ones. Scratch movements over the skin were preserved in all rats during the whole period of training, though sometimes they performed also the scratch movements in the air or simply raised the limb. The conditioned scratch movements were weaker, of lesser amplitude, frequency and number than the unconditioned ones. But the most important difference was that, even when an animal performed very strong conditioned scratch movements over the skin, these were never followed by biting or licking the nails what usually happened at the end of the unconditioned scratch reflex.

2. The instrumental scratch reflex after deafferentation

The results of experiments both on cats and rats have shown consistently that deafferentation does not result in the loss of a previously established instrumental scratch reflex. In the experimental situation all the animals in which deafferentation was successfully performed executed the movements analogous to those of the preoperative period (though in a somewhat changed form), appearing with the same regularity and at the same time after the end of eating (Table II). In spite of frequent repetition the movements did not decay. They were preserved on the same level throughout all the period of observation.

Cats. Deafferentation caused a change in relative frequency of different kinds of instrumental movements (rhythmic and single beats). The animals raised the limb without any rhythmic component more frequently than before the operation. Usually animals changed from the group they belonged to (see Fig. 2) and passed to a worse group, i.e. to a group performing movements less similar to the unconditioned ones.

Moreover after the operation the quality of movements was deteriorated. The conditioned scratch movements, as well as unconditioned ones, were executed only in the air. The character of the

movements was also changed and often not all joints but only some of them were involved. Fig. 3 shows schematically the conditioned movements most frequently performed. In Fig. 4 examples of some of these movements are given. It is striking that the conditioned movements of the deafferentated limb are very often performed only in one joint, and that they may consist either in flexion or in extension. This is most distinctly seen in the movements of the knee joint.

Table II

Number of movements performed in 1 minute before and after deafferentation

Cat No	Before deafferentation	After deafferentation	
		initial period	later period
24	7.66	5.30	12.09
26	5.25	5.08	4.77
28	3.45	2.56	2.25
29	6.28	3.55	6.00
39	6.66	1.92	4.07
49	2.88	1.65	2.32
53	4.30	4.92	5.80
Mean	5.21	3.44	5.33

Means from 5 experiments before deafferentation, 5 experiments just after deafferentation and 5 at a later stage.

For an outside observer some of the movements performed by the deafferentated animals had very little, if anything, in common with the scratch movements. But systematic observation suggests that they are the residual instrumental scratch movements and we treated them as such on the grounds that: 1) scratch movements in a simplified form, as single, often abortive flexions, appeared also in the normal cats; the movements of the deafferentated limb were sometimes very similar to these abortive movements; 2) the movements under consideration appeared only in the experimental situation and with the same regularity as the doubtless instrumental scratch movements before operation; 3) the abortive movements of the same character appeared repeatedly in many cats; 4) the weakest and most questionable movements alternated with stronger ones that were more similar to the unconditioned scratching. The same was observed in normal cats in which the movements

of raising the limb alternated with scratch movements in the air or over the skin.

The character of movements performed after the operation depended, as in normal animals, on the starting position of the limb. This is seen in extreme form in the examples of movements *a* and *n* or *c* and *h* in Fig. 3. In each pair, the same kinds of movements were performed in the same joint, but the final result was different because their starting positions were not the same. The quality of the movements also depended, as in the preoperative period, on the state of animals. When more hungry they performed stronger movements, involving a greater number of joints and more often of rhythmic character.

No dependence between the degree of worsening of the movements and the extent of the deafferentation and its uni- or bilaterality was observed. Both after less and more extensive deafferentation some cats performed relatively very good movements and some others rather bad. Besides deafferentation there are, however, many other factors which could influence the worsening of the movements. Among the most important are undoubtedly: the tendency to keep the limb in extension, which made flexion more difficult and the curving of the spine impeding the proper posture of the body.

Rats. In all rats after deafferentation both the movements of raising the limb and the rhythmic scratch movements in the air were present. Both were, in comparison with those in cats, much more similar to the unconditioned scratch movements performed with the deafferentated limb and to the conditioned movements of the preoperative period.

The establishment of the instrumental scratch reflex in animals after deafferentation

The training of the instrumental scratch reflex in deafferentated animals was similar to that in normal ones. Some differences in procedure were due to the fact that after deafferentation the unconditioned scratch reflex is more difficult to evoke and can be easily inhibited.

Cats. In deafferentated cats, as in normal ones, the training was started by evoking the unconditioned scratch movements. For

this purpose cotton wool was put into the ear on the side of the deafferentated limb. The cotton wool was not, however, left in the ear for long, but was taken off immediately the animal performed the scratch movements. As the scratch movements appeared somewhat irregularly (with different and sometimes very long latent periods) and could be easily inhibited by alimentary reaction, they were not reinforced by food for the first few (8—10) days of training. Such was the preliminary training. The training proper, consisting in the reinforcement of the scratch movements, started only when these movements began to appear regularly some seconds after cotton wool was put into the ear. 5 cats were trained in this way (3 after unilateral deafferentation and 2 after the bilateral one).

In order to establish conditioned scratching it is important to follow this procedure. When a different training method was tried in 3 cats it did not succeed. They were trained before the described procedure was worked out, and had the scratch movements reinforced from the very begining of training. During an experimental session 10—20 movements were reinforced. In consequence, on the 2 nd or 3rd day of training a very strong alimentary reaction appeared in the experimental situation. It made the evoking of any unconditioned scratch movements impossible, and further training had to be discontinued.

In 2 cats training proved impossible, because the scratch movements could not be evoked at all (even during the preliminary training) or else were very irregular.

The course of training for all 5 cats in which the instrumental scratch reflex was established was practically the same both after unilateral and bilateral deafferentation. The first scratch movements without cotton wool (after its removal) appeared in different cats between the 1st and 5th experimental days. They began to appear regularly in 4 cats from the very beginning of the experimental session, without cotton wool having been previously put in the ear between the 3rd and 10th day. In one cat it was always necessary first to stimulate the ear using cotton wool or pressing pressing the external side of the concha.

At the beginning all movements when the cotton wool was in the ear as well as those without it, were similar to the unconditioned scratch movements. Then, in both circumstances, single move-

ments of raising the limb and the abortive flexions in different joints, such as are shown on Fig. 3 and 4, began to appear. In about two weeks from the start of the proper training the instrumental movements had taken on a definite pattern with regard to their quality and the time at which they were performed after the end of eating. This pattern was maintained without pronounced changes troughout all the period of experiments. The degree of worsening of the instrumental movements during training was similar to that observed in normal animals. The final state of the reflex was also similar.

Rats. 5 rats were used for these experiments. Cotton wool was not put in their ears because of a strong defensive reaction preventing the appearance of the scratch movements. This reaction was more pronounced than in normal animals. The only way to establish the instrumental scratch reflex was to reinforce the spontaneous scratch movements, which appeared from time to time. The conditioned scratch movements performed regularly immediately after the end of drinking, appeared after a few reinforcements of the spontaneous movements, during the same experimental session or on the following day. During further experiments the scratch movements gradually underwent the same change as in normal animals and the final state of the reflex, with regard to quality of movements and their regularity, was similar to that of deafferentated rats trained before deafferentation.

DISCUSSION

The results of experiments described in this paper show that an animal is able to perform with the deafferentated limb the instrumental scratch movements which were trained before deafferentation. He is also able to form new reflexes after deafferentation. Thus using the instrumental scratch reflex we have got different results from those described by earlier workers who observed an almost complete loss of instrumental movements after deafferentation.

Before analysing the possible reasons of this discrepancy we must say a few words about the criterion by which the occurrence of instrumental movements of the deafferentated limb was judged in the present study. It did not matter in our experiments whether

the movements performed attained a degree of perfection and skill necessary for efficient work. Our criterion was the presence of movements either identical with or similar to those performed by normal animal in the same situation, i.e. movements of similar character and affecting the same joints but without taking into account the strength or skill of the reaction.

It is possible that if the other authors had used such criterion they would formulate their statements about the loss of instrumental movements in not so peremptory way and would agree that some elements of these movements could be detected in the reflexes they investigated. Should this be the case the difference between our data and those of earlier authors would not be so great.

In looking for the cause of difference between the fairly good state of instrumental scratch reflex of deafferentated limbs as compared with the marked impairement of reflexes investigated by previous authors, several possibilities have to be considered. The unconditioned scratching is independent of the sensory information from the limb. So it is possible that the conditioned scratch reflex can also do without such information. If so, this peculiar property of the scratch reflex would explain the difference between the present results and those of other workers. But at the same time it would make necessary a more detailed examination of conditioned connections of this reflex. According to the theory of the instrumental conditioned reflexes put forward by Konorski and Miller (1933, 1936) information about performance of a movement is playing an essential part in the establishment of these reflexes and in the execution of learned movements. If this information is indispensable in all instrumental reflexes except the instrumental scratch reflex this would suggest a different mechanism of this latter. Some experiments performed in this laboratory suggest, however, that other reflexes may resemble in this respect the instrumental scratch reflex. Should this be the cause of divergence ought to be sought elsewhere.

SUMMARY

It has been shown that an instrumental scratch-reflex, established in normal cats and rats, is not abolished by the deafferentation of the limb. It can be also established in deafferentated

animal which have not been trained before operation. Some explanations of these facts have been discussed.

I wish to thank Mgr. Teresa Górska for carrying some of the experiments in rats and for help in preparing this paper. I am also very indebted to Prof. Jerzy Konorski for his valuable criticism and advices and to Prof. Lucjan Stępień for performance of some operations.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

ON THE CONDITIONED REFLEX OF THE CESSATION OF THE ACT OF EATING
III. EXTINCTION OF THE CONDITIONED CESSATION REFLEX

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In the previous papers of this series the conditioned cessation reflex was described and analysed (A. W. Zbrożyna 1958). This reflex develops when the indifferent stimulus is applied during the act of eating and is followed by the withdrawal of food. It consists in the animal discontinuing the act of eating to the stimulus itself. It was further shown that the cessation reflex can be differentiated if a stimulus similar to the conditioned cessation stimulus is not reinforced by the withdrawal of food.

The present paper is concerned with the problem of extinction of the conditioned cessation reflex which should occur, when the stimulus continues to be applied during the act of eating but the reinforcement — withdrawal of food is discontinued.

PROCEDURE

Four dogs used in our previous experiments (A. W. Zbrożyna, 1958), in which the conditioned cessation of eating was established were submitted to the extinction series. In this series the conditioned cessation stimulus was applied during the act of eating without reinforcement i.e. the animal was allowed to continue the meal during and after the operation of the stimulus.

RESULTS

Dog "Burek". The conditioned cessation of eating in this dog was well established (A. W. Zbrożyna 1958). The conditioned cessation stimulus was the buzzing of the loudspeaker. As it is shown in Fig. 1, the first effects of the extinction procedure began to appear in this dog after 4 experiments comprising 16 trials of the cessation stimulus nonreinforced by the withdrawal of food. These effects were: in some trials the dog did not interrupt the eating during the action of the stimulus, at the same time a new phenomenon was observed, namely the dog sometimes interrupted the eating spontaneously without stimulus. It is worth noticing

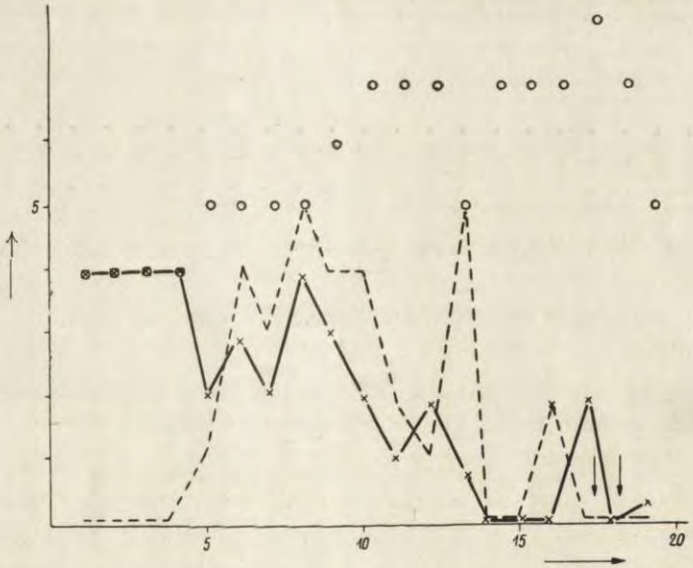

Fig. 1. The extinction of the conditioned cessation reflex in dog "Burek" Ordinates — the number of trials, Abscissae — successive experimental days. Circles — number of conditioned cessation stimuli applied (buzzing of a loudspeaker). Constinuous line — number of effective cessation stimuli. Broken line — number of spontaneous interruptions in the act of eating. Arrows denote the experiments in which the conditioned cessation stimulus was applied before the presentation of food and prolonged during the act of eating.

that the same phenomenon was seen in the early period of establishing the cessation reflex. These spontaneous interruptions appeared very often during the first stage of this series, and disappeared completely when the cessation reflex to the buzzing was

well extinguished. The full extinction of the conditioned cessation reflex to the buzzing was obtained after 13 experimental days consisting of 70 nonreinforced trials of the buzzing. At the end of this series the buzzing was applied in an intertrial interval, the food presented during the operation of this stimulus and its action was prolonged up to the end of the act of eating. In this situation the conditioned cessation reflex proved to be disinhibited. This disinhibition appeared only in one such trial (the first arrow in Fig. 1.).

Dog "As". The conditioned cessation reflex was in this dog well established (A. W. Zbrożyna 1958). In the first experiments with extinction the dog withdrew from food immediately at the beginning of the action of the conditioned cessation stimulus and returned to the meal some seconds after discontinuation of the stimulus. After 40 experiments comprising 92 extinction trials of the cessation conditioned reflex the reaction of the dog did not undergo any change: the animal withdrew from the foodtray immediately after application of the stimulus during the meal and resumed eating when the stimulus was switched off (Fig. 2.) The prolongation of the action of the conditioned cessa-

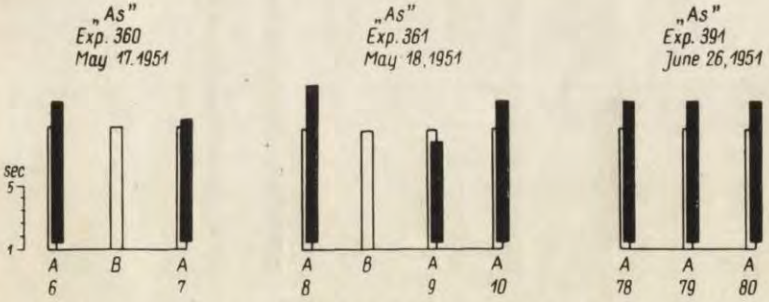

Fig. 2. The extinction of the conditioned cessation reflex in dog "As"
The white columns represent the action of stimulus during the act of eating, The black columns represent the duration of the pause in the meal. A, the conditioned cessation stimulus, B, differentiated stimulus. Figures under the stimuli symbols denote the total number of trials of the conditioned cessation stimulus applied in the extinction series. Notice that in the 391th experiment, after 77 trials of the conditioned cessation stimulus in the extinction series the cessation reflex is completely undisturbed.

tion stimulus was then examined. The stimulus was applied for 45 to 75 sec. In most cases the animal withdrew from the bowl in the firsts seconds of the stimulus and refused to eat as long as it was in operation (Fig. 3. Exp. 402). In other trials however the

dog hesitated and after some seconds of interruption in eating he returned, had some gulps and withdrew again (Fig. 3. Exp. 400). In the last experiments of this series the conditioned cessation stimulus was applied repeatedly during the same act of eating.

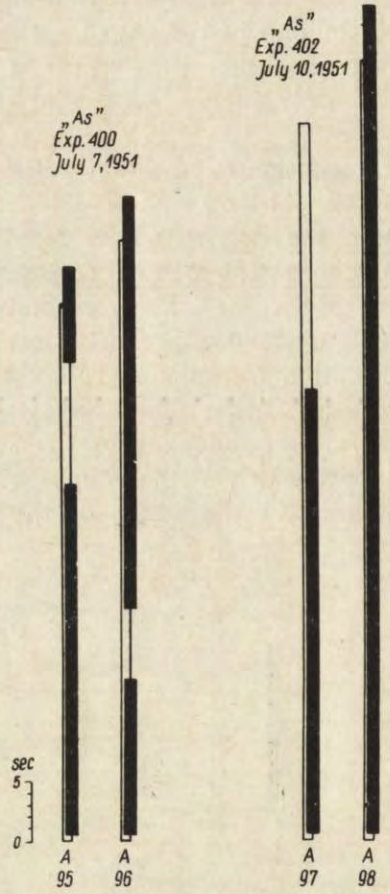

Fig. 3. The effect of prolonged operation of the cessation stimulus tested at the end of the extinction series in dog "As"

The white columns represent the action of the stimulus during the act of eating, the black columns represent the duration of the pause in the meal. A, the conditioned cessation stimulus. The figures under the stimulus symbol denote the total number of trials of the cessation stimulus applied in described exinction series.

The periods of operation of the stimulus and the intervals were from 5 to 10 seconds (Fig. 4. Exp. 404, 405). In this situation only the first two applications of the stimulus were effective, then the

cessation reflex was acutely extinguished but reappeared in the next act of eating.

Dog "Kiel" The conditioned cessation of eating was in this dog irregular (see A. W. Zbrożyna 1958). The stimulus used as a withdrawal signal was the buzzing of a loudspeaker. Complete extinction of the conditioned cessation reflex occurred in this dog during one experimental session. Then in 13 trials the action of the conditioned cessation stimulus was examined during the meal. It was found that the dog did not react in any visible way to the action of this stimulus.

Dog "Kacper". In this dog the extinction of the conditioned cessation reflex occurred as the result of four experiments in

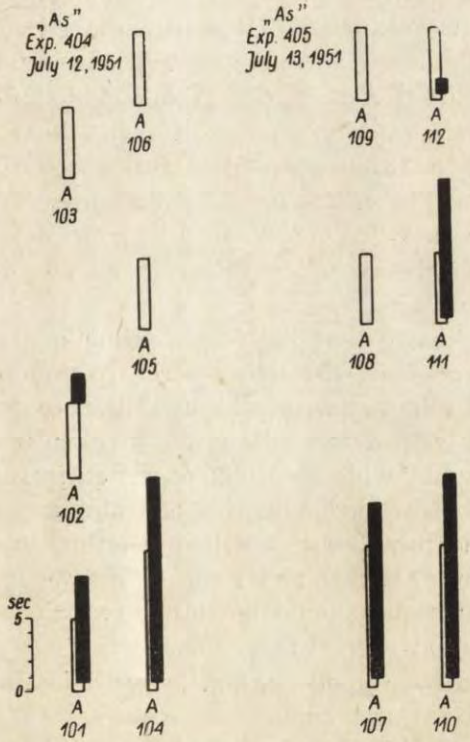

Fig. 4. The effect of repeated application of the cessation stimulus during the same act of eating in dog "As"

These experiments were performed at the end of extinction series. The white columns represnet the duration of the contitioned cessation stimulus, the black columns represent the pauses in the meal. The columns in each vertical line represent the stimuli action and pauses in one act of eating. A, the conditioned cessation stimulus, the figures under the stimulus symbol denote the total number of trials of the conditioned cessation stimulus applied in the described extinction series.

which the conditioned cessation stimulus (bubbling of water) was applied five times without reinforcing by withdrawal of food. Thereafter a series of 20 experiments comprising 30 trials of the conditioned cessation stimulus with reinforcement by withdrawal of food was carried out. In spite of this retraining the conditioned cessation reflex did not reappear: the animal did not discontinue eating as it did before to the action of the stimulus, but ate right up to the moment of withdrawal of food.

DISCUSSION AND CONCLUSIONS

In four dogs after the conditioned cessation of eating had been established extinction of this reflex was carried out. In one animal the conditioned cessation reflex remained quite unaffected despite over a 100 extinction trials. In another dog this reflex was eventually extinguished but only after a long extinction series. On the other hand in two other dogs the cessation reflex was extinguished extremely rapidly. In one of them the extinction was so strong that the conditioned cessation reflex could not be restored. As the resistance to extinction is a measure of the strength of a conditioned reflex this means that in spite of more or less the same training of the cessation reflex in all our dogs its firmness was very different.

It must be stressed that the term „extinction" is rather unsuitable to the procedure described here. In our experiments we had to do with elimination of the inhibition of the act of eating: the animal was trained to continue the meal in spite of the operation of the stimulus, which had before the extinction series signalled the withdrawal of food. This sort of training is similar to transformation of the inhibitory conditioned reflex into the excitatory one described by J. Konorski and G. Szwejkowska (1952). The term „extinction" should be rather reserved for process consisting in inhibition of excitatory conditioned reflexes.

According to the hypothesis put forward by the author (A. W. Zbrożyna 1958) the suppression of the conditioned cessation reflex obtained by the procedure described here could be considered as the result of the formation of new connections between the stimulus center and the positive alimentary centers (in lateral hypothalamic region). In this way the new established connections may counterbalance those previously developed with the inhibitory alimentary center (nucleus hypothalami ventromedialis).

SUMMARY

In this paper the course of extinction of the conditioned cessation of eating in dogs is described.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

THE EFFECT OF SENSORY CORTEX ABLATIONS ON INSTRUMENTAL (TYPE II) CONDITIONED REFLEXES IN DOGS

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From the time of Hitzig and Fritsch it has been well known that lesions localized in the region now referred to as the sensori-motor cortex produce an impairment in the so-called voluntary movements. The motor behaviour of animals becomes inefficient, and after extensive lesions the skilful movements are totaly lost.

These conclusions have been principally based on general observations of "natural" behaviour of animals. There are only a few papers concerned with the analysis of particular motor reactions established in instrumental conditioning.

The present paper is concerned with the question of the effects of lesions in the sensory area of the cerebral cortex on the instrumental conditioned reflexes.

The general experimental technique employed was such that in animals, instrumental (type II) alimentary conditioned reflexes were firmly established and then the sensori-motor area, or particular parts of it, were removed. After the operation, the conditioned-reflex activity of the animals was carefully tested over a long period.

Although there are numerous data concerning the ablations of sensory cortex on tactile sensibility (Woolsey and Bard 1936; Kennard and Kesler 1940; Peele 1944; Ruch and Kasdon 1943), on the ability to discriminate weights (Ruch 1935; Ruch, Fulton and German 1938), texture of rough surfaces (Zubek 1951, 1952) information concerning the effects of such ablations on motor activity is scarce. It was shown that partial

ablations on the sensory cortex in monkeys cause impairment of the ability to discriminate by palpation between different three-dimensional solids (Ruch 1935; Cole 1952; Cole and Glees 1951, 1954). Skilled movements remain however almost unchanged after such lesions (Pinto Hamuy 1956). The ablations of the sensory areas 3, 1 and 2 cause temporary disuse of hands and a persistent slowness of movements (Cole and Glees 1951). After partial ablations of the sensory area, the instrumental conditioned reflex remains entirely unaltered (Whatmore and Kleitman 1946). It was shown also that bilateral removal of both somatic areas in dogs and monkeys abolishes completely inhibitory tactile conditioned reflexes (differentiation) while the positive conditioned reflexes remain almost normal (Ruch, Fulton and Kasdon 1937; Allen 1947). According to Rosental (1938), Allen (1947), Abuładze and Rozental (1948), Adrianov (1953), the instrumental conditioned reflexes can be established in dogs after bilateral ablations of the sensory cortex.

MATERIAL AND METHODS

The experiments were performed on 9 mongrel dogs 2—4 years of age, weighing about 10—20 kg. In all dogs the instrumental conditioned reflexes (type II) were established. They consisted in the animal putting his right foreleg on the foodtray in front of him at the sound of various acoustic conditioned stimuli. The performance of this movement was reinforced by presentation of pieces of bread soaked with broth. After several days of training, the dogs learned to perform this movement immediately after the applications of the stimulus, while the reactions performed in the intervals, not being reinforced, gradually disappeared.

In some dogs, besides the positive conditioned reflexes also inhibitory reflexes were established by application without reinforcement of stimuli resembling to the positive ones.

In 7 dogs, a different conditioned reflex of the locomotor character was also established: the animals were trained to run through a very simple maze to a place where they received food. An acoustic stimulus was used as the conditioned signal of this reaction. The food was not visible from the starting point. Some of the dogs trained in the maze were also taught to perform an instrumental movement after arriving at the feeding place. In dogs S-1, S-3, S-4 and S-7 this movement consisted in lying down on the floor; in dog No S-6, in pressing a lever located 70 cm above the floor (with his forelegs).

Usually, about 50—100 experiments were performed with every dog during the preoperative training, each experiment consisting of 8—10 trials.

In addition to these experiments, observations concerning the general behaviour of the animals were made, as well as their behaviour under

certain special conditions, referred to hereafter as "additional tests". The following additional tests were made: a) Going over a barrier. A dog was put in front of a horizontal barrier placed 25 cm above the floor. He had to cross over the barrier in order to reach food placed on the opposite side. b) Climbing with the forelegs on a table. This was provoked by placing a piece of meat on table. The animal had to put his forelegs on the table and take food with his mouth. c) Withdrawing from a cul de sac. A dog was brought into a narrow passage closed on three sides so that he could get out of it only by moving backwards. Every normal dog in such situation moves backwards quite easily and quickly.

When the conditioned reflexes were firmly established and the additional tests had been repeatedly carried out, the more or less extensive ablations of the sensory cortex were performed.

Surgical procedures were done under aseptic conditions in general anesthesia (Nembutal 32—35 mg/kg). After the incision of the skin in the medial line, and pushing aside the temporal muscles, the frontal and parietal bones were trephined. The dura mater was divided and the cerebral tissue was removed by subpial aspiration. Care was taken to avoid excessive bleeding. The dura, muscles, subcutaneous tissue and skin were sutured in layers.

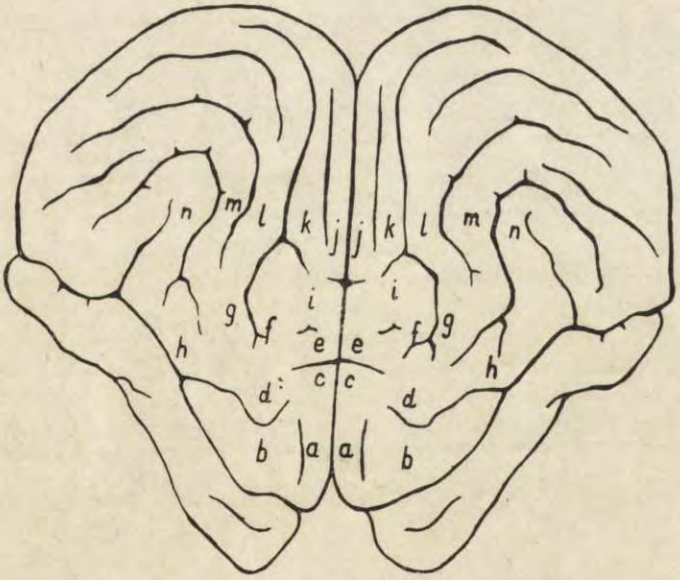
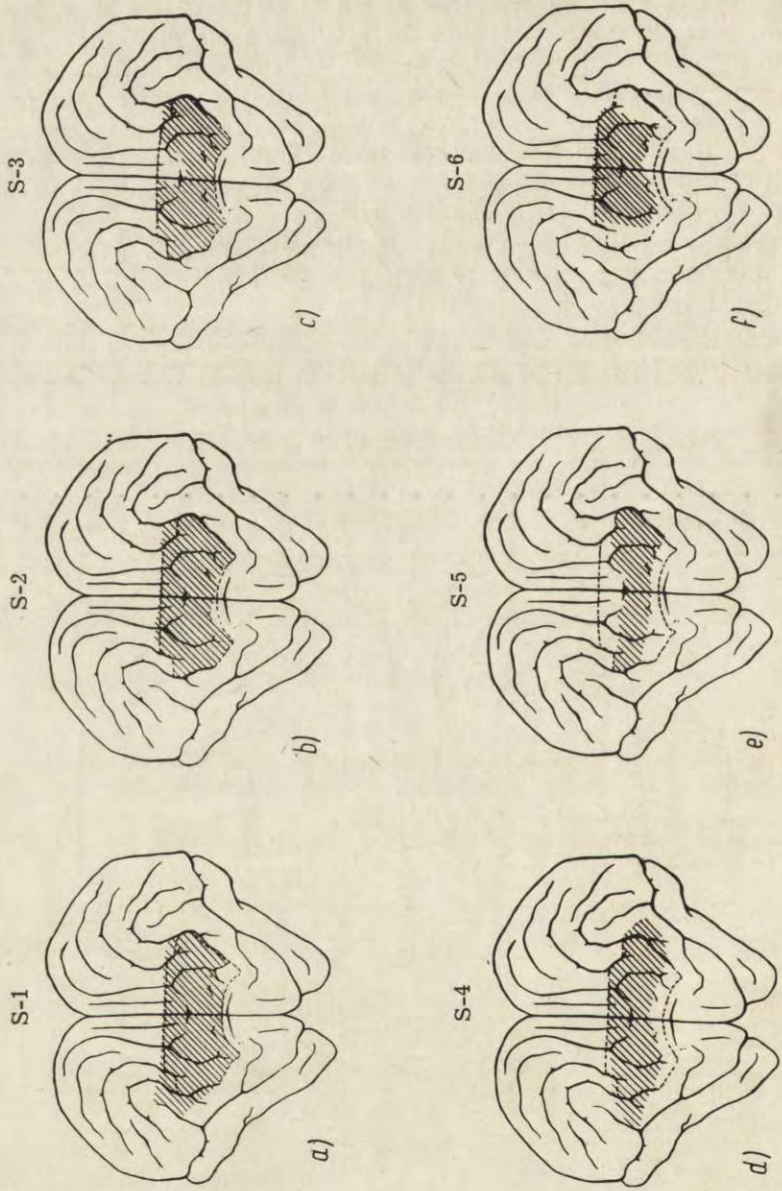


Fig. 1. Dorsal and lateral views of a brain of dog shown on one plane
Gyri are indicated as follows: a — proreus; b — orbitalis; c — picruciatus; d — sigmoideus anterior; e — postcruciatus; f — sigmoideus posterior; g — coronalis; h — compositus anterior; i — postcentralis; j — suprasplenialis; k — entolateralis; l — ectolateralis; m — ectosylvius; n — sylviacus



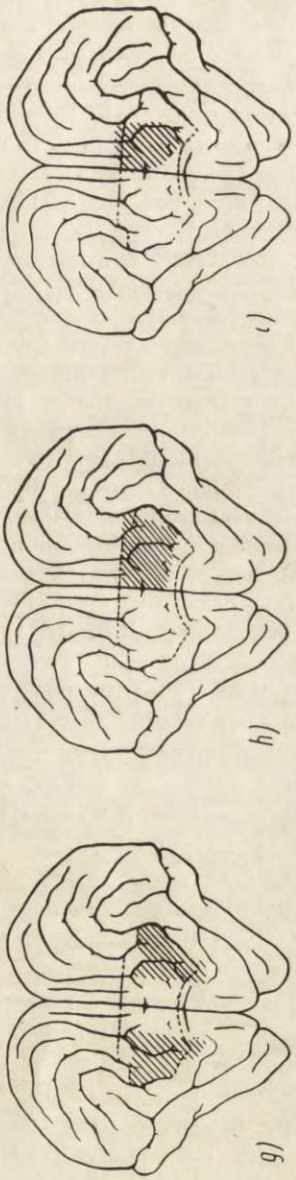


Fig. 2. Charts of brain lesions in dogs S-1 to S-9 described in text. Destroyed areas are marked by hatched surfaces, the boundaries of somatic sensory areas I and II (after Pinto Hamuy, Bromiley and Woolsey, 1956) marked by dashed lines

After the operation the dogs were given 200—300 ml of saline, and 200—300 thousands units Penicillin daily. The post-operational period was uneventful. The general condition of the animals operated on was excellent; no epileptic fits were observed. When the post-operational series of experiments were completed the animals were either sacrificed and their lesions verified, or in some cases further operations were performed.

The procedure of post-operational experimentation requires some comment. The aim of the study was to see whether or not the conditioned reflexes were abolished after the ablations and, if so, whether the recovery was „spontaneous" or needed some additional training. In those cases in which the conditioned reflexes were not totally lost but only disturbed, any positive response was reinforced, even if the movement performed, was not quite perfect. The problem was however more difficult when no leg movement appeared to conditioned stimuli. The reinforcement of the stimulus in spite of the absence of active movement might lead to formation of a classical (type I) conditioned reflex, i.e. of a reflex in which active instrumental movement is not required. On the other hand, the non-reinforcement of the stimulus would lead to the extinction of the conditioned reflex not only to such stimulus, but even to the whole experimental situation. Our experience in this matter has shown that the danger of extinction of the conditioned reflexes by non-reinforcement is greater than the danger of reversal training if the stimulus is reinforced. It is necessary, however, to make a limited number of trials at each experiment and not repeat them too often. Accordingly, the experiments were performed only every few days, and when the conditioned reaction was lacking, only 2—3 trials were given. Usually, the conditioned stimuli were prolonged to 10—20 seconds in order to give the animal chance to perform the movement, and if he failed to do so, in some trials the food was none the less presented. We found that such a procedure did not in any way hamper the spontaneous recovery of the temporarily abolished conditioned reflexes.

According to the localisation and extent of lesions, our experimental animals can be divided into three groups.

Group 1: Bilateral ablations of whole sensory areas I and II. This lesion was performed bilaterally in one stage in dogs S-1, S-2, S-3. In dog S-4, first the left and after 11 days the right sensory cortex was removed (Fig. 2a, 2b, 2c, 2d).

Group 2: Bilateral partial ablations of the sensory cortex. In dog S-5, both areas were removed, except the caudal and rostral parts of area I (Fig. 2e). In dog S-6, the sensory area I was almost totally removed while the sensory area II was left intact (Fig. 2f). In dog S-7, sensory areas II, with some adjacent portions of the cortex, were removed (Fig. 2g).

Group 3: Unilateral ablations (left). In dog S-4, the whole sensory areas I and II were removed (Fig. 2d). In dog S-8, the total area I and the upper part of area II were removed (Fig. 2h). In dog S-9, the medial part of sensory area I was removed (Fig. 2i).

RESULTS

General behaviour and neurological symptoms

In all the animals, the general behaviour remained unchanged. When brought into the room for observation, the dogs were quite quiet; they sniffed objects on their way, and after some time sat down on the floor. No tendency to hyperactivity or to stereotype movements was observed. The animals reacted quite adequately and promptly to such stimuli as calling them, opening the door, etc.

The proprioception of the limbs was, however, strongly impaired. The animals put the dorsal aspect of the foot to the ground, slid on the smooth floor, put one leg on the other, crossed their legs etc. These symptoms were most marked shortly after operation and then gradually, although only partially, disappeared. But even atfer a long period of time it was possible sometimes for the experimenter to place incorrectly the leg of the animal when it was eating and the dog maintained this position without correcting it. The defects in group 1 were more prolonged than in groups 2 and 3 (Table I).

Table I

Duration of impairment of proprioception and motor activities after bilateral ablations of sensory cortex in dogs. In weeks

Dog No	Period of observation after oper.	Period of impaired reactions		Period of absence of		
		postural	placing	climbing on a table	going over a barrier	withdrawing from a cul de sac
S—1	30	6	Throughout the period of observ.	6	8	Normal
S—2	29	2		2	4	
S—3	11	6		2	9	
S—4	15	6		4	Throughout the period of observ.	
S—5	4	4		4		
S—6	5	2		5	4	
S—7	7	4		—	4	

Placing reactions were parmanently abolished in all our dogs. After unilateral lesions, these symptoms were seen only in the contralateral side.

In dogs S-2, S-3, and S-4 a marked defect in the upper portion of the visual field was also observed.

In the additional tests, we found that climbing on the table and going over the barrier were strongly impaired in all bilateral dogs, for several weeks after operation. At the sight of a piece of meat placed on the table, the dogs tried to get it with their mouths, without, however trying to climb on the table. When put in front of a barrier, they stretched their necks forward in the direction of the food, pushed the barrier, but they did not lift their feet over it. At the same time, they jumped without difficulty over the barrier if they were at some distance from it.

The unilateral dogs performed the movement of climbing on the table only with their left „normal" leg, while the right one hung loose. Going over the barrier proved possible only when the dog was not too near of it so that he could see it, or when the animal touched it with its left shoulder.

After several weeks both climbing on the table and going over the barrier were gradually restored in all the dogs. The movement of withdrawing from a cul de sac remained normal in all dogs (Table I).

Conditioned-reflex experiments

When brought to the experimental chamber the dogs behaved quite adequately. To the conditioned stimuli they manifested a very clear and prompt general alimentary reaction — turning towards the foodtray and salivation — but for a longer or shorter period they were completely unable to perform the learnt movement.

The lack of learnt movement persisted from 4 to 10 weeks after operation in dogs of group 1, whereas in dogs of groups 2 and 3 it did not exceed 4 weeks (Table II).

Group 1

Dog S-1, in spite of a very pronounced general alimentary reaction did not perform the movement for 78 days, with the exception of a single incomplete attempt to do so on the 56th day after the operation. The instrumental conditioned reaction reappeared on the 79th day, first to strong conditioned stimuli, and then to other ones also. From that time on, the movement was

performed quite regularly and as efficiently as before the operation.

In dog S-2 the impairment of the motor conditioned reflex lasted till the 57th day after operation. For te first 20 days, the instrumental reaction was totally lost. To the sound of the conditioned stimulus the animal immediately ran up to the food-tray, looked into it, and stamped the floor with his forelegs, and then even whined or howled. When the duration of the conditioned stimulus was somewhat prolonged, the general alimentary reaction grew stronger and stronger but the learnt movement did not appear. Between the 21st and 40th days, the dog occasionally performed the trained movement at the moment when the bowl of food was moved into position, but not to the conditioned stimulus. Between the 40th and 57th days, the dog performed two abortive movements to the conditioned stimulus, and from the 57th day on the movement was regular and differed hardly from that before operation.

Table II

Duration of absence or impairment of motor conditioned reflexes after ablations of sensory cortex in dogs. In weeks

Dog	Extent of lesion	General alim. r.	Movement of foreleg	Lying down on the floor	Locomotor reaction
S—1	SI and SII bl	Normal	11	11	Normal
S—2	SI and SII bl		8	—	
S—3	SI and SII bl		6.5	Less than 6	
S—4	SI and SII left except rostral part		1	Normal	
	SI and SII right except rostral part		4	Throughout the period of observ.	
S—5	SI and SII bl except rostral and caudal parts of SI		2	—	—
S—6	SI bl		3	—	Normal
S—7	SII and later. parts of SI bl		Normal	2	
S—8	SI and medial part of SII left		More than 20 days	—	
S—9	SI left		Normal	—	—

SI — Somatic sensory area I; SII — Somatic sensory area II (after Pinto Hamuy, Bromiley and Woolsey 1956); bl — bilateral ablation.

The experiments with dog S-4 were made for three weeks after operation, and then were stopped for 4 next weeks. During the initial three weeks, the conditioned stimuli evoked only a general alimentary reaction, without any movement. When the experiments were resumed 45 days after operation, the dog, after a few unsuccessful trials, began to perform the instrumental reaction, but its latent period was prolonged to 5—6 sec. during several days. The movement was atactic and awkward and was preceded by 1 or 2 abortive movements. This condition remained unchanged throughout the period of observation i.e. 80 days after operation. The inhibitory conditioned reflex was totally preserved: to the sound of the inhibitory stimulus the dog turned away from the food-tray.

In dog S-4, in which the sensory areas were removed successively, the learnt movement was absent for about two weeks after the second operation. The first abortive movements appeared on the 17th day. From the 29th day on, the movements were fuller and more regular, but they remained permanently atactic and clumsy. The inhibitory conditioned reflex was completely preserved.

Group 2

In dog S-5 the learnt movement reappeared on the 15th day after operation, quite suddenly. It was however atactic and awkward during the whole period of observation, i.e. for 30 days after operation. The inhibitory reflex was normal.

Dog S-6 did not perform the learnt movement for two weeks. After the conditioned stimulus had been given, a normal general alimentary reaction and a strong trembling of the right foreleg was seen but no lifting. The first abortive movements appeared on the 15th day after operation. The full movements were performed on the 23rd day, although after a considerable latent period (about 5 seconds). Such a delayed reaction was preceded by a trembling of muscles in the right foreleg or by small abortive movements. The inhibitory reflex was totally preserved.

In dog S-7, in which the sensory areas II were ablated the instrumental conditioned reflex was preserved.

Group 3

In dog S-9 the learnt movement was totally preserved, although it was atactic and clumsy.

In dogs S-4 and S-8 the instrumental reaction was absent, in dog S-4 for 6 days and in dog S-8 for a least 20 days. After this period the experiments with dog S-8 were discontinued for seven weeks; when they were resumed, it was found that the animal was again able to perform the learnt movement, but it was for a long time abortive and only gradually became normal.

The movement of taking the leg off the foodtray in dogs S-1, S-2 and S-9 was performed in the same way as before operation. In dogs S-3 to S-8 however, this movement was performed only passively when the animal turned away from the foodtray.

The general behaviour during the inter-trial intervals was quite normal in all dogs. No disinhibition or hyperactivity was observed.

Maze experiments

The locomotor conditioned reflexes were completely preserved in all dogs, but the special movement to be performed after arrival at the goal was disturbed in all bilateral dogs in the same way as the movement described above.

Group 1

In dog S-1 the movement of lying down on the floor was absent for 80 days. During this time the dog, after coming to the food-place, stayed there and waited for food but made no effort to perform the learnt movement. Later, the dog began to perform this movement in a particular way: he first bent quickly the forelegs, and then very slowly and gradually (5—6 seconds) the hindlegs. The full and normal movement of lying down did not return until 200 days after operation, it was after a long interruption of experimental sessions.

In dog S-4 the movement of lying down on the floor did not return at all after the second operation throughout the whole period of observation i.e. 105 days.

Group 2

In dog S-7 the movement of lying down was absent for two weeks. After this time, the dog performed this movement, although in a very slow manner, resembling a slow motion.

In dog S-6 the learnt movement consisted in pushing a lever with the forelegs. It was absent for 11 days. After this period, the movement was not so skilful and correct as before operation.

In a dog with an unilateral lesion (dog S-4 after the first operation) the movement of lying down on the floor was quite normal.

DISCUSSION

According to our results, after lesions of the sensory area in dogs, the instrumental conditioned reflexes of manipulatory character (such as putting the foreleg on a food-tray, pushing a lever, or lying on the floor) are in almost every case absent at the beginning and then more or less impaired, whereas the locomotor conditioned reflexes in all our animals were totally preserved. The general alimentary reaction to the conditioned stimuli was also completely normal in all our dogs from the very first experiments after the operation.

After bilateral total ablations of sensory area I and II in a single session, the instrumental conditioned reflexes were absent for 6—10 weeks (dogs S-1, S-2, and S-3). In the dog in which the sensory areas were ablated in two stages (dog S-4), as well as in those dogs in which the lesion was less extensive, the learnt movement was abolished for a much shorter period — 2—3 weeks — (dogs S-5 and S-6), and in one dog (S-7) it was completely preserved. So it may be assumed that the degree of disturbance of the instrumental conditioned reflexes after bilateral sensory ablations depends upon the extent of the lesion. Early reappearance of the movement in dog S-4 was due to the fact that ablation was done in two stages. The data obtained in our laboratory as well as the results of experiments made by A d e s and R a a b (1946) and T r a v i s (1956), indicate that the removal of the brain tissue in several consecutive operations causes less disorder of function than the same lesion produced in a single operation.

On the basis of our experiments we may assume that as regards the execution of the instrumental movement, the sensory area I is much more important than the area II. Dog S-6 for example, with only a partial ablation of the sensory area I was for two weeks unable to perform the learnt movement, whereas dog S-7 after total removal of area II, preserved his conditioned reflex completely. These results are in agreement with P i n t o H a m u y's (1956) observations that the removal of somatic area II in monkeys does not impair the manipulatory learnt movement.

The reappearance of the abolished instrumental conditioned reflexes was in our experiments always „spontaneous", i.e. it occurred without any additional training. Moreover in dogs S-1, S-3 and S-8 the movement reappeared immediately when the experiments were resumed after an interruption of several weeks.

Two problems arise: 1) what is the cause of the absence of instrumental conditioned reflexes after sensory ablations; and 2) why are they recovered spontaneously after a lapse of time without any additional training?

There is a vast body of evidence showing that in all instrumental ("voluntary") behaviour the feed-back, i.e. the flow of proprioceptive stimuli generated by the performance of the movement and by the posture of the extremity, plays a very important or, perhaps a quite indispensable role. Speaking freely, we can say that the animal is able to perform a "voluntary" movement only when the sensory reception of the posture of the extremities involved in the movement is normal. Konorski and Miller (1933) and Konorski (1948), when analysing the conditioned reflexes of the 2nd type, heve proved that there exists a very close interdependence between the performance of the motor conditioned reaction and the sensory feed-back generated by this reaction. According to some experimental and clinical data which have been lately fully confirmed in the neuronographic study by Mountcastle (1957), the particular sensation of the extremity is represented in the sensory cortex. Therefore, after removal of the whole sensory area, a large part of the sensory feed-back information connected with postural stimuli is destroyed. In fact, observation of our animals after operation gave the impression that they "are not aware" of the position of their legs and therefore cannot use them for any instrumental movement. The general reaction towards food and the locomotor conditioned reflexes, on the other hand, are preserved, because they are based chiefly on visual and labirinthine sensations which remain quite normal after these lesions. As some part of proprioception of the extremities is also represented in the so-called motor and premotor cortex, the animals re-learn, probably in their normal life outside the experimental chamber, to perform the instrumental movement in the experimental situation.

The compensation of the impaired functions is most likely the

result of the take-over of these functions by the neighbouring undamaged regions of the brain, mainly by the so-called motor cortex. Therefore, after bilateral ablation of the sensori-motor cortex in dogs, the compensation of the instrumental conditioned reflexes and proprioception is much less complete and slower than after the removal of the sensory areas only. The improvement of function may also be based on sensory reception from other sources. For example, in experiments with going over a barrier, for some time after the operation the animals put just in front of the barrier pushed it but did not put their legs over it since this movement could be provoked by tactile stimuli only. On the other hand, when put at some distance from the barrier they jumped over it without any difficulty. In this case, the visual impulses (seeing the barrier) elicited the motor reaction.

The striking fact is that the reappearance of the learnt movement is quite independent of the placing reaction, which was abolished in all the animals for the period of observation i.e. up to 7 months.

In contradistinction to the results of Zubek (1952), who observed "amnesia for general test situation" after bilateral ablation of the sensory areas I and II in cat, our dogs recognised the experimental situation perfectly well even after the most extensive ablations.

As the general behaviour of our animals after operations was normal, and their general reactions remained adequate, it is quite understandable that the inhibitory conditioned reflexes were in no way disturbed. Just as before the operation the animals turned away from the foodtray and did not perform any leg movement to the conditioned inhibitory stimuli.

SUMMARY

1. After bilateral or unilateral ablations of sensory cortex in dogs the instrumental conditioned reflexes of the manipulatory character (putting foreleg on foodtray, pressing a lever with forelegs) were abolished whereas the locomotor conditioned reflexes were preserved.

2. After a lapse of time the conditioned manipulatory reflexes are restored „spontaneously" i.e. without any special training. They became quite normal in some dogs whereas in others they

remained more or less impaired: they were abortive and awkward, the latent period was increased.

3. In dogs with total ablation of sensory area I and II the lack of instrumental reactions lasted for 6—10 weeks, whereas in dogs with less extensive ablations it was shorter.

4. The climbing onto a table and going over a barrier was also abolished after sensory lesions and then gradually reappeared. On the other hand the contact placing reaction was permanently lost.

5. The general behaviour of the animals remained quite normal and adequate and their inhibitory conditioned reflexes were preserved.

6. The mechanism of the disturbances of manipulatory conditioned reflexes and their „spontaneous" recovery is discussed.

We should like to thank Prof. J. Konorski for his help and advice in this work.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

RETURN REACTION AS A TEST OF THE SPACE ORIENTATION OF WHITE RATS IN THE HORIZONTAL AND PERPENDICULAR PLANE

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The ability of the animal to find its way home after shorter or longer excursions for hunting, water drinking, food collection etc. is a well known fact extensively studied by ethologists. The physiological mechanism, however, of this phenomenon is almost completely unknown because of the lack of the appropriate experimental investigations carried out under laboratory conditions. To fill this gap it seemed necessary to submit this sort of reactions to the experimental analysis, not in complicated conditions existing in natural environment but in specially devised and much simpler situation in which the return reaction could be provoked and analysed.

This sort of investigations was recently undertaken and its results will be a subject of another paper. Here we shall discuss only one side of this problem concerning the ability of the rat to return home in the horizontal and perpendicular plane.

MATERIAL AND METHOD

Experiments were carried out on white rats, both sexes, from 3 to 9 months old. The rats were confronted with the problem of returning to the starting place by the same route they had just taken to reach their goal. Two roads from two different directions led to a platform where a bowl containing food was placed. The starting place was a small cage situated at the beginning of one of the two roads. The rat had to go out of the cage, run to the goal, snatch the food and return to the cage, where it was allowed to eat it. When returning with food to the starting cage the rat had

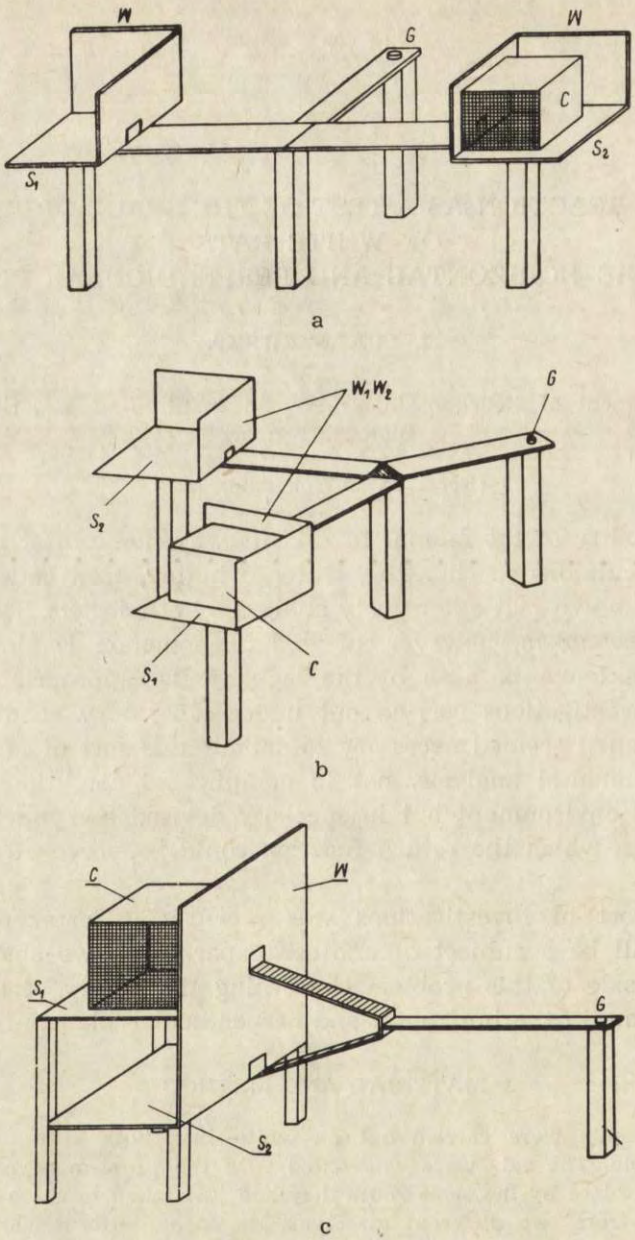

Fig. 1. Various types of mazes: a) Horizontal T maze; b) Horizontal Y maze; c) Perpendicular maze

S_1, S_2 — starting platforms; C — cage; g — goal; W, W_1, W_2 — wooden walls

to choose between two roads and use the same one he had taken going to the bowl.

For experiments in horizontal plane the T shaped elevated maze (Fig. 1a) was built of boards 14 cm. wide and 75 cm. long placed on poles 75 cm. high. The cage was placed on one of the starting platforms at the ends of the arms of the maze (S_1, S_2), while a bowl with food was placed at the end of the third board. The starting platforms were screened by the wooden walls. A swing door in the wall allowed the rats to leave and reenter the cage, while the door in the opposite wall was closed. The perpendicular maze (i.e. a maze in which all paths are situated in one perpendicular plane, Fig. 1c) consisted of two shelves placed one over the other, 40 cm. apart. From these shelves two ladders 50 cm. long, placed at the angle of 50° led to a board which was situated half way between the two shelves. The cage stood on one of the shelves, the bowl with food was on the board at the distance about 40 cm. from the ladders. The shelves were screened by wooden walls so that the cage could not be seen from any point of the maze.

The rats were divided into 4 groups of 6 or 7 animals in each group. Two groups were used for experiments in the horizontal maze and two in the perpendicular. Experiments proper were preceded by a preliminary training in which animals were habituated to the experimental situation and taught to leave the cage and return to it after having snatched the food. Pieces of biscuit about 2 g were used as reinforcement.

RESULTS

Experiments in the horizontal maze

After the preliminary training was completed the following experimental procedure was applied. The experiments were performed every day and consisted of 3 or 4 trials. During each experimental session the cage was placed on the same starting platform. Each day the place of the cage was changed. Ten experimental sessions were carried out in this way. In Table I the percentage of correct return runs made by all rats in each trial is

Table I

The percentage of correct return runs in the horizontal maze in each trial in 10 successive experiments

Groups	No of rats	I run in %	II run in %	III run in %	IV run in %
1	6♀	98	100	100	100
2	7♂	94	100	100	—

presented. As seen from this table the percentage of correct runs in the first trial of each experiment is very high: this means that almost in all cases the rats were able to find their way to that platform from which they started. In trials No 2, 3, 4 of each experiment all return runs were correct. This was due to the fact that the task to return to the starting platform was made even more easy by repetition of the same run. When after the end of this series the starting cage was placed on the same platform as on the preceding day, this did not cause any disturbance in the animals' correct return to the cage.

Experiments in the perpendicular maze

The course of this series of experiments was similar to that in the horizontal maze, i.e. one day the animal started from platform S_1 and on the next day from platform S_2. Every day 3 or 4 runs were performed on the same road. 10 such experiments were performed. Results are given in Table II. The low percentage of correct solutions in the first run proves that in this case the rats were not able to find their way back to the starting platform. The gradual increase of correct returns in 2nd, 3rd and 4th runs indicates that by repetition of the same task in a given experiment the animal learned by trial and error to choose the proper way to the starting platform. It should be noticed that in group 2 the percentage of correct runs in the first trial of each experiment is below a chance level. This suggests that the animals have a tendency to repeat the same reaction which they learned in the preceding day.

Table II

The percentage of correct return runs in the perpendicular maze in each trial in 10 successive experiments

Groups	No of rats	I run in %	II run in %	III run in %	IV run in %
1	7♀	50	75	83	97
2	6♂	35	66	80	—

To prove that this is so, we performed another series of experiments in which the place of the starting cage was changed

every two days. Results of these experiments are shown in Table III. It is seen that in those experiments in which the same road leads to the starting platform, as in the preceding experiment, the first return is nearly always correct. On the other hand if the road is changed the animals almost always go to the wrong platform.

Table III

Course of experiments in the perpendicular maze when the road was changed every two experiments (7 rats)

No of experiments	Road leading to the bowl	Number of correct returns			
		I run	II run	III run	IV run
0	top	3	5	7	7
1	top	7	6	7	7
2	bottom	1	6	7	7
3	bottom	7	6	7	7
4	top	1	3	5	6
5	top	6	7	7	7

Since in the horizontal maze the angle between two roads leading to the starting platforms was 180° and in the perpendicular maze only 50° (c.f. Fig. 1c) it might be supposed that this was the very reason of the greater difficulty of the perpendicular maze

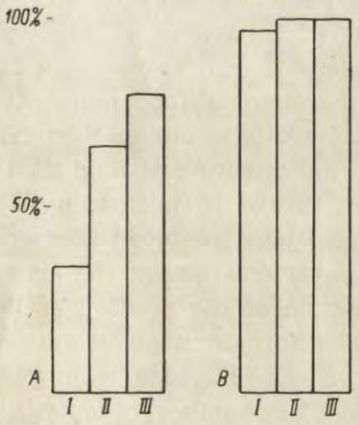

Fig. 2. Comparison of experiments in perpendicular maze (A) and in Y horizontal maze (B)

I, II, III — successive runs. Each column represents the percentage of the correct return runs in the respectivetrial (6 rats and 10 experiments)

over the horizontal one. To elucidate this point new series of experiments was performed in a horizontal maze, in which the angle between the roads was reduced to that between the ladders (Fig. 1b). The same group of rats was used which previously served in experiments in the perpendicular maze. It was found that in this case the rats managed very well in returning to the cage. The mistakes were very few and occurred only in the first trials of each experiment. A comparison of the results of experiments in this and in the perpendicular maze is given in Fig. 2.

Attention should be drawn to other differences in the behaviour of animals in the horizontal and perpendicular mazes. If the rat made a mistake in the horizontal maze and ran to the screen where the door was closed, it immediately corrected its mistake and ran straight to the cage. In the perpendicular maze, on the other hand, when the rat missed the way and ran to the wrong shelf, it seemed very often completely helpless: it ran back to the bowl and then once again to the same shelf, often repeating its mistake several times. Sometimes, it was even necessary to put the rat on the right road or take it into the cage. Because of the great difficulty of the task some animals refused to go to the bowl and had to be withdrawn from the experiments.

DISCUSSION

It is quite a long time now since, at first by accidental observations, examination of behaviour of rats in a horizontal maze have proved their orientation in space. Such facts as: running into blind alleys in the direction of food more often than in opposite direction (Dashiell 1920), climbing the walls of the maze and taking a short-cut in the direction of food (Hubbert and Lashley 1917, Lashley 1929), running straight to the food when the walls of the maze have been removed (Dennis 1929) show that a rat while learning a certain road in the maze, gains, at the same time a general orientation of the position of the goal.

The more detailed information about rats' orientation in space has been given by Dashiell (1930) who performed experiments in a maze consisting of a number of open alleys giving the rat a free choice of road to its goal. It was found that the rat took several different roads to the goal but always correctly orientated its position in relation to it.

All these facts go to show that the rats are able to grasp a relation between the two points of the space, one representing the starting point, the other the goal. The problem set before the rats in the present work needs also a certain kind of space orientation, consisting in the reversal of relation between the start and the goal. In order to get the cage on the return journey the rat has to go in the opposite direction to the one taken to the bowl (going right changes on the return journey to going left and an upward direction to a downward).

As it was found in our experiments the rats showed ability to reverse space relations in the horizontal plane, and in consequence, to return correctly to the starting point. On the other hand in the perpendicular maze this ability was completely absent. They had to learn the return road during each experiment and relearn it again in the next. This allows us to assume that the orientation of rats in the perpendicular plane is much worse than in the horizontal one. It is even possible that the rats lack completely orientation in the perpendicular plane, unfortunately, we have available, up to now, very little information on this subject. While the horizontal mazes of various kinds were extensively used in many experimental works, the perpendicular maze was applied, to our knowledge, only by Hunter (1929); but even his maze was a three dimensional one i.e. it combined elements of both planes.

As in the training to run correctly in a horizontal maze the orientation in space plays undoubtedly a great role, it may be expected that learning in the perpendicular maze should present to the rat a much greater difficulty than learning in the horizontal maze. This problem requires, however, a special investigation.

SUMMARY

1. The ability of the rats to return to the starting point in a maze with the double-choice return way was investigated.

2. It has been found that the rats are able to find the correct return way in the horizontal T and Y shaped mazes whereas they are totally unable to do so in a perpendicular maze.

3. It is suggested that the rats have very poor if any orientation in space in the perpendicular plane in contrast to their very good orientation in the horizontal plane.

I wish to express my thanks to prof. J. Konorski for his constant encouragement and helpful advice throughout this work.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

EFFECTS OF PREFRONTAL ABLATIONS ON SALIVATION DURING THE ALIMENTARY UNCONDITIONED REFLEX AND AFTER ITS CESSATION

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(Received 29 October 1958)

Several investigators have established that one of the frequent consequences of prefrontal lesions in animals is a more or less pronounced „increase in appetite" and connected with it an enhanced food intake (Afanasev 1913, Fulton, Jacobsen and Kennard 1932, Langworthy and Richter 1939, Ruch and Shenkin 1943).

Stanley and Jaynes (1949) in their review of papers concerning the functions of frontal lobes consider the frontal hyperphagia as a separate symptom and indicate that it supports their hypothesis that frontal lobes are mainly concerned with a cortical act-inhibition. According to these authors protracted food intake of prefrontal animals indicates that such inhibition is impaired. Stanley and Jaynes' conclusions acquire special significance in the light of our own results indicating that prefrontal ablations in dogs produce a disinhibition of alimentary inhibitory conditioned reflexes (Brutkowski et al. 1956, Brutkowski 1957, Ławicka 1957).

In observations on many prefrontal dogs we have also noted that the operated animals returned to an empty bowl from which they previously have eaten all the food, that they carefully licked out the bowl and smelt it thoroughly on all sides. These dogs exhibited great voraciousness, too, consisting in rapid and violent intake of food. Similar observations were made recently also by Shustin (1958).

In connection with these observations a series of experiments was undertaken in which the effect of prefrontal ablations on the salivary unconditioned reflexes and salivation in the intertrial intervals was investigated. The results of these experiments are reported in the present paper.

EXPERIMENTAL MATERIAL AND METHODS

Experiments were performed on 3 dogs in a sound-proof conditioned-reflex chamber. All dogs had a parotid gland fistula performed by the Glinski-Pavlov method.

In each experimental session usually 6—7 positive stimuli were applied and among them 1—2 inhibitory stimuli. The excitatory stimuli were always reinforced by the presentation of 40 gm meat-bread powder, moistened with broth. The conditioned stimuli were applied in a stereotyped manner. The intervals between stimuli lasted 4 minutes.

The salivary secretion was recorded by Kozak's method (1950).

Above the bowl in the foodtray a small mirror was fixed in which the whole course of the food intake could be followed.

When the full conditioned-reflex training was completed (which took usually one year) the following bilateral ablations were performed*:

1. In dogs 3 and 4, gyrus proreus and gyrus orbitalis anterior, rostrally to sulcus presylvius, were removed („limited prefrontal lesion").

2. In dog 1, gyrus proreus, the anterior part of gyrus orbitalis, gyrus precruciatus (a few mm anterior to sulcus cruciatus) and gyrus sigmoideus anterior were ablated („extensive prefrontal lesion").

16 months after the first removal an additional ablation was performed in dog 3 in which the anterior part of gyrus precruciatus, gyrus sigmoideus anterior and the rostral part of gyrus compositus anterior were removed.

3. In dogs 3 and 4, besides the ablations described above, the medial parts of parietal lobes, including gyrus suprasplenialis, gyrus entolateralis and gyrus ectolateralis, were removed. These lesions were performed to serve as control for the prefrontal ablations.

In order to compare the amounts of salivary secretion to the unconditioned stimuli (food) and in intertrial intervals the ten successive experiments before and after the operations were analysed. The mean values of secretion for the first three trials (before application of inhibitory conditioned stimuli) of each experiment were calculated.

RESULTS

The amounts of saliva produced to the unconditioned stimuli and in intertrial intervals before and after limited prefrontal lesions are shown in Fig. 1. It indicates that removal of gyrus proreus

* Part of the results obtained in these dogs as well as the details of the surgical procedure were presented in other papers of this series (Brutkowski 1957, 1959a, 1959b).

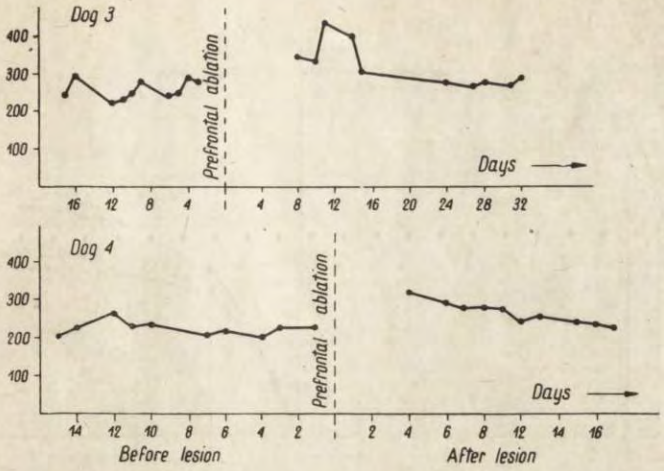

Fig. 1. Salivation during alimentary unconditioned reflex and after its cessation in the last pre-operative period and immediately after the limited prefrontal ablations in dog No 3 and dog No 4

Abscissae: consecutive days before and after operations. Ordinates: mean values of salivary secretion (to the unconditioned stimuli and in interial intervals) in the first three trials of every experiment

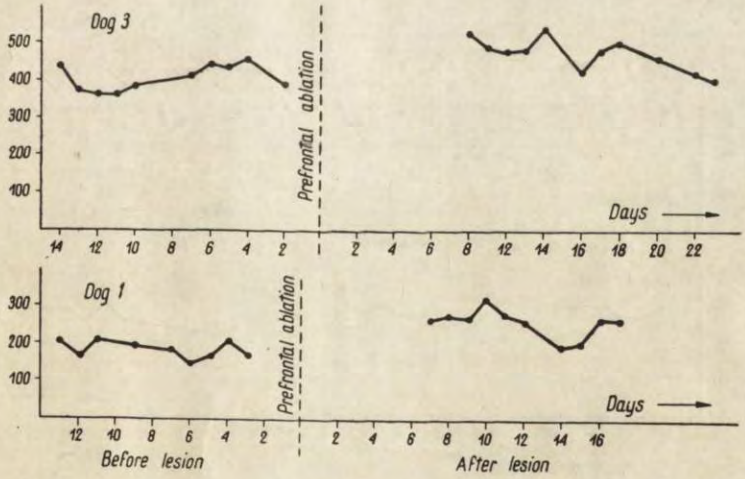

Fig. 2. Salivation during alimentary uconditioned reflex and after its cessation in the last pre-operative period and immediately after the extensive prefrontal ablations in dog No 3 and dog No 1

Abscissae: consecutive days before and after operations. Ordinates: mean values of salivary secretion (to the unconditioned stimuli and in interial intervals) in the first three trials of every experiment

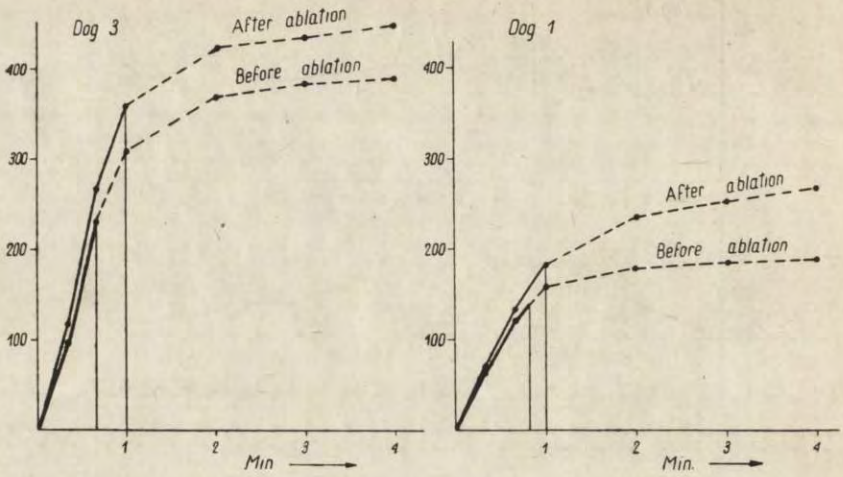

Fig. 3. Course of salivation in a single trial during and after food intake (unconditioned stimulus) immediately before (heavy lines) and after prefrontal ablations (light lines)

Abscissae: duration of salivary secretion in minutes. Ordinates: amounts of saliva producer to the unconditioned stimulus (continuous lines) and in the intertrial interval (broken line).

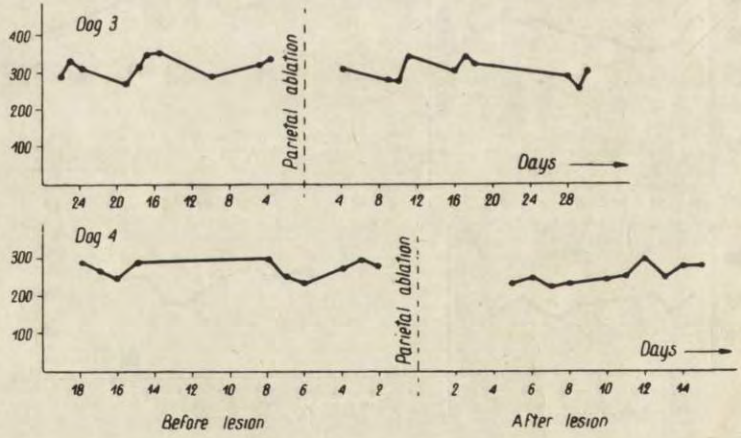

Fig. 4. Salivation during alimentary unconditioned reflex and after its cessation in the pre-operative period and immediately after the parietal ablations in dog No 3 and dog No 4

Abscissae: consecutive days before and after operations. Ordinates: mean values of salivary secretion (to the unconditioned stimuli and in the intertrial intervals) in the first three trials of every experiment

and the rostral part of gyrus orbitalis results in unequivocal, though rather transient, augmentation of salivation: in dog 4 the mean increase was about 22% and in dog, 3, about 30%; in single trials the increase was even greater, reaching up to about 100% of the preoperative level. The rate of salivation was also markedly increased.

Similar results were seen after extensive prefrontal lesions (Fig. 2). These lesions were also followed by an augmented salivation. The extent and course of disturbances were almost the same as in the case of limited prefrontal removals.

In Fig. 3 the course of salivation in a single trial during and after food intake immediately before and after operation is presented. This figure indicates that the process of eating in prefrontal dogs lasted longer than in normal dogs. This protraction, however, did not result from any damage to the motor activity of the mouth but was due to the fact that the animals licked again and again the empty bowl (that was seen very well in the mirror hanging above the foodtray). The dogs licked out and smelt the bowl also in intertrial intervals, sometimes rummaging in the bowl or in its neighbourhood almost incessantly.

As seen in Fig. 3 this prolongation of the act of eating is the main cause of the augmentation of salivary secretion in prefrontal dogs. Besides, the salivary after-effect is also increased in these dogs that is particularly well seen in Fig. 3, dog 1.

Removal of the parietal lobes does not produce any change of either unconditioned salivation or salivation in intertrial intervals (Fig. 4). As seen in Fig. 4b the salivation in the first postoperative period is even diminished as compared with the salivation before operation.

DISCUSSION

The present experiments have shown that after prefrontal bilateral ablations in dogs the secretion of saliva during eating and afterwards is augmented. This phenomenon is transient and not very marked. Its degree is more or less the same both after extensive and limited prefrontal lesions. Control lesions of parietal lobes do not result in such an effect.

As can be seen in Fig. 4 the enhanced salivation in prefrontal dogs is mainly produced by protraction of the act of eating. This

protraction is in turn connected with very careful emptying of the bowl, licking it out many times and rummaging inside it. Therefore the increased salivation in the intertrial intervals may be considered as prolongation of the unconditioned reaction.

The prolongation of the act of eating and augmented unconditioned salivation seem to indicate that in prefrontal animals the very mechanism of the cessation of the act of eating when the bowl is already empty is impaired. Thus, the symptom described in this paper may be also considered as due to the impairment of inhibition of alimentary activity.

In view of these facts the prefrontal area can be considered as a cortical representation of the alimentary unconditioned inhibitory reflex, i.e. a centre that controls inhibition of the alimentary activity. This suggestion seems to be consistent with numerous findings of other investigators indicating that frontal lobes, and especially their orbital part, are concerned with many autonomic functions, excitatory as well as inhibitory (Bailey and Sweet 1940, Delgado 1948, Delgado and Livingston 1948, Livingston 1948, Livingston et al. 1948, Babkin and van Buren 1951). These physiological facts are supported by well known anatomical evidence showing indirect connection between the frontal cortex and hypothalamus: as Le Gross Clark and M. Meyer (1950) have established there exists an important cortico-subcortical projection from the posterior part of gyrus orbitalis and premotor cortex to ventro-medial nucleus of the hypothalamus. On the other hand, it is well known that this nucleus has an inhibitory effect on the alimentary activity of animals (Brobeck 1946; Anand and Brobeck 1951a, 1951b; Delgado and Anand 1953; Larsson 1954; Anand et al. 1955; Brobeck 1956; Andersson 1956).

Since in our dogs the orbital surface of the frontal lobes, with or without pre-motor cortex, was partly destroyed, it can be assumed that our lesions in the cortical inhibitory region were rather extensive. Consequently, the excitatory alimentary centre took an upper hand over the inhibitory centre and this resulted in an increased alimentary reactivity and in enhanced salivation.

The problem of compensation of postoperative disorders also requires some comment. As we have seen, the increase of the unconditioned salivation in prefrontal dogs in rather transient. This

shows that there are in the cerebral cortex other centres controlling alimentary functions when the frontal lobes are removed. There is ample evidence (Wheatley 1944; Hess, Brügger and Bucher 1949; Adey and Meyer 1952) that the ventro-medial nucleus of the hypothalamus is connected not only with the frontal areas but also with the tips of temporal lobes (area peri-amygdaloidea). Green et al. (1957) have recently reported that after removal of the anterior part of gyrus pyriformis some cats exhibited hyperphagia. This shows that in our case only a part of the extensive cortical representation of this nucleus was destroyed and it explains satisfactorily the rather slight and shortlasting effects resulting from our lesions.

SUMMARY

1. The present paper is concerned with the effects of removal of prefrontal lobes in dogs on the salivation during the alimentary unconditioned reflex (act of eating) and after its cessation.

2. It has been found that after bilateral removal of the frontal lobes involving either gyrus proreus and gyrus orbitalis anterior, or encroaching also upon the anterior part of gyrus precruciatus, gyrus sigmoideus anterior and gyrus compositus anterior, the salivary secretion to the unconditioned stimuli, and in intertrial intervals, is augmented.

3. This augmented salivation is chiefly due to the protraction of the act of eating, consisting in a very careful emptying of the bowl, licking it out and rummaging inside it, though the food is already eaten.

4. The augmented unconditioned and intertrial salivation lasts about 7—14 days following the ablation, and amounts to about 125% of the preoperative level.

5. The transient increase of salivation in prefrontal dogs is presumably brought about by a partial injury in the cortical representation of the alimentary inhibitory unconditioned reflex, located in the prefrontal area.

6. After parietal ablations no change in unconditioned and intertrial salivation is observed.

I am grateful to Prof. J. Konorski for his advice and encouragement in this work.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

COMPARISON OF CLASSICAL AND INSTRUMENTAL ALIMENTARY CONDITIONED REFLEXES FOLLOWING BILATERAL PREFRONTAL LOBECTOMIES IN DOGS

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(Received 29 October 1958)

As shown in our previous papers (Brutkowski et al. 1956; Brutkowski 1957; Ławicka 1957) the removal of the prefrontal lobes in dogs results in a pronounced disinhibition of instrumental as well as classical alimentary inhibitory conditioned reflexes. The findings obtained, however, seemed to indicate a more marked disturbance of the instrumental (motor) than of the classical (salivary) reflexes. The inhibitory salivary conditioned reflexes were not entirely disinhibited after the ablations, whereas the motor conditioned reactions to the inhibitory stimuli did not differ from the reflexes to the excitatory stimuli.

It may be, however, that this disparity resulted from different method of training used in each case. While in instrumental reflexes the motor reaction was reinforced immediately after its performance and therefore the isolated period of the conditioned stimulus lasted not more than 1—2 seconds, in salivary reflexes the isolated period of conditioned stimulus lasted always 20 seconds. In consequence, whereas in instrumental reflexes the conditioned stimuli were „purely" excitatory, in salivary reflexes they possessed a strong admixture of the inhibitory delay.

The purpose of the present work was to study the disinhibitory effect of the prefrontal lesions on the salivary and motor reflexes elicited under identical experimental conditions.

EXPERIMENTAL PROCEDURE

The subjects of the present study were two dogs: No 1, a mongrel, and No 3, a half-bred doberman*.

In both dogs chronic fistulae of parotid gland were made. Then alimentary instrumental conditioned reflexes were trained. They consisted in lifting the right foreleg and putting it on the foodtray to various acoustic stimuli. When these reflexes were firmly established the isolated period of conditioned stimuli was gradually protracted up to 10 seconds in dog 3; in dog 1 it usually did not exceed 5 seconds.

In both dogs inhibitory reflexes were established by differentiation and conditioned inhibition. The conditioned inhibitor preceded the conditioned stimulus by about 10 seconds. Inhibitory stimuli lasted 10 seconds in dog 3, and 5 seconds in dog 1.

The intervals between conditioned stimuli lasted from 3 to 7 minutes.

In both dogs prefrontal ablations were carried out in two stages. In the first operation the prefrontal poles only were removed (gyrus proreus and gyrus orbitalis anterior); in the second operation, the lesion involved also gyrus sigmoideus anterior, the rostral part of gyrus precruciatus and gyrus compositus anterior. All operations were bilateral.

RESULTS

In both dogs the results following each of the two removals were fairly similar. They were as follows:

1. Immediately after operation the instrumental conditioned reflexes were very often abolished. However after several reinforcements of the conditioned stimuli, the motor reaction reappeared spontaneously. On the other hand, salivary reaction was present from the very beginning (Fig. 1), although its magnitude was sometimes diminished. The salivation in intertrial intervals was markedly augmented (B r u t k o w s k i 1957, 1959a).

2. After reestablishment of the instrumental reaction, it appeared not only to the conditioned stimuli but also in the intertrial intervals (B r u t k o w s k i et al. 1956). Each motor reaction in intervals was accompanied by strong salivation.

3. As seen in Fig. 2, 3, 4 and 5 the postoperative disinhibition of the inhibitory conditioned reflexes affects to almost exactly the same degree both the salivary and motor conditioned reactions. In some cases an augmented salivation to the inhibitory stimuli was not accompanied by instrumental reaction. Instead, however, more

* Some results obtained in these dogs were described in other papers of this series (B r u t k o w s k i 1957, 1959a 1959b).

primary alimentary reactions appeared. They were such as licking out an empty bowl on all sides, looking into it, climbing the foodtray and so on. Occasionally, a reverse picture was observed, a very poor salivation being accompanied by the proper instrumental reaction (Fig. 6). This happened mainly when the dog was

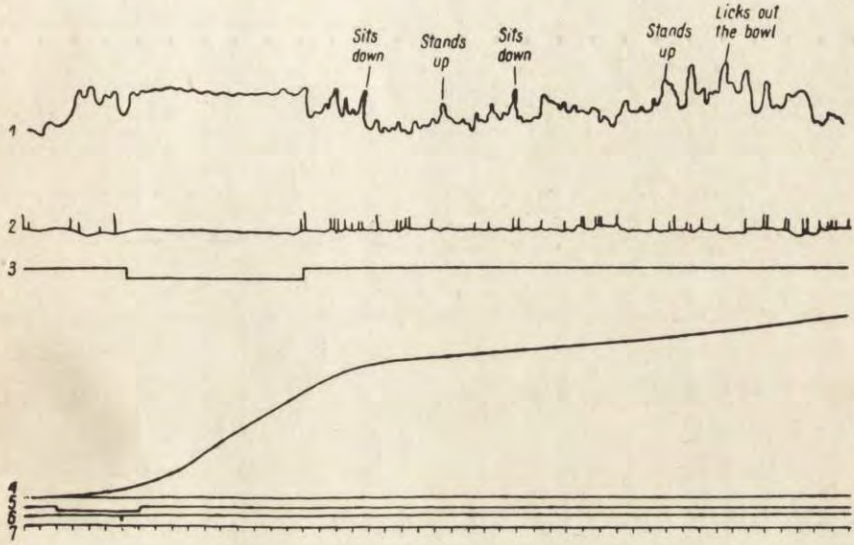

Fig. 1. Records of the motor and salivary reactions in dog 3 on 12th day after the extensive prefrontal ablation

1 — movements of the head; 2 — movements of the right foreleg (no instrumental reactions are performed; minute movements of this leg result from the over-activity of the prefrontal dog); 3 — duration of the act of eating (unconditioned reflex); 4 — salivation: 5 — conditioned stimulus; 6 — the moment of food presentation (unconditioned stimulus); 7 — time (5 seconds)

overactive: among many chaotic movements of the whole body and all four legs, instrumental reaction was also produced.

4. With the lapse of time the inhibitory conditioned reflexes were reestablished, the motor and salivary effects to the inhibitory stimuli disappearing at exactly the same time (Fig. 4 and 5).

DISCUSSION

The method used in the present paper consisted in parallel observation of salivary and motor effects elicited by both excitatory and inhibitory conditioned stimuli. As stated previously by Konorski and Miller (1936), there is in normal dogs a strong

parallelism in the two effects, both in excitatory conditioned reflexes and in the course of training of inhibitory reflexes. According to our data the same parallelism generally holds also after prefrontal ablations when inhibitory conditioned reflexes are markedly disturbed. However, there are some important exceptions from this rule which should be briefly discussed.

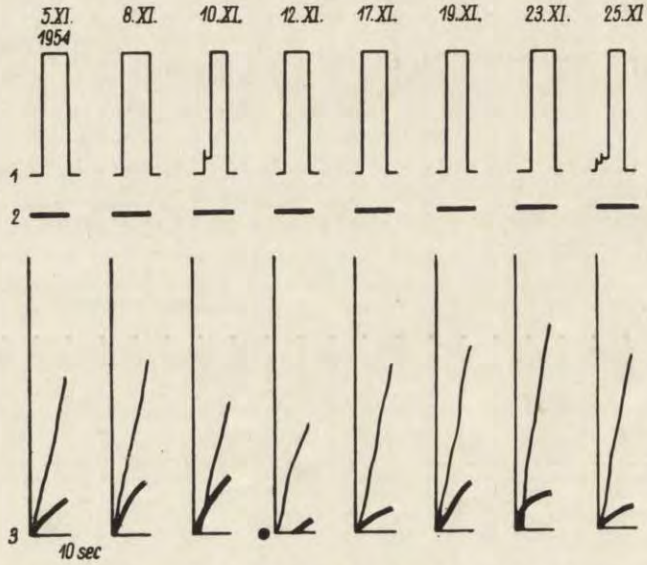

Fig. 2. Kymographic records of the motor and salivary conditioned reflexes in dog 3 in successive pre-operative experiments in which differentiated stimulus was applied

Instrumental reaction to the excitatory stimulus immediately preceding the inhibitory stimulus — 1, and to the inhibitory stimulus (no instrumental movement is performed) — 2. Salivary conditioned reaction to the inhibitory stimulus (heavy line) and to the excitatory stimulus (light line) which preceded it immediately — 3. Abscissae: duration of the conditioned stimulus (in seconds). Ordinates: amount of secreted saliva

Immediately after operation positive conditioned stimuli evoke a slightly diminished salivary reaction whereas the instrumental motor reaction does not appear at all. Observing the general behaviour of the dog we can easily find the cause of this discrepancy. The animal exhibits a very strong d i r e c t alimentary motor reactions to the conditioned stimuli consisting in "attacking" the foodtray, climbing it, incessantly fidgeting at the stand, licking out the empty bowl etc. (Fig. 1). The manifestation of these reactions after prefrontal lesions may be considered as one of the syndroms

of disinhibition of more primary alimentary reactions which inhibit the more "artificial" instrumental movement.

The period of depression of the instrumental conditioned reflex passes, however, very rapidly and the learnt movement appears again. Owing to the postoperative alimentary disinhibition there appears a parallelism between the motor and salivary reflexes: an augmented salivation in the intertrial intervals is accompanied by frequently repeated instrumental movements and, further, at

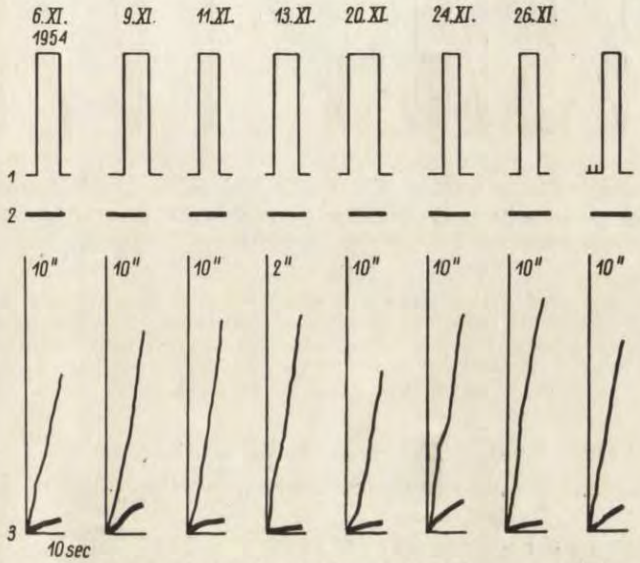

Fig. 3. Kymographic records of the motor and salivary conditioned reflexes in dog 3 in successive pre-operative experiments in which conditioned inhibition was applied

Instrumental reaction to the positive stimulus immediately preceding the conditioned inhibition — 1, and to the same conditioned stimulus following conditioned inhibitor — 2 (no instrumental movement is performed). Salivary conditioned reaction to the conditioned stimulus following conditioned inhibitor (heavy line) and to the same stimulus (light line) which preceded the conditioned inhibition immediately — 3. Abscissae: duration of the conditioned stimulus (in seconds). Ordinates: amount of secreted saliva. Seconds noted at each record refer to the duration of the inhibitory interval between the conditioned inhibitor and conditioned stimulus

every application of conditioned stimuli (both excitatory and inhibitory) the salivary as well as the instrumental reactions are elicited.

In the following weeks or months the disinhibition of the inhibitory conditioned reflexes gradually disappears and compensatory changes may be observed in both conditioned reactions: there

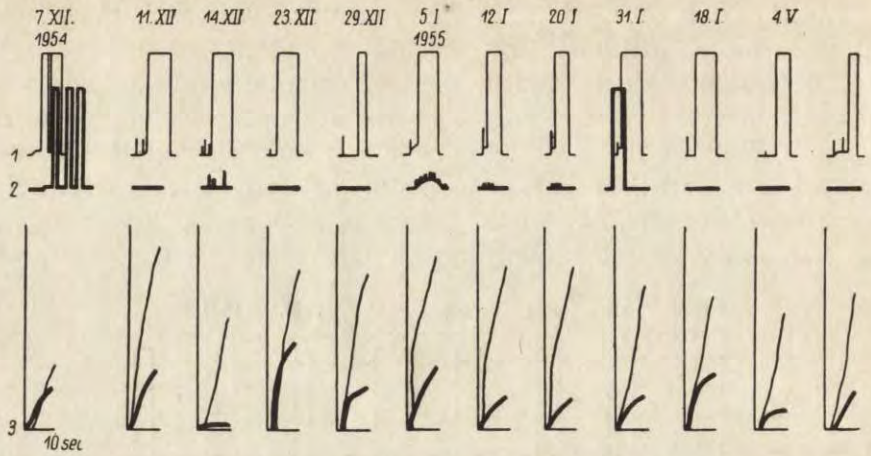

Fig. 4. Kymographic records of the motor and salivary conditioned reflexes in dog 3 in successive post-operative experiments in which differentiated stimulus was applied. (The prefrontal ablation was performed on November 29th 1954)

Instrumental reaction to the excitatory stimulus immediately preceding the inhibitory stimulus — 1, and to the inhibitory stimulus — 2. Salivary conditioned reaction to the inhibitory stimulus (heavy line) and to the excitatory stimulus (light line) which preceded it immediately — 3. Abscissae: duration of the conditioned stimulus in seconds. Ordinates: amount of secreted saliva

is a decrease of salivation as well as a disappearance of instrumental movements to the conditioned inhibitory stimuli and in the intervals.

Sometimes in this period, however, the conditioned inhibitory stimulus may produce either an abundant salivation without any instrumental movement, or, on the contrary, a very poor salivation accompanied by the instrumental movement.

How these discrepancies between both conditioned reactions may be interpreted?

In the first case, in which only the salivary reaction occurs and the instrumental movement is inhibited, the latter is replaced by the other alimentary reactions, such as looking into the bowl, smelling it and intensive licking it out.

The second case in which only the instrumental reaction unaccompanied by the salivary reaction occurs is more complex. As the salivation does not appear here, this seems to suggest that the movement performed is not alimentary in nature. Such a supposition is based upon the fact that prefrontal dogs perform many

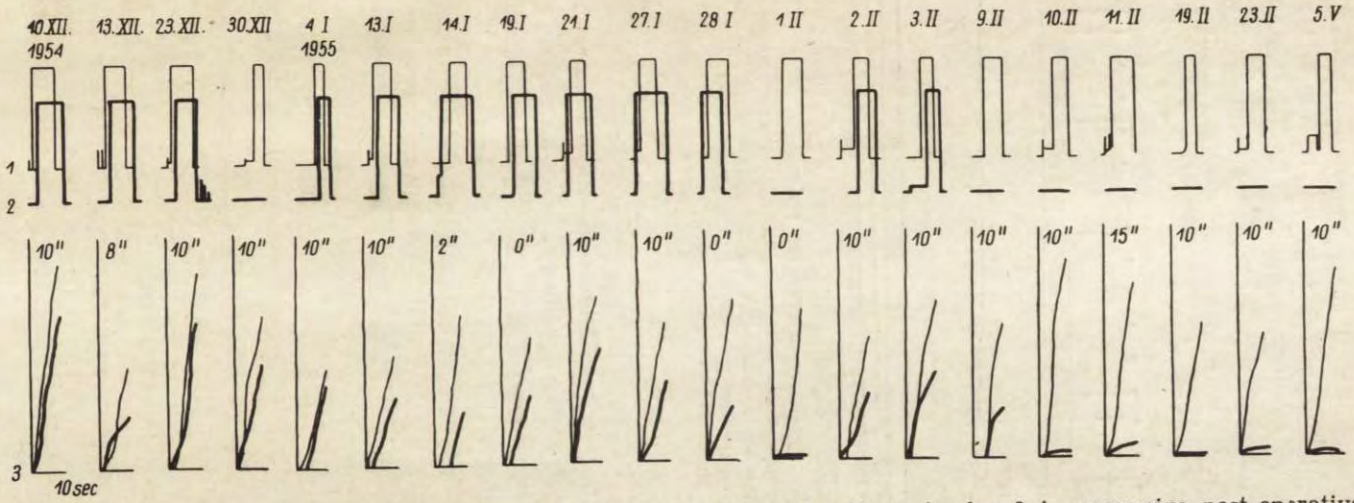

Fig. 5. Kymographic records of the motor and salivary conditioned reflexes in dog 3 in successive post-operative experiments in which conditioned inhibition was applied. (The prefrontal ablation was performed on November 29th 1954)

Instrumental reaction to the positive stimulus immediately preceding the conditioned inhibition — 1, and to the same conditioned stimulus following the conditioned inhibitor — 2. Salivary conditioned reaction to the conditioned stimulus following conditioned inhibitor (heavy line) and to the same stimulus (light line) which preceded the conditioned inhibition immediately — 3. Abscissae: duration of the conditioned stimulus (in seconds). Ordinates: amount of secreted saliva. Seconds noted at each record refer to the duration of the inhibitory interval between the conditioned inhibitor and conditioned stimulus

forced movements which are frequently repeated in spite of their nonreinforcement. These movements may be: lifting and drooping the head, movements of all legs, climbing the foodtray etc. All these obsessive movements do not seem to be connected with the alimentary functions, for they are not directed to the bowl and they are not accompanied by salivation.

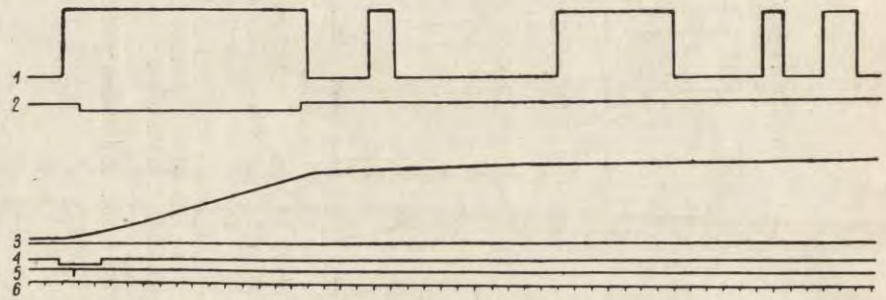

Fig. 6. Records of the motor and salivary reactions in dog 1 on 14th day after the extensive prefrontal ablation

1 — performance of the instrumental reaction (lifting the foreleg and putting it on the foodtray); 2 — duration of the act of eating (unconditioned reflex); 3 — salivation; 4 — conditioned stimulus; 5 — the moment of the food presentation (unconditioned stimulus); 6 — time (5 sec.)

SUMMARY

1. The effects of prefrontal lesions on the salivary and instrumental components of the alimentary conditioned reflexes were investigated both to excitatory and inhibitory conditioned stimuli as well as in intertrial intervals.

2. It was found that postoperative disinhibition affects in nearly the same degree the salivary and motor conditioned reactions, and that they return simultaneously to normal after a lapse of time.

3. There are some cases in which the salivary and motor reactions do not run parallelly, the one of them or the other being disinhibited. The mechanism of these discrepancies is discussed.

I am grateful to Prof. J. Konorski for his encouragement and helpful criticism in the preparation of this paper.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

THE SOLUTION OF A DIFFICULT INHIBITORY TASK (ALTERNATION) BY NORMAL AND PREFRONTAL DOGS

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In the previous papers of this series (Brutkowski et al. 1956, Brutkowski 1957, Ławicka 1957) it has been shown that the impairment of inhibitory conditioned reflexes following the prefrontal ablations in dogs depends on the difficulty of the given inhibitory task. Therefore it was expected that one of the most difficult inhibitory tests used in our experiments consisting in reinforcing the same conditioned stimulus on every second application only (so-called alternation), should be most heavily and even irreversibly impaired. In fact, it was shown that in two dogs after the prefrontal lesions the alternation test was permanently abolished: both dogs after the lobectomy reacted positively to almost every application of the conditioned stimulus.

Since these papers appeared, further experiments concerning the alternation test were performed both on normal and on prefrontal dogs. As our previous conclusions have proved not to be quite correct the new data are presented in this paper and the problem concerning factors influencing the inhibitory capacity of animals is re-examined.

MATERIAL AND METHODS

Experiments were performed on 4 dogs* in a typical sound-proof conditioned-reflex chamber. The dog stood during the experiment on the

* In discussion the findings obtained previously on two other dogs described elsewhere (Brutkowski et al. 1956, Brutkowski 1957) will be also analysed.

stand, the foodtray was placed before him. Food was presented from small bowls moving into position in each positive trial.

Breeds of these dogs, their training and location of lesions are presented in Table I.

Table I

Dogs	Breeds	Conditioned reactions	Operations
No 7(„Iskra")*	mongrel	instrumental	prefrontal
No 3(„ Oberon")*	half-bred doberman	salivary	prefrontal and parietal
No 5(„Misio")*	mongrel	instrumental	prefrontal and parietal unoperated
No 8(„Medzio")*	spaniel	instrumental	

* Some of the results obtained on these dogs were reported in previous papers (Brutkowski 1957, 1959a, 1959b; Ławicka 1957).

The preliminary training of instrumental reflex was as follows. The dog was urged to lift his right foreleg (dogs No 5 and 7) or hindleg (dog No 8) and to put it on a tray at the sound of a conditioned stimulus, the performance of this movement being reinforced by the presentation of food. After several days each applications of the signal almost immediately elicited the learnt movement and was followed by food presentation.

In dog No 3 salivation from glandula parotis was recorded. In order to record salivation the isolated periods of the conditioned stimuli lasted in this dog 10 seconds.

When the preliminary training had been completed the elaboration of alternation began. For this purpose a new auditory stimulus was introduced and reinforced only at every second application. In each experimental session the conditioned stimulus was applied 7 times in dogs No 3 and 8 and 15 times in dogs No 7 and 5, the first application being always reinforced. The intertrial intervals lasted three minutes in dog No 3 and one to two minutes in other dogs.

Operations. In dogs No 5 and 7 gyrus proreus and rostral part off gyrus orbitalis were bilaterally ablated (limited prefrontal ablations). In dog No 3 the lesion included also the anterior part of gyrus precruciatus, gyrus sigmoideus anterior and rostral part of gyrus compositus anterior (extensive prefrontal ablations). In dogs No 3 and 5 the parietal areas weree additionally removed: the lesions included gyrus suprasplenialis, gyruss entolateralis and the anterior part of gyrus ectolateralis.

RESULTS

Prefrontal dogs that succeded in alternation (dog No 7 and No 3)

Two of our prefrontal dogs solved successfully alternation. In both these dogs limited prefrontal ablations resulted in a marked but transient disinhibition of preoperatively acquired differentiations and conditioned inhibition (Brutkowski 1957, Ławicka 1957). After inhibitory reflexes were re-established, the dogs were taught alternation.

Dog No 7*. The training of alternation began in this dog in

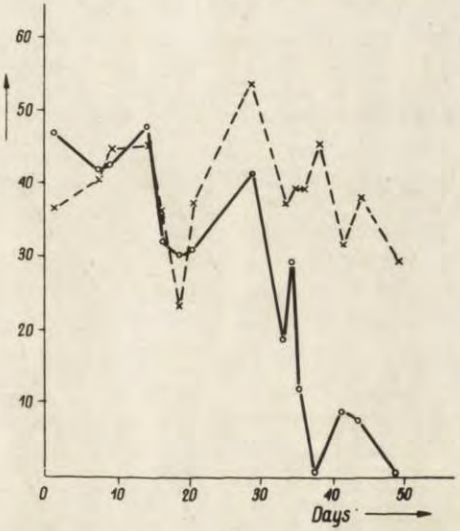

Fig. 1. Preliminary training of the alternation test in the prefrontal dog No 3

Abscissae: successive experimental sessions. Ordinates: mean values of salivary reactions to the conditioned stimulus reinforced by food (broken line) and to the same stimulus without food reinforcement (continuous line)

the 6th month after operation. Already after several days of the alternation training the dog began to inhibit the motor reactions to the unreinforced stimuli. Soon it appeared that the dog was able to react correctly when the intertrial intervals lasted 1 minute but failed to do so when they were protracted to 1 minute 15 seconds. When the dog succeeded at last in mastering alternation

* I should like to thank W. Ławicka for her kind permission to report here the experiments on this dog which were performed by her.

at intervals of 1 min. 15 sec. it was sufficient to prolong the intervals between the stimuli to 2 minutes to produce a new dramatic disorder. When in the following experiments this task was mastered a further prolongation of the intertrial intervals led to a heavy neurotic state.

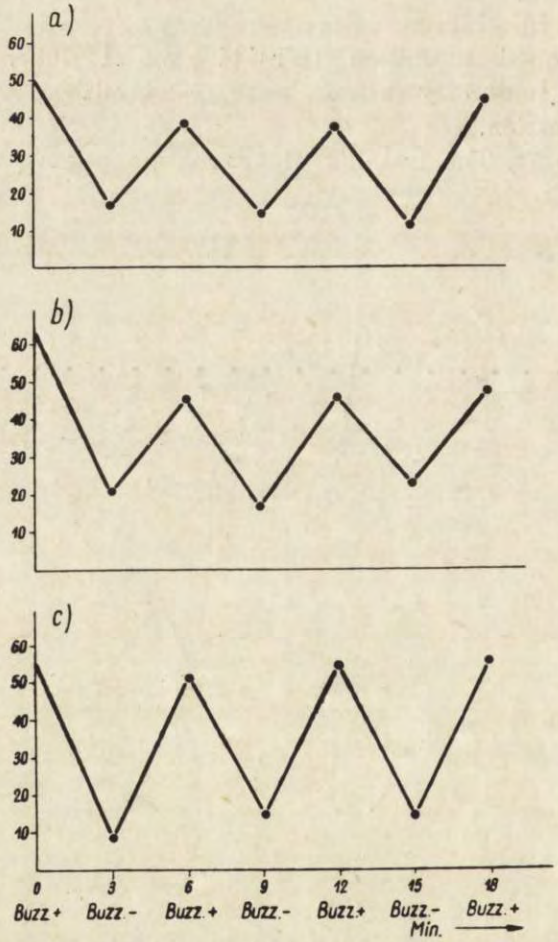

Fig. 2. Alternation in the prefrontal dog No 3

a — in the last period before parietal operation (mean values from 20 experiments); b — immediately after parietal operation (20 experiments); c — after second (more extensive) prefrontal operation when the initial postoperative disturbances were compensated (20 experiments)

Abscissae: time in minutes. Dots correspond to consecutive applications of the conditioned stimulus (Buzzer) alternately reinforced (+) and not reinforced (−) by food. Ordinates: mean values of conditioned salivary reactions obtained in successive experiments

Dog No 3. The alternation training began in the 3rd month after operation. The intertrial intervals were 3 minutes. As seen in Fig. 1 the alternation test was solved by this dog in about 1 month (90 trials). When it was firmly established (Fig. 2a) 5 months after prefrontal ablation the lesion in parietal area was performed. This operation did not change at all alternation (Fig. 2b). After one year a second, more extensive prefrontal ablation was carried out. Similarly to the first prefrontal removal the second one resulted in a marked disinhibition of all well-established inhibitory conditioned reflexes. The alternation was completely abolished (Fig. 3). However, it was sufficient to train this test for about 1 month (90 trials) to restore it again (Fig. 2c).

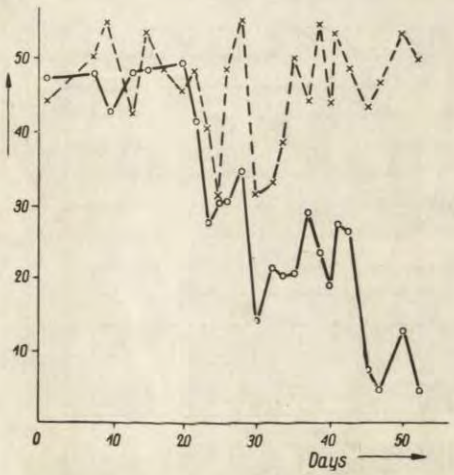

Fig. 3. Alternation in dog No 3 immediately after second (more extensive) prefrontal operation

Abscissae: successive experimental sessions. Ordinates: mean values of salivary reactions to the conditioned stimulus reinforced by food (broken line) and to the same stimulus without food reinforcement (continuous line)

It should be noticed that both these dogs belonged to the Pavlovian "well-balanced type of the nervous system". Their behaviour was always quite adequate and matter-of-fact. Before the operations they mastered very easily several difficult conditioned reflex tasks (differentiations, conditioned inhibition), reacting quietly and readily and never being neurotic. Their general behaviour did not change significantly after operations.

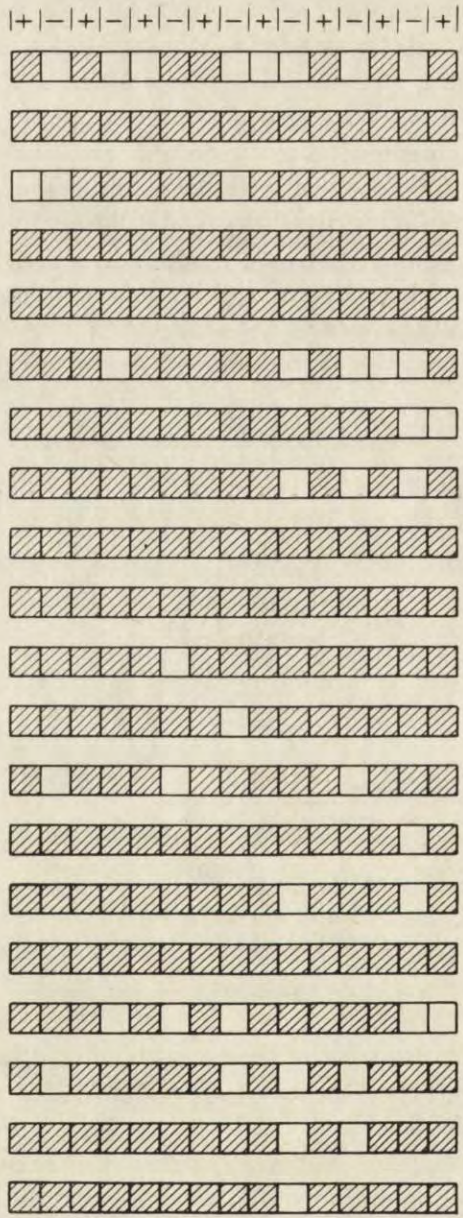

Fig. 4. Alternation in the prefrontal dog No 5 in the last period of training

Black squares denote the positive instrumental reaction to the conditioned stimulus, hollow squares denote inhibitory reaction (no reaction) to the same conditioned stimulus; + denotes reinforced trials; − unreinforced trials

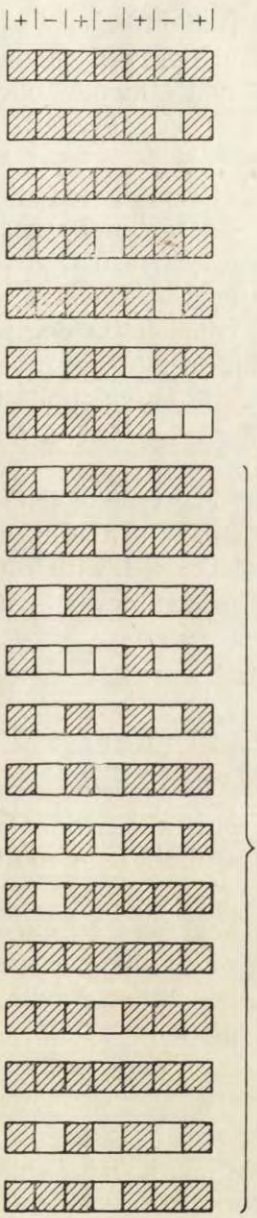

Fig. 5. Alternation in the normal dog No 8 in the last period of training

Black squares denote the positive instrumental reaction to the conditioned stimulus, hollow squares denote inhibitory reaction (no reaction) to the same conditioned stimulus + denotes reinforced trials; — unreinforced trials

Prefrontal dog that failed in alternation (dog No 5)

The experiments with alternation began in this dog in the 21th month after the prefrontal ablation and in the 16th month after the parietal ablation. They lasted 4 months during which the conditioned stimulus was applied about 500 times. The intertrial intervals were 1 minute.

In the first 4 weeks of the training conditioned reactions were positive both in excitatory and inhibitory trials. Afterwards, the reactions of the animal were very irregular and changed in various periods: from time to time the instrumental reflexes disappeared both in positive and negative trials or the dog performed the learnt movement not in positive but just in negative trials. Most often, however, he reacted positively to every application of the stimulus and only in his "bes days" he succeeded in inhibition in the half of the negative trials (Fig. 4).

This dog belonged to the "weak type of the nervous system". He was timid and adapted very slowly to the experimental conditions. Conditioned reflexes were elaborated with difficulty. His excitatory reactions were small or, sometimes, absent. All these features were observed before the brain damage as well as after the prefrontal and parietal ablations.

Normal dog that failed in permanent alternation (dog No 8)

In this dog experiments with alternation lasted 4 month, conditioned stimulus being applied more than 500 times. The intertrial intervals were 2 minutes.

Fig. 5 represents the results from 20 experiments in the last period of training. As seen from it the dog was not able to master alternation permanently. Although from time to time (especially in the period when bromides were administered) the responses were correct, in most trials they were wrong: the dog either performed the learnt movement to the unreinforced stimuli, or, on the contrary, he did not perform it to the reinforced stimuli.

Dog No 9 belonged to the Pavlovian "unbalanced type of the nervous system": the excitatory processes markedly predominated in him over the inhibitory processes. From the very beginning of experiments the dog was extremely excited, though never

aggressive. During the experimental sessions he incessantly whined, barked and howled; these reactions especially increased during the inhibitory stimuli. The instrumental movement (consisting in lifting the right hindleg and putting in on a tray) was repeatedly performed in intervals in spite of very prolonged training. From time to time he developed neurotic disorders in which he refused to take food and became even more restless. Neither differentiations nor conditioned inhibition could be firmly established. It is worth mentioning that under administration of bromides (1—4 grm NaBr at 15 kgm weight of the dog) the dog became quiet and able to solve some tasks.

The training of alternation was accompanied by very marked restlessness which was so strong that further continuation of experiments proved to be impossible.

DISCUSSION

Of the three prefrontal dogs (with or without parietal lesions) presented in this paper two dogs (No 3 and No 7) were able to master alternation. The third prefrontal dog (No 5) failed to do so, similarly to the two other dogs, described elsewhere (Brutkowski et al. 1956, Brutkowski 1957). Thus our previous supposition that alternation is too difficult to be solved by prefrontal dogs proved to be erroneous. On the other hand among 6 normal dogs of this laboratory in which alternation test was trained (experiments performed by S. Brutkowski and W. Ławicka) one dog (No 8 of this paper) was unable to cope with this test at all while another one (dog No 6, described in another paper) solved it with great difficulties.

In view of all these facts the problem arises as to how is it possible that there are normal dogs for which the alternation test is unsolvable, whereas some of the prefrontal dogs (i.e. dogs with severe impairment of the inhibitory processes) are able to establish permanent alternation even after extensive lesions.

As mentioned above alternation is a very difficult inhibitory task. Ławicka has shown that it is a variety of conditioned inhibition in which the presentation of food preceding a conditioned stimulus plays a role of the conditioned inhibitor. The difficulty of this test is due to the fact that a long interval of at least one minute intervenes between the conditioned inhibitor (food intake)

and the conditioned stimulus. For this reason alternation can be easily trained only in those dogs in which the balance between excitatory and inhibitory processes is good. On the other hand, if in a dog the excitatory processes strongly predominate over the inhibitory processes this task is very hard or even impossible to solve.

The dominance of excitatory over inhibitory processes may be due to the innate properties of the animal. It may also be produced by limited or extensive prefrontal ablations. Therefore, in our operated animals the solution of this task depends, on the one hand, on their innate inhibitory capacity and, on the other hand, on the effect of operation.

It seems that our experimental results are in accordance with this explanation:

1. Among our normal dogs there was one which did not master alternation at all (dog No 8) and another which succeded in it only after many months of training (dog 6, B r u t k o w s k i 1957). Both dogs were half-bred spaniels; it is well known that these dogs are by nature very active and excited and suffer an inborn deficit of inhibition. It is, therefore, quite understandable that these dogs had great difficulties with all inhibitory tasks, and particularly with alternation.

2. Among prefrontal dogs there were two in which alternation, established before operation, was totally abolished, and one in which this task could not be mastered after operation. The first of these dogs was No 6, just mentioned, in which alternation before operation was set up with greatest difficulties. The second dog was very lively, brisk and excitable (B r u t k o w s k i et al. 1956). Although before operation he acquired inhibitory conditioned reflexes easily, after operation they were severaly impaired. While differentiation and conditioned inhibition were restored the restitution of alternation proved to be impossible. The third dog (No 5 of this paper) belonged to the so-called weak type. This type is characterised by weakness of excitatory and inhibitory processes, the latter being even more defective than the first. Therefore, the fact that he was not able to master this task seems quite understandable.

3. We now pass to the consideration of the two prefrontal dogs which were able to master alternation, one of them even after extensive lesion. These were the dogs No 3 and No 7 of this paper.

They belonged to the Pavlovian strong and balanced type. Both excitatory and inhibitory processes were excellent in them. After prefrontal ablations their inhibitory processes were temporarily impaired but after a short period they were completely compensated.

In the light of these facts it may be concluded that prefrontal ablations do not change fundamentally the general typological features of the animals. The permanent effect of such ablations consists only in the deepening of inhibitory insufficiency in those dogs in which this insufficiency was present. It may be supposed that the general typological properties of the animals depend rather on subcortical structures and, therefore, the general behaviour remains more or less intact after „purely" cortical lesions. The extensive description of decorticated dogs given by Asratian (1938) seems to support this view.

SUMMARY

1. The present paper is concerned with the problem of how the normal and prefrontal dogs solve the alternation test, i.e. a test in which every second application of the same conditioned stimulus is reinforced by food.

2. The data obtained have shown that:

a) the majority of normal dogs is able to master alternation with greater or lesser difficulty; however, there are some normal dogs which are not able to solve this test even after a very prolonged training;

b) in some dogs which were able to master this test this ability was totally and irreversibly lost after prefrontal ablations;

c) some prefrontal dogs were able to solve this test; in one of these dogs the enlargement of the prefrontal lesion led to temporary abolishment of the alternation which was restored after about a month.

3. The successful performance of the alternation test seems to depend on individual features of animals: the dogs belonging to the Pavlovian strong and balanced type are able to master alternation even after extensive prefrontal lesions; on the other hand, the dogs belonging to the unbalanced or weak type are not able to do so even without surgery.

4. The ablation of prefrontal lobes does not change fundamentally the general typological properties of the dogs.

I am greatly indebted to Prof. J. Konorski for his encouragement throughout this work.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

ELABORATION AND MUTUAL RELATIONS BETWEEN ALIMENTARY AND WATER INSTRUMENTAL CONDITIONED REFLEXES IN DOGS

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(Received 18 November 1958)

The problem of elaboration in animals of two instrumental conditioned reflexes reinforced by food and water respectively, was investigated by a number of authors (Hull 1933, Leeper 1935, Wolfe 1936, Kendler 1946, Heron 1949, Rethlingshafer, Eschenbach and Stone 1951, Bolles and Petrinovich 1954, Mamming 1956). With the exception of Wolfe who performed experiments on chimpanzees, all other experiments were carried out on rats by using a single choice maze with a food and water goal. These experiments have shown that the differentiation of two respective locomotor instrumental responses is possible, i.e. the rats can acquire the ability to react in accordance with the dominant drive. The authors, however, did not investigate the precise interrelations between alimentary and water responses. In this paper we have undertaken a detailed analysis of these interrelations. For this purpose we have used the Konorski and Miller method of instrumental reflexes (Konorski and Miller 1933, Konorski 1948), in which the motor acts performed by the animal are relatively simple and do not involve any change of the position of the body in relation to the goal.

MATERIAL AND METHOD

The experiments were performed on four dogs. The dogs were fed in the animal house twice a day: in the morning, one hour before the experiment, and at midday. They were entirely deprived of water in the

animal house and were given an additional portion of salt in their morning meal. The amount of food and salt given was balanced so that during an experimental session the animal could take the same number (20—30) of standard food and water portions: one food portion consisted of a 20 gr. bread cube, one water portion of 25 ml of water. Just before an experiment the dogs were allowed either to drink ad libitum (hunger-driven experiments) or to eat ad libitum (thirst-driven experiments), or both (no-drive experiments), or else neither food nor water was given (double-drive experiments).

The experiments were performed in a normal experimental chamber. The portions of bread and water were conveyed through a tube to a dish placed before the animal. The dish was so constructed that it could be immediately emptied if the dog refused to accept either food or water. A particular movement performed by the animal was reinforced by food, while another movement was reinforced by water. The training of a given movement was carried out by reinforcement either of passive flexion of the leg or of spontaneous barking.

RESULTS

Single-drive experiments

The course of elaboration of the two movements (alimentary and water) were as follows. During the first stage of experiments all four dogs were trained to perform the alimentary movement. In this period the dogs were given water ad libitum just before every experimental session (hunger-driven experiments). When the alimentary movement was firmly established the dog performed it with maximal frequency, i.e. as soon as a cube of bread was eaten the next movement appeared. Only at the end of the experiment when the dogs were partially satiated were the movements performed with longer intervals.

After several weeks of such training the hunger-driven experiments were stopped and the thirst-driven experiments were begun. Now the animals were given food ad libitum before every experimental session and another movement was provoked and reinforced by water. In the beginning of this series the animals often performed alimentary movements although they refused to take food presented after their performance. These movements appeared especially at the beginning of each session, or when the water movement, not being fullsized, was not reinforced. Gradually the alimentary movements in the thirst-driven experiments became less frequent and eventually they disappeared altogether.

When the water movement was firmly established the thirst-driven experiments were again replaced by hunger-driven experiments. Now in the beginning of the series the water movements tended to appear (especially in the beginning of each experiment) although water was not accepted by the animal.

Such hunger-driven and thirst-driven experiments continued to be performed in each dog in longer or shorter series or alternately. The experiments on each dog will be described separately.

Dog No 1 (alimentary instrumental reaction was barking; water instrumental reaction was lifting of the right foreleg). After a few hunger-driven and thirst-driven series of experiments both instrumental reactions were firmly established and differentiated. In consequence, when the dog was thirsty he performed from the very beginning of the experiment only the water movement (lifting of the leg), and when he was hungry, he performed only the alimentary movement (vocal reaction). Only in those experiments which succeeded after a long series of experiments with the other drive, did the dog perform at the beginning of the session the wrong movement which was very soon replaced by the proper one.

Dog No 2 (alimentary movement was barking; water movement was lifting of the right hindleg). The differentiation of the two instrumental reactions was in this dog very difficult and in spite of a prolonged training it was easily disturbed. From time to time either alimentary or water movement predominated and tended to appear also in experiments with the opposite drive. In order to restore the "balance" between the two movements long series of experiments were conducted with the drive corresponding to the weaker movement, or the intensity of the corresponding drive was increased. After such procedure the differentiation between two movements could be significantly improved (Fig. 1.).

Dog No 3 (alimentary movement, lifting of the right foreleg; water movement, lifting of the left hindleg) and dog No 4 (alimentary movement, barking; water movement, lifting of the right hindleg). In both these dogs the differentiation of two movements, in spite of a very long training, was never attained. Moreover in the course of experiments one of the two movements began to predominate over the other one: in dog No 3, lifting of the right foreleg (alimentary movement) prevailed over lifting of the hind-

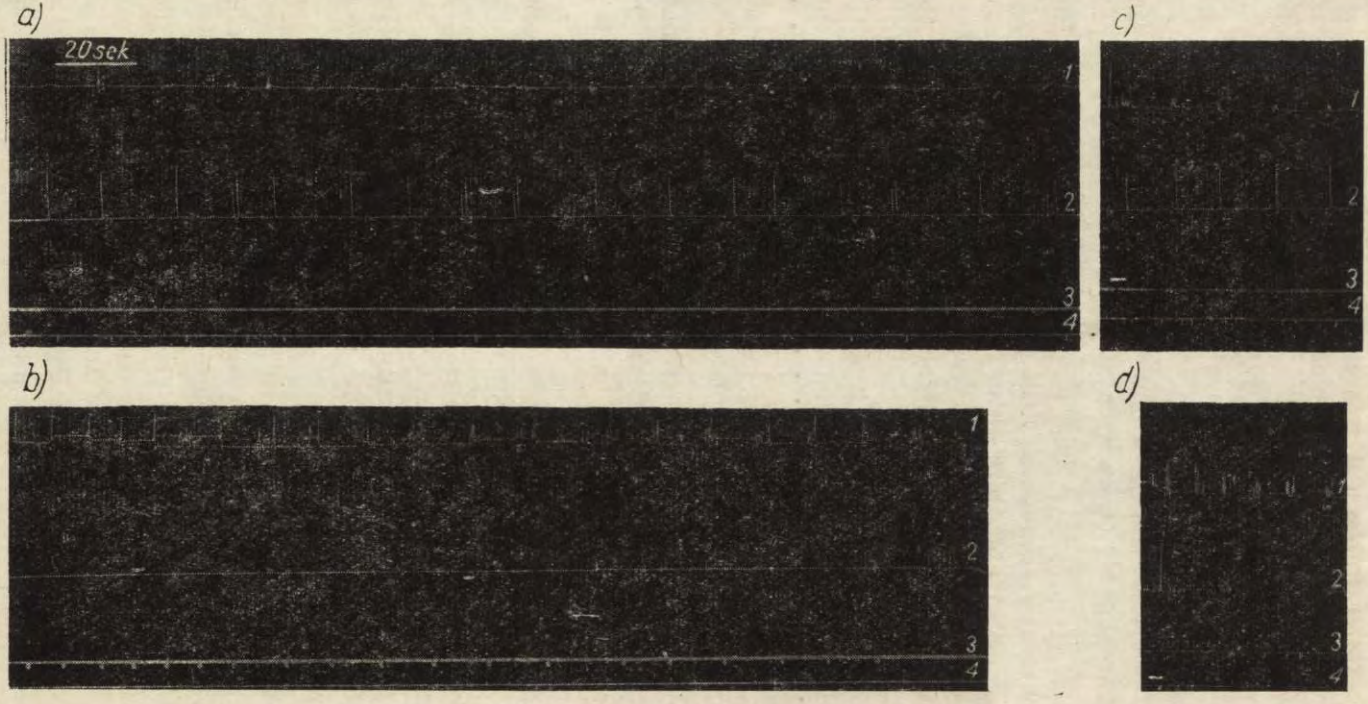

Fig. 1. Single-drive experiments in dog No 2

Each graph represents one experiment or an initial part of it. 1 — water movement (lifting of hindleg); 2 — alimentary movement (barking); 3 — water reinforcement; 4 — alimentary reinforcement. a and c — hunger-driven experiments; b and d — water-driven experiments. In a and b — only proper movements appear. In c and d — at the beginning of the experiment a wrong movement appears and the animal does not accept reinforcement (−). In a and c — small undulations of 1 line correspond to jumping movement accompanying each barking

leg (water movement); in dog No 4 lifting of the right hindleg (water movement) prevailed over barking (alimentary movement). The dogs performed usually the predominant movement in the beginning of experiments of both kinds and, when it was a wrong movement, it was usually replaced by the proper one only after one or more trials. The intensive training of the weaker movement (by conducting experiments with the corresponding drive) had in these dogs only a temporary effect.

Here it must be added that in all dogs however firmly the two movements were differentiated one from the other, the wrong movement immediately appeared when the proper movement was once or a few times not reinforced. Reinforcement offered after the performance of this movement was, as a rule, not accepted, and the animal again returned to the proper movement.

It must also be noted that sometimes in the end of the experiment the dogs started to perform the movement connected with the other drive. The corresponding reinforcement was then in most cases accepted.

No-drive and double-drive experiments

In the no-drive experiments the dogs performed only a few movements of both kinds (food and water not being usually accepted) or did not perform any movement at all.

In the double-drive experiments the dogs performed both movements. In dog No 1 the priority of alimentary or water movement was quite accidental. In dog No 2 the first movement was usually that which was recently trained. Dogs No 3 and 4 began a session with their dominant movements.

As a rule, the movement which first appeared (independently of whether it was alimentary or water movement) was repeated many times in succession, and only then was it replaced by the second movement which again was repeatedly performed. Usually, the whole experiment consisted only of two such series of movements: first, the animal performed only one movement till complete alimentary or water satiation, then it passed to the second movement till the complete satiation of the second drive (Fig. 2a). Sometimes however each series of movements was not so protracted and the animal shifted several times from one movement to the other.

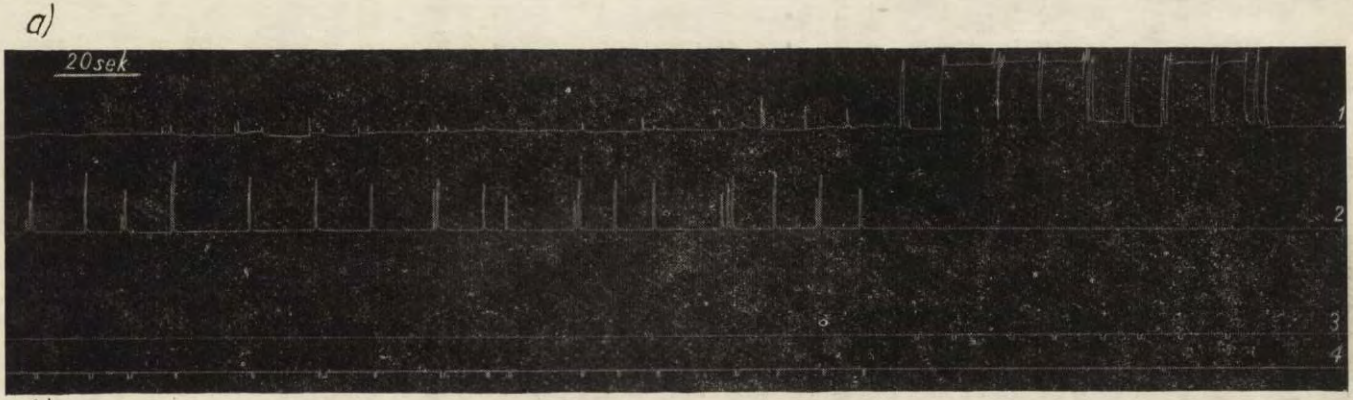

Fig. 2. Double-drive experiment in dog No 1 (a) and middle part of double-drive experiment in dog No 4 (b)

1 — water movement (in a lifting of the foreleg and putting it on the foodtray, in b lifting of the hindleg); 2 — alimentary movement (barking); 3 — water reinforcement; 4 — alimentary reinforcement. In a the transition from alimentary series to water series is almost abrupt. In b the transition from water series to food series occurs after two not accepted water reinforcements

In those dogs in which differentiation between two movements was precise the transition from one movement to another was rather easy and it took place either abruptly (Fig. 2a) or after a short interval in which either the animal stood quietly or he performed some abortive movements of both kinds. Those animals in which the differentiation of movements was bad usually began to perform the other movement only when they had not accepted several times the reinforcement connected with the first movement (Fig. 2b).

The just described natural course of an experiment could be deliberately changed in two ways. First, if a given movement performed by the animal was once or several times not reinforced, the dog very easily shifted to the second movement, and when the respective reinforcement was accepted, which was as a rule

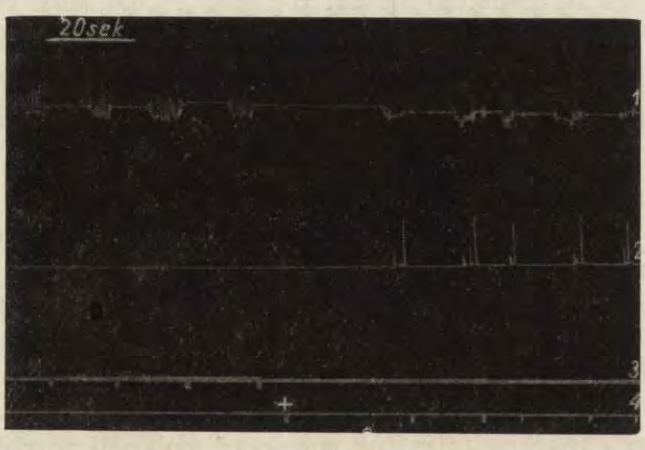

Fig. 3. Part of double-drive experiment. Deliberate shifting from water series to alimentary series

1 — water movement (lifting of the hindleg); 2 — alimentary movement (barking); 3 — water reinforcement; 4 — alimentary reinforcement. The presentation of food gratis (+) causes transition from water series to alimentary series

what happened *, the other series of movements was started. Secondly, if immediately after the dog had accepted the reinforcement, reinforcement of another kind was given ("gratis") and accepted *, the dog began to perform the series of movements con-

* Although the animal was in these experiments under both food and water deprivation, it sometimes happened that shifting of the reinforcement caused the animal not to accept it at once.

nected with the last reinforcement (Fig. 3). This trick was almost always unfailing in the dogs with precise differentiation of movements but it failed about 50% of times in dogs with poor differentiation.

It was interesting to see, whether or not the repetitive performance of one and the same movement would be also preserved, when some intervals were interposed between them. In this order in two dogs (No 1 and No 3) the sporadic conditioned stimulus (buzzer) was introduced. The preliminary training took place in single-drive experiments. When the instrumental reactions to buzzer were strongly established and in intervals they almost completely disappeared, the double-drive experiments were conducted. It appeared that the repeated performance of the given movement was under these conditions fully preserved, i.e. with the new application of the stimulus the animal returned to the same movement it performed at the preceding trial. Again after being satiated in respect to one drive he began to perform the other drive movement.

DISCUSSION

There is ample experimental evidence which shows that in instrumental conditioned reflexes the conditioned connections are formed on the one hand between the centres of exteroceptive stimuli (sporadic stimuli, experimental situation) and the centre of the motor response, and on the other hand between the centre of the reinforcing stimulus* and the motor centre (Wyrwicka 1952 and others). According to this it may be accepted, that in our experiments in the course of elaboration of both alimentary and water instrumental reflexes the following conditioned connections are established (Fig. 4a): 1) between the centre of exteroceptive stimuli (E) and the centres of both movements M_a and M_w); 2) between the alimentary centre (A) and the centre of alimentary movement (M_a); 3) between the water centre (W) and the centre of water movement (M_w), (cf. Hull 1943). As seen from this figure the connections $A \rightarrow M_a$ and $W \rightarrow M_w$ have a differentiating significance for the animal and will be further called specific connections, while the connections $E \rightarrow M_a$ and $E \rightarrow M_w$ have

* In our considerations the alimentary and water centres are regarded as a whole and their anatomical and functional complexity is neglected.

no differentiating character and will be called unspecific connections.

In the above scheme it has been assumed that the alimentary centre and the water centre are quite separate i.e. that they do not possess any common part. There is however experimental evidence that this is not so. If we compare the results of our experiments with those reported by Konorski and Miller (Konorski and Miller 1936, Konorski 1939) on the alimentary and defensive conditioned reflexes, we see, that there is a significant difference between the interrelations between the respective groups of reflexes. While there is hardly any transfer between alimentary and defensive reflexes, the transfer between alimentary and water reflexes is quite considerable. In fact the differentiation between two movements was in nearly all our dogs rather difficult and in some of them it was never firmly established. The generalization of these two kinds of reflexes which has also been stressed by other authors (Webb 1949, Brandauer 1953, D'amato 1955)

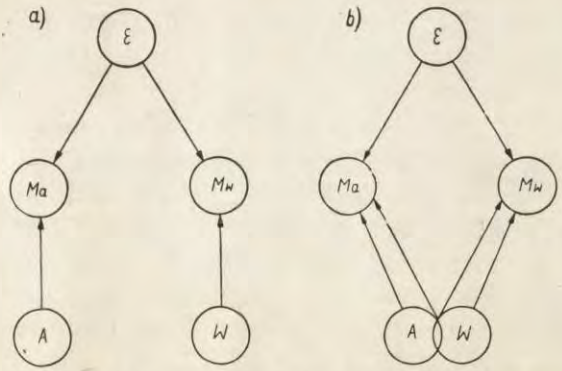

Fig. 4. The hypothetical mechanism of interrelations between alimentary and water conditioned reflexes

E — centre of exteroceptive stimuli; Ma — centre of alimentary movement; Mw — centre of water movement; A — alimentary centre; W — water centre. Arrows — conditioned connections

is most probably due to the fact that both reinforcements have a positive character. In consequence, we may suppose that two centres responsible for the alimentary and water reflexes respectively are partially overlapping (Fig. 4b): the connections from the common part of these centres are unspecific and contribute to the generalisation of both conditioned reflexes.

The schemes represented in Fig. 4 seem to explain satisfactorily all the facts reported in this paper. To begin with, suppose that in a given experiment the animal is hungry and not thirsty. In this case the water centre is blocked and no impulses are sent from it to the centre of the water movement. Therefore the centre of the alimentary movement is bombarded by impulses both through the specific connections from the alimentary centre and through unspecific connections from the centre of the exteroceptive stimulus, whereas the centre of water movement is bombarded only by unspecific connections coming from the centre of the exteroceptive stimulus and from the common part of the alimentary and water centres. The result is that, other things being equal, the centre of the alimentary movement is excited stronger than the centre of the water movement and, therefore the alimentary movement will appear. If, however, for one reason or another the impulses running through the unspecific connections to the centre of the water movement are much more abundant than those running to the centre of the alimentary movement, the water movement will appear instead of the alimentary movement. This happens either if the water movement was previously trained for a long time, or if the animals has a prevailing tendency to perform this and not that movement because of its greater easiness.

If the animal under the hunger drive performs the water movement he will not accept the food reinforcement. Therefore, this movement is partially extinguished, i.e. some inhibitory connections are added to the excitatory ones. In consequence, the connections to the centre of the alimentary movement gain predominance and this movement is performed. The performance of the alimentary movement and its reinforcement leads on the one hand to the further fixation of this movement and on the other hand to the stronger excitation of the alimentary centre. As a result the tendency to perform the alimentary movement is further increased and the probability of the performance of the water movement diminishes. This movement can now appear only in this case when the alimentary movement will not be reinforced.

Of course the same considerations may be applied in the case of water-driven experiments.

In the case of no-drive experiments both unconditioned centres are blocked. In consequence the centres of movements are

excited only by unspecific connections from the centre of the exteroceptive stimulus. This may be either insufficient to provoke the movements, or if they appear, the nonaccepting of reinforcement leads to their rapid extinction.

On the other hand in the double-drive experiments both unconditioned centres are active and the centres of both movements are excited by the unspecific connections as well as by the specific ones. In these conditions the performing of the first movement depended either on the better fixation of one of the movements, or, when the fixation of both movements was more or less equal, it was quite accidental. But when the first reinforcement was accepted, the excitation of the corresponding unconditioned centre grew and this led to repetition of the same movement. The dog, as a rule, stopped performing this movement and shifted to the other one only if a given drive was satiated. When the dog had a preferential movement, the shifting to the other one occurred only after the reinforcement to this movement was not accepted, and it was partially extinguished. The natural sequence of the movements could be disturbed at any time either by non reinforcing of the performed movement or by excitation of the second uconditioned centre by presenting gratis of corresponding reinforcement. The last method however worked without fail only in those dogs in which specific connections were strong.

SUMMARY

1. In four dogs in the same experimental situation two different instrumental conditioned reflexes were established by food and water reinforcement.

2. In two of these dogs the differentiation between both movements became perfected and they performed in hunger-driven experiments only the alimentary movement and in thirst-driven experiments only water movement.

3. In two other dogs the differentiation was never very precise due to „natural" preference of one of the trained movements.

4. In the no-drive experiments the dogs either did not perform any movement at all or performed only a few movements.

5. In double-drive experiments both movements were performed. The dogs started with one of them, repeated it in a long

series, then shifted to repeated performance of the other movement. When in the course of such series the second reinforcement was presented, the animal started to perform repeatedly the corresponding movement.

6. The non-reinforcement of a given movement led to the appearance of the other one.

7. The mechanism of the interrelations between the two reflexes is discussed.

The authors wish to thank Prof. J. Konorski for his most valuable criticism and helpful advice.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

FATIGUE OF ACID CONDITIONED REFLEXES

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(Received 10 November 1958)

The problem of whether or not conditioned reflexes, when elicited repeatedly in short intervals, are subjected to fatigue was investigated extensively in Pavlov's laboratories by a number of authors about 30 years ago (Fedorov 1944, Galperin 1941, Lindberg 1949, Petrova 1937, Solovejcik 1940, Stroganov 1929, Zuravlev 1954). The routine experimental procedure was that a given conditioned stimulus was applied (with reinforcement) many times in succession and its conditioned reaction was observed. It was shown in these experiments that in the course of repetition of the conditioned stimulus conditioned reactions gradually decreased and eventually dropped to zero. This phenomenon has been called "extinction with reinforcement" and was considered as due chiefly to the fatiguability of the conditioned reflex (Pavlov 1940, 1949).

Since all the experiments concerned with this problem were performed on alimentary conditioned reflexes, the diminution of the conditioned reflex could be understood as the result of the gradual satiation of the animal during the experiment (Stroganov 1954, Wyrwicka 1950 and others). Therefore it seemed necessary to reinvestigate this problem taking into consideration such conditioned reflexes which by repetition do not modify considerably the excitability of the centre of unconditioned reinforcing stimulus. For this reason in the present study introduction of acid into the animal's mouth was used as the reinforcing stimulus. According to the large experience of the Pavlovian laboratories introduction of acid evokes a strong salivary defensive reaction which can become as easily

conditioned as salivary alimentary reaction. Because in our experiments salivation was used as a conditioned test-reaction, a comparison of our results with those of the previous authors was possible.

GENERAL COURSE OF EXPERIMENTS

The experiments were performed on three male dogs, one of them castrated, in a usual half-sound-proof conditioned-reflex chamber. Conditioned reflexes were established to various acoustic and visual stimuli. These stimuli were reinforced by introduction of 10 ml of 1% acetic acid into the animal's mouth. The introduction of acid lasted a few seconds and was accomplished by the small metal tube fixed to the mouth of the dog by the Mendeleiev wax (cf. Podkopaiev 1936). Salivation was recorded by means of Kozak's volumetric apparatus (Kozak 1950).

When conditioned reflexes were firmly established the series of experiments proper concerning their fatiguability were carried out. In these experiments one and the same conditioned stimulus was repeatedly applied with intervals of either 6 or 3 minutes or 1.5 minutes. The number of trials in each experiment was either 8 or 24. The isolated period of conditioned stimuli lasted in different series of experiments from 15 to 45 seconds.

RESULTS

When the intervals between conditioned stimuli were either 6 min. or 3 min. the level of the conditioned reactions remained the same throughout the experimental session, even in those experiments in which 24 trials were given (Fig. 1a)*.

When the intertrial intervals were 1.5 min. the results were different in different dogs. In two of them conditioned reflexes remained also on the same level throughout the experiment, except that the reaction in the second and third trial was usually hypernormal. The rate of conditioned salivation was the same as with longer intervals and it did not change when the isolated period of conditioned stimuli was protracted from 15 sec. to 30 sec. In the third dog (the castrated one) the salivation in the first few trials diminished rapidly till about 65% of the normal value and remained on the depreseed level till the end of the experiment (Fig. 1b). This phenomenon was repeatedly observed in many experiments during several months, and then it disappeared. When the isolated period of conditioned stimuli was protracted from 15 to 30 seconds, the

* The conditioned reflex to the first stimulus is usually somewhat reduced, due to the previous inactivity of the salivary glands (cf. Bruner and Kozak 1954).

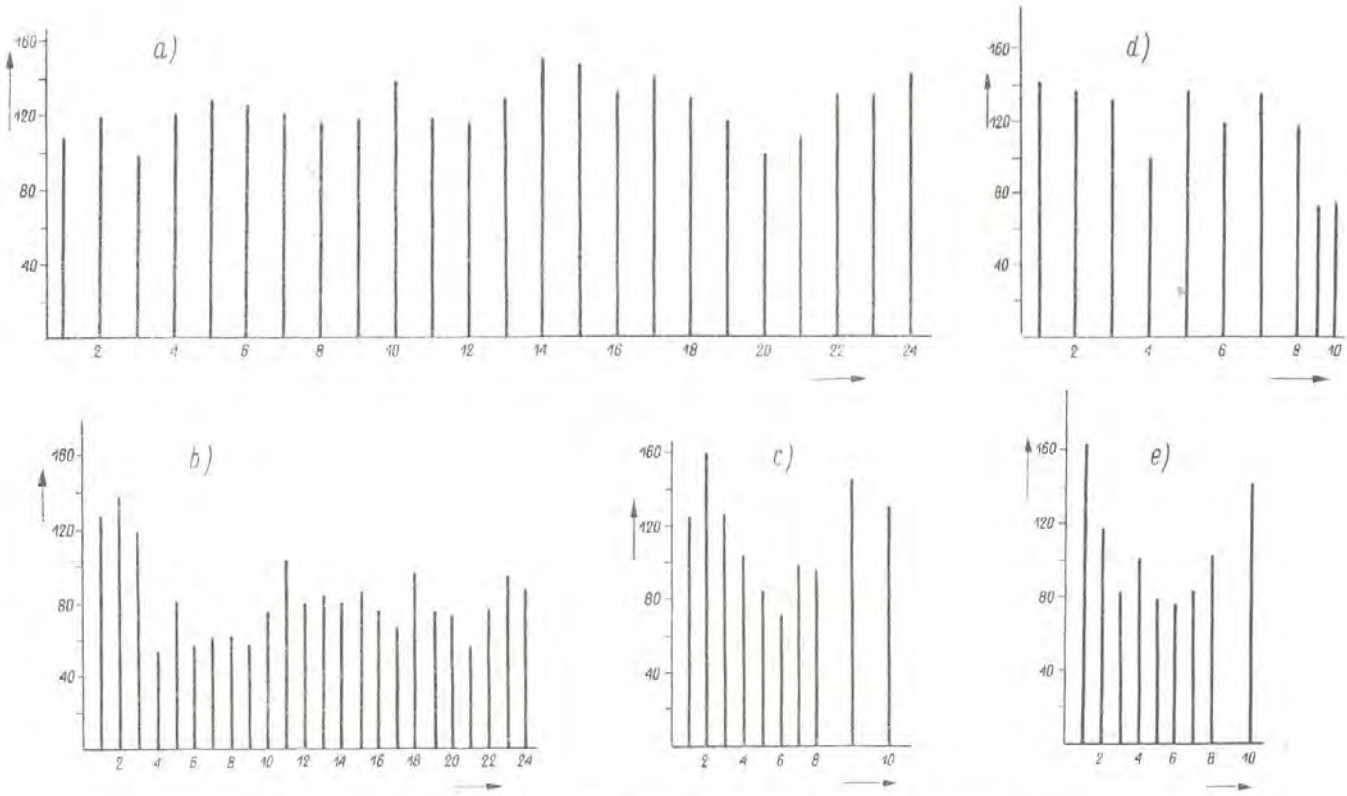

Fig. 1. Magnitude of conditioned reflex to the repetitive application of conditioned stimulus in relation to the intertrial intervals

Each graph represents the course of one experiment. Abscissae: trials. Ordinates: amount of salivation in grades of scale — 134 grades of scale corresponds to 1 ml of salive. Each vertical line represents conditioned salivation in particular trial. In all experiments the strong stimulus (rattle) was used. Isolated periods of conditioned stimuli, 30 sec. Distances between lines correspond to 3 min. or 1,5 min. intervals. a — typical experiment with 3 min. intervals and 24 trials; b — typical experiment with 1,5 min. intervals and 24 trials; c — prolongation of the intervals from 1,5 min. to 3 min. from the 9th trial; d — shortening of the intervals from 3 min. to 1,5 min. from 9th trial; e — experiment with 1,5 min. intervals, in 9th trial unconditioned stimulus alone was given.

diminution of conditioned reflexes reappeared, and again it lasted for several months. After this time the reflexes again became normal, and then further protraction of the isolated period to 45 sec. was ineffective.

Because the two periods, in which the repetition of the conditioned stimulus with 1.5 min. intervals led to the diminution of the reflex, were longlasting, it was possible to study this phenomenon in some detail. First, it was noticed that when in the course of an experiment with 1.5 min intervals, these intervals were protracted till 3 min., the conditioned reflex immediately regained its normal value; in the first trial it could be even hypernormal (Fig. 1c). On the other hand, if in the experiment with 3 min. intervals (in which conditioned reflexes were normal) the 1.5 min. intervals were

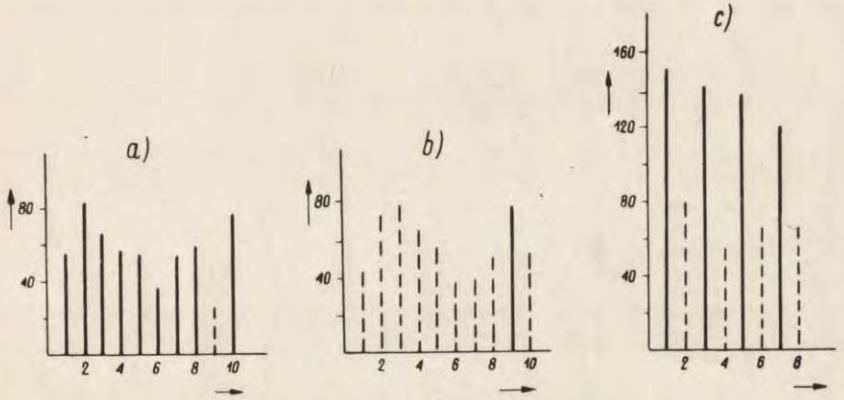

Fig. 2. The magnitude of conditioned reflexes in relation to the strength of stimuli

Continuous lines — strong stimuli (rattle). Dashed lines — weak stimuli (rotating fan). Other explanations as in Fig. 1. a — experiment with strong stimuli, in 9th trial the weak stimulus is given instead of the strong one; b — experiment with weak stimuli, in 9th trial the strong stimulus is given instead of the weak one; c — strong and weak stimuli are alternately applied .In a and b isolated periods of conditioned stimuli are 15 sec., in c — 30 sec.

introduced, the magnitude of the reflexes was immediately diminished (Fig. 1d). If in the experiments with 1.5 min. intervals, in one trial acid was introduced into the mouth without any conditioned stimulus, in the next trial the conditioned reflex was also normal (or hypernormal) (Fig. 1e).

As in this dog two conditioned stimuli were applied, namely the sound of the rattle (strong stimulus) and the sight and sound of the rotating fan (weak stimulus), it was possible to compare the properties of these two stimuli in respect to their repetitive application. If the rattle was repeatedly applied with 1.5 min. intervals and then fan was given, instead, in a single trial, its effect was also diminished, but the next application of the rattle produced a normal effect (Fig. 2a). On the other hand if fan was repeatedly applied, and in one trial rattle was substituted, the effect of this stimulus was normal, or hypernormal, while the effect of fan applied in the next trial continued to be diminished (Fig. 2b). In consequence of these relations, if rattle and fan were applied alternately

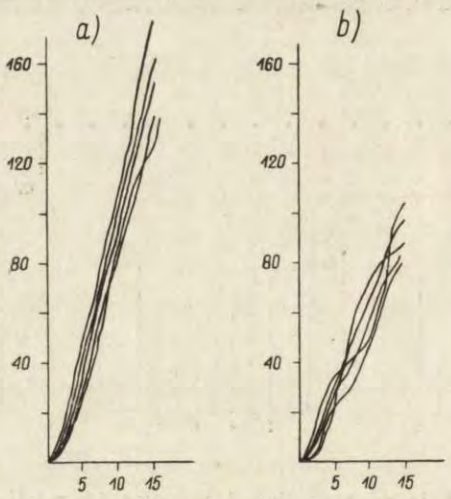

Fig. 3. Course of salivation during the conditioned reaction in a normal (a) and fatigued (b) state

Abscisae: time in seconds. Ordinates: salivation in grades of scale. In a and b fire curves of salivation are superimposed from an experiment with 3 min. intervals and with 1.5 min. intervals respectively

in 1.5 min. intervals, the reflex to rattle remained normal while the reflex to fan was diminished (Fig. 2c).

When analysing in this dog the course of salivation to the conditioned stimulus applied with 1.5 min. intervals in particular trials, the following differences from the normal course of salivation could be observed: i, the rate of salivation was generally slower than in trials with longer intervals; ii, while normally the rate of saliva-

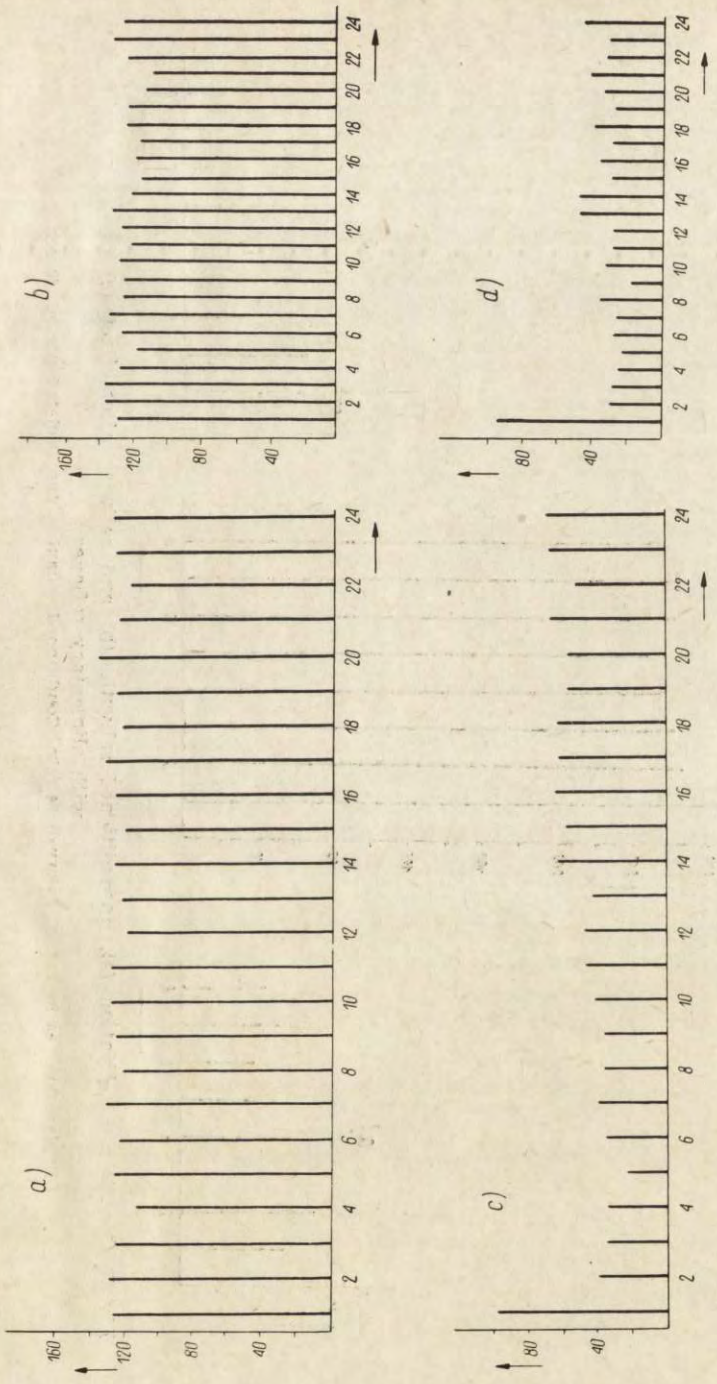

Fig. 4. Unconditioned salivation and salivary after-effect in relation to intertrial intervals

Explanations as in Fig. 1. a) unconditioned salivation with 3 min. intervals; b) unconditioned salivation with 1,5 min. intervals; c) salivary after-effect with 3 min. intervals; d) salivary after-effect with 1.5 min. intervals

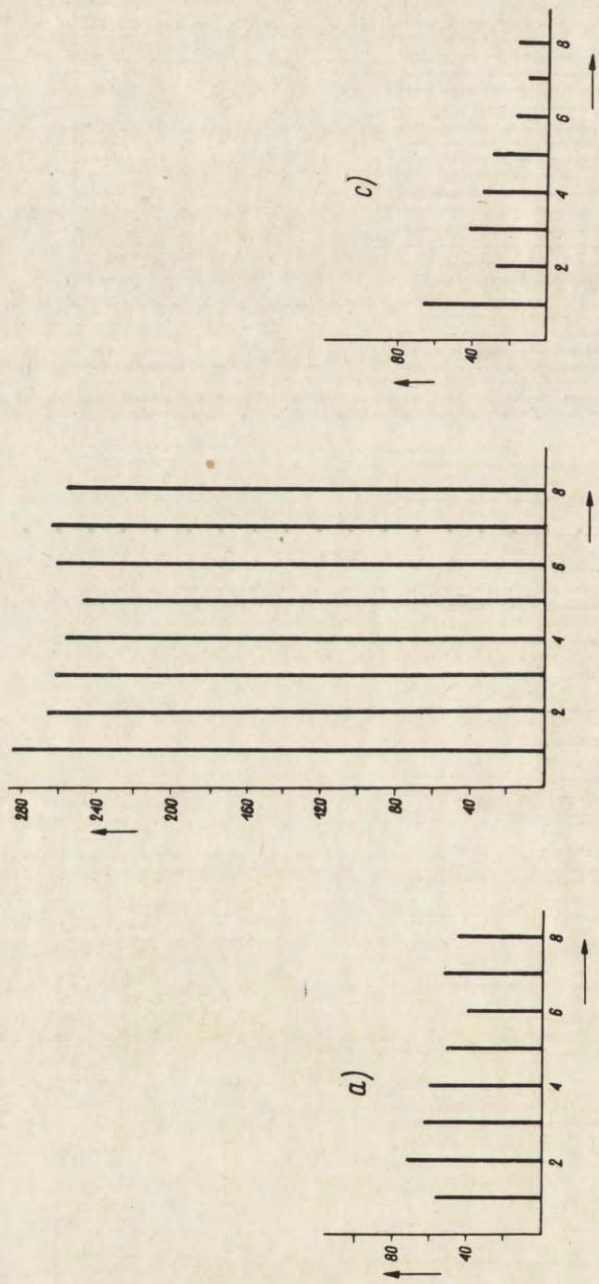

Fig. 5. Conditioned salivation (a), unconditioned salivation (b) and salivary after-effect (c) in an experiment with alimentary reflexes

Explanations as in Fig. 1. Intervals, 4 min. Conditioned stimulus, ton. isolated period, 30 sec.

tion increased during the course of the stimulus, here this increase was not observed; iii, the course of salivation was generally more irregular than with 3 min. intervals (Fig. 3).

As far as unconditioned reflexes are concerned, their course was much the same in all three of our dogs. The unconditioned reflexes were as a rule not diminished, irrespective of the intervals used; only in some experiments with 1.5 min. intervals a slight decrease of unconditioned reflexes took place. On the other hand the salivary after-effect of the unconditioned reflex * underwent in all our dogs considerable changes in the course of the experiment. It was the strongest in the first trial and then rapidly diminished, often reaching its minimum in the third trial. Then, in experiments with 6 and 3 min. intervals, the salivary after-effect slowly increased not attaining, however, the initial value. In experiments with 1.5 min. intervals it remained as a rule on the low level till the end of the experiment (Fig. 4 a and b).

DISCUSSION

According to our experiments the acid conditioned reflexes measured by their salivary effect must be considered as very resistant to fatigue. In spite of the fact that conditioned stimulus was repeated at short intervals many times, and its isolated period lasted as much as 30 sec., two of our dogs did not show any signs of fatigue. The third dog (the castrated one **) manifested fatigue only as a temporary phenomenon when the intertrial intervals were very short (1.5 min.).

The course of the diminution of the conditioned reflexes due to fatigue was in this dog very characteristic. The conditioned reflexes fell to the level of about 65% of their normal value almost immediately, and did not decrease further even after many aplications of the stimulus. The prolongation of the interval immediately restored the reflex. On the other hand, the transition from 3 min. intervals to 1.5 min. intervals caused an abrupt decrease of the conditioned reflex.

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* According to the observation of the animal's behaviour (smacking and licking movements) it can be accepted that the action of the unconditioned stimulus (acid in the mouth) lasted about 20 sec., while its after-effect lasted another 20 sec.

** The fatiguability of the reflexes in the castrated dog is consistent with the characteristic of such animals given by Petrova (1936).

It must be stressed that this course of events is very similar to that observed by Holliger (1923), Schmid (1923) and Zakaraja (1933) in neuro-muscular preparation in situ in rabbits (repetitive stimulation with intervals of a few seconds) and by Lloyd and Wilson (1957) in monosynaptic spinal reflexes (the intervals being less than 1 sec.).

As far as the course of salivation is concerned during the single fatigued conditioned reflex it has been shown that not only the original rate of salivation is diminished but also the usual acceleration does not take place. This means that the growth of fatigue is detectable not only from trial to trial but also during the course of the conditioned reflex.

The character of diminution of the conditioned reflexes with short intertrial intervals seems to suggest the possible mechanism of this phenomenon: it may be assumed that in the course of the conditioned reflex the transmitter involved in its occurrence is destroyed, and thereafter it is gradually restored in the interval. When the interval is too short, this restoration is not complete, hence the following conditioned reflex is diminished. Thus the steady lowered level of the conditioned reflexes (when repeated with short intervals) corresponds to the amount of the transmitter which can be restored after each trial.

It seems that our data allow for drawing of some conclusions concerning the site of the fatiguability of conditioned reflexes. There is no doubt that the unconditioned salivary reflex is not prone to fatigue even with its frequent repetition. Moreover, when the unconditioned stimulus alone is applied, and so the conditioned stimulus is omitted, its effect in the next trial is restored (cf. Fig. 1e). All this goes to show that the phenomenon of fatigue takes place somewhere in the synapses linking the conditioned centre with the unconditioned one. The fact that repeated application of the strong stimulus produces fatigue in the reflex to the weak stimulus, but not vice versa, seems to indicate that the weak conditioned reflex uses partially the same connections which are activated by the strong one.

The diminution of the salivary after-effect which takes approximately the same course as that of the conditioned reflex suggests that the same process of fatigue takes place in it. Since a similar diminution of salivary after-effect was also observed by B. Żer-

nicki (Fig. 5) in alimentary reflexes (unpublished experiments) and was also found by W. Kozak (unpublished experiments) on the neuroglandular preparation in situ, it may be possible that this phenomenon is totally or partially of the peripheral origin.

The repetition of the conditioned reflexes led sometimes not only to the phenomenon of fatigue but also to the opposite phenomenon of enhancement. This phenomenon was seen particularly clearly in the following cases: I. in those experiments in which fatigue was not observed the frequent repetition of the conditioned stimulus led in the first trials to augmentation of the reflexes; II. the application of the strong stimulus after prolongation of the interval or after a number of weak stimuli often led to its hypernormal effect; III. the original diminution of the salivary after-effect was in experiments with 3 min. intervals followed by its gradual increase. The first fact is probably due to the summation of the salivary after-effect of the preceding trial (particularly profused at the beginning of the experiment) with effect of the conditioned stimulus. The second fact is a manifestation of the postactivation potentiation and is analogous to that observed by Sherrington (1947) and Forbes (1912) in the spinal reflexes. The third fact seems to be due to the increasing defensive excitability produced by many repetitions of the noxious stimulus; this phenomenon is best seen with 3 min. intervals, but is masked by fatigue when the intervals are 1.5 min.

As indicated in the preceding section, the fatiguability of conditioned reflexes, if at all present, was only temporary and after some time it could not be further detected. The explanation of this fact may be that in the course of experiments the given conditioned reflex is more and more firmly established, and therefore its resistance to fatigue is increased.

Our experiments seem to throw light on the results obtained by the aforementioned authors concerning the fatiguability of the alimentary conditioned reflexes. It is most probable that the gradual decrease of these reflexes during the whole experimental session is chiefly due to the gradual satiation of the animal and has, therefore, nothing to do with fatigue. This issue is further substantiated by the fact that the intertrial intervals used in those experiments were as a rule much longer that in ours, but in spite of this the gradual decrease of the reflexes was very considerable. In this connection it is worth mentioning that in those experiments in

which short intervals were used (Solovejcik 1940) a rapid diminution of the conditioned reflex which took place in the first trials was very similar to that obtained in our experiments.

SUMMARY

1. The problem of fatiguability of conditioned reflexes was investigated by using the salivary defensive reflexes reinforced by introduction of acid into the animal's mouth.

2. When the intertrial intervals were either 6 min or 3 min. no signs of fatigue of the conditioned reflexes was observed. When the intertrial intervals were 1.5 min., fatigue of conditioned reflexes was clearly manifested in one dog.

3. The fatigue of the conditioned reflexes was manifested by a decrease of the effect of the repeatedly applied conditioned stimulus till about 65% of the normal value. This decrease occurred more or less abruptly in the very first trials and remained on the same level throughout the experiment.

4. When the intertrial interval was prolonged, or the strong conditioned stimulus was applied instead of the weak one, the conditioned reflex immediately returned to normal.

5. The fatiguability of conditioned reflex was only temporary and with prolonged training it gradually disappeared.

6. The unconditioned salivary reflex was, as a rule, not fatiguable in our experimental conditions, but its after-effect was fatiguable in the same way, as the conditioned reflex. This phenomenon was observed in all our dogs and may be due to the peripherical mechanism.

7. The mechanism and the localisation of fatigue is discussed.

8. The present results seem to indicate that the so called phenomenon of "extinction with reinforcement" obtained in alimentary reflexes is chiefly due not to fatigue but to the gradual diminution of alimentary excitability connected with satiation.

The authors wish to thank Dr. T. Mandybur for his assistance in conducting of some experiments.

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Acta Biologiae Experimentalis, Vol. XIX, 1959

GLYCOGEN AND CHITIN METABOLISM DURING DEVELOPMENT OF THE SILKWORM (*Bombyx mori* L.)

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There are many publications concerned with carbohydrate metabolism in insects (cf. Chauvin 1949, Kuźniecow 1953, Roeder 1953, Wigglesworth 1950). Two aspects of it are particularly interesting: carbohydrates, esp. glycogen are examined 1° as a source of energy and 2° as a supposed building material for the synthesis of chitin. Both aspects have been taken into account in the present research, which deals with carbohydrate metabolism in the silkworm, *Bombyx mori* L.

Such metabolism has been so far studied mainly either during development of the egg (Moulinier 1956, Pigorini 1924, 1925, 1929), or during metamorphosis (Ludwig, Rothstein 1949; Vaney, Maignon 1905 and others), whereas only few papers are devoted to the larval period, principally to the last stage of larval growth (Białaszewicz 1937; Comenge, Ojeda 1948).

The aim of the present investigation was to examine both the glycogen and chitin metabolism during the whole period of development from egg to the fully mature form.

A preliminary communication of some of the results concerning glycogen was presented at the 4th Congress of the Polish Physiological Society (Niemierko, Kąkol, Załuska 1954).

MATERIAL AND METHODS

The experiments were done on yellow silk worm (*Bombyx mori* L.). Ascola race. Analyses were performed: 1° on eggs in prediapause and postdiapause period; 2° on larvae in all stages of growth (considering especially

moulting periods); 3° on pupae in successive days of metamorphose, and 4° on imagines.

Since there were rather significant individual variations in rate of growth, the insects were sorted at moulting periods in groups as uniform as possible. The number of specimens used in each analysis depended on their size.

The stage of embryonic development was determined in the postdiapause eggs by examination under microscope. From the third stage of growth up, the analyses were performed separately on males and females.

In all experiments glycogen and chitin were determined in the same samples of material by the method of Good, Kramer, and Somogyi (1939), which was slightly modified.

The procedure was as follows: The samples were put into hot potassium hydroxide (30%) — 2 ml for 1 g of body weight — and hydrolysed for about twenty minutes. The skin was then cut through and the heating continued for twenty to forty minutes to complete digestion of tissues (excluding the chitin fragments). These fragments were taken out and boiled three times in small amounts of water. This water was added to the hydrolysate. Glycogen from the hydrolysate was precipitated with alcohol. After centrifugation the sediment contained glycogen and the rest of the chitin. Glycogen was dissolved in hot water, freed of alcohol (by evaporation), and the remaining chitin fragments were drained off and washed with hot water. Glycogen was again precipitated, centrifuged and hydrolysed for 3 hours with 0.6n HCl. The glucose obtained, was estimated by the method of Fujita and Iwatake (1931). All chitin, separated from glycogen as above, was digested in sulfuric acid and estimated by the micro-Kjeldahl method. To calculate the amount of chitin the values for nitrogen were multiplied by 14.5.

RESULTS

Period of embryonic development

In the period of embryonic development eggs were examined during four successive days of prediapause and in the last days of postdiapause.

Changes in the content of glycogen and chitin during the development of silkworm eggs are presented in Table I.

The Table shows that the initially high content of glycogen (3.86%) in eggs diminishes to about 1/4th during the third and fourth day of prediapause. (Similar results were found recently by Moulinier, 1956). As the eggs from the prediapause period dissolved completly in hot alkali, they did not presumably contain chitin. The synthesis of chitin does not take place until the postdiapause period.

The content of glycogen in the egg just before hatching of larvae falls to below 0.09% of the fresh weight. During this period in one egg 4—5 μg of glycogen are consumed and 6 μg of chitin are produced. One can therefore conclude that the total amount of glycogen consumed by the embryo in postdiapause stage would not be sufficient for the synthesis of the chitin produced in the same period.

Hence, the results obtained show indirectly, that other substances must take also part in the synthesis of chitin.

Period of larval growth

During the first three stages of growth, larvae were examined only just before and after ecdysis. In the fourth and fifth instar the analyses were made every day and moreover females and males were examined separately.

Changes in the content of glycogen and chitin in the silk worm larvae during all stages of growth are presented in Table II.

Table I

Percentage of glycogen and chitin during embryonic development of the silkworm. Fresh weight of one egg: about 0.7 mg. Fresh weight of one newly hatched larvae: about 0.4 mg. Mean value for 5 samples and standard deviation

Stage of development	Glycogen		Chitin	
	mg%	Stand. dev.	mg%	Stand. dev.
Prediapause				
1st day	3.86	±0.08	0	
2nd day	3.52	±0.09	0	
3rd day	3.15	±0.14	0	
4th day	0.74	±0.08	0	
Postdiapause				
6th day	0.83	±0.10	0.22	±0.014
9th day	0.40	±0.02	0.51	±0.045
12th day (just before hatching)	0.086	±0.01	1.15	±0.090
Newly hatched larvae	0.15	±0.02	1.97	±0.200

The results indicate that during feeding in all stages of growth an accumulation of glycogen in larval body is observed. Percentage

Table II

Glycogen and chitin in one individual during the growth of the silkworm larvae. Mean value for 5 to 7 samples and the range

Period of growth	State of development	Body weight	Glycogen		Chitin	
		mg	mg	% of body weight	mg	% of bhody weight
I	Larvae (♀ and ♂) just after hatching	0.4	0.0006	0.15	0.008	2.00
	Larvae (♀ and ♂) just before the 1st moult	7.56 (7.43—7.78)	0.063 (0.055—0.068)	0.88	0.06 (0.051—0.068)	0.80
II	Larvae (♀ and ♂) just after the 1st moult	6.80 (6.60—6.93)	0.014 (0.009—0.017)	0.20	0.06 (0.052—0.064)	0.89
	Larvae (♀ and ♂) just before the 2nd moult	44.7 (44.0—45.2)	0.32 (0.31—0.35)	0.73	0.17 (0.15—0.17)	0.40
III	Larvae (♀ and ♂) just after the 2nd moult	44.3 (43.0—45.2)	0.044 (0.037—0.050)	0.10	0.20 (0.18—0.21)	0.45
	Larvae (♂) just before the 3nd moult	218 (198—244)	1.60 (1.36—1.77)	0.75	1.22 (1.07—1.36)	0.56
IV	Larvae (♂) just after the 3rd moult	197 (172—219)	0.15 (0.12—0.20)	0.07	1.29 (1.06—1.45)	0.65
	Larvae (♂) just before the 4th moult	1139 (1037—1294)	9.70 (9.40—9.90)	0.80	5.70 (5.4—6.0)	0.50
V	Larvae (♂) just after the 4th moult	1060 (945—1125)	1.0 (0.90—1.20)	0.10	6.0 (5.6—6.3)	0.59
	Larvae (♂) just before spinning	4500 (4240—4740)	67.5 (62.2—70.9)	1.50	25.0 (24.0—25.4)	0.55

of glycogen in all stages just before the moulting period reaches about 0.8% of fresh weight (in the last instar it reaches up to 1.5%). After each ecdysis the content of glycogen decreases rapidly to 0.11—0.2%. Thus during the moulting period about 80—90% of glycogen is consumed. It should be mentioned that the decrease of body weight during moulting period is very small.

The chitin content in one individual (Table II) increases from 8 μg (newly hatched larvae) to 25 mg (males) or 30 mg (females) in larvae beginning to spin cocoons.

The percentage of chitin is considerably greater in newly hatched larvae (2%) and during the first moulting period (0.9%). Afterwards it decreases and during the next periods of growth it remains on more or less the same level (from 0.42 to 0.65% of the fresh weight). This decrease of percentage of chitin is probably due to a distinct accumulation of water in larval body in the first two stages of growth (Niemierko, Włodawer, and Wojtczak).

In spite of a decrease the amount of chitin, due to casting off of the skin, (the experiments shows that chitin accounts for 10 to 12% of skin weight) the content of chitin in larval body after each ecdysis generally increases. One can therefore conclude that the synthesis of chitin takes place both in feeding and moulting periods.

As can be seen from Table III, both in the IVth and the Vth stages of growth, an important increase of chitin in the first day of feeding is apparent. In the subsequent days of feeding the increase is much smaller. Similar results concerning the Vth stage of silk worm's growth were obtained by Białaszewicz (1937).

The increase of glycogen is the highest however in the last days of the feeding period, that is, in the days when the increase of chitin is smaller. One can suppose that there is a certain interrelation in metabolism of glycogen and chitin during feeding periods (the days when more chitin is formed the sythesis of glycogen is less intensive). This interrelation is specially visible in the moulting period.

The 4th moulting period preceding casting off of the skin, lasts about 50 hours.

During this period three subperiods can be distinguished differing in the intensity of glycogen and chitin metabolism (Table IV).

In the first subperiod, lasting about 10 hours, the changes of content of glycogen and chitin in larval body are not considerable.

In the second subperiod, lasting more or less up to the thirty fifth hour, the disappearance of nearly the total amount of glycogen (up to 90%) is seen. In the meantime the synthesis of considerable amounts of chitin is observed.

In the third subperiod, lasting up from the thirty fifth hour to the moment of casting off of the skin, the amount of glycogen diminishes slightly, but the quantity of chitin is greatly decreased.

Data concerning the changes in the amount of glycogen and chitin during the 4th moulting are shown also in Fig. 1.

Silk worm larvae, as is known, do not feed during moulting periods. Glycogen consumption shown in this period, can be partly

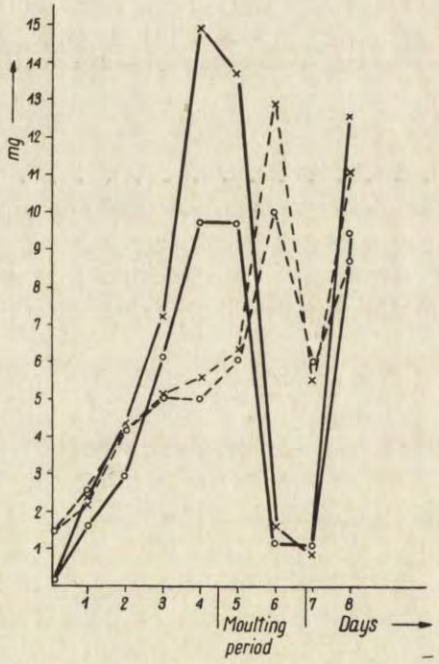

Fig. 1. Glycogen and chitin during the IV-th period of larval growth

Glycogen:
o—o—o—o males
x—x—x—x females

Chitin:
o - - - o - - - o - - - o males
x - - - x - - - x - - - x females

at least the result of starvation. Special experiments were made to show whether utilization of glycogen, during the moulting period, differs from that caused by experimental starvation.

The results were as follows:

Each larvae (females) utilized during moulting period about

Table III

Daily increase of glycogen and chitin in one silkworm larvae during 4th and 5th instar. Mean value for 5 samples and the range

	Females			Males		
	Body weight	Glycogen	Chitin	Body weight	Glycogen	Chitin
	mg	mg	mg	mg	mg	mg
Value just after the 3rd moulting	212 (188—235)	0.15 (0.10—0.19)	1.4 (1.20—1.45)	197 (152—219)	0.15 (0.12—0.2)	1.3 (1.06—1.45)
Increase during the 1st day of feeding	210 (186—236)	2.3 (2.0—2.5)	0.6 (0.48—0.63)	186 (143—213)	1.45 (1.1—1.9)	1.1 (0.7—1.8)
Increase during the 2nd day of feeding	236 (166—316)	1.9 (1.0—2.7)	2.1 (1.9—2.5)	212 (162—263)	1.3 (1.1—1.5)	1.7 (1.5—2.0)
Increase during the 3rd day of feeding	227 (203—254)	2.9 (2.0—3.7)	1.0 (0.8—1.3)	208 (107—217)	3.2 (3.0—3.5)	0.9 (0.7—1.1)
Increase during the 4th day of feeding	243 (212—307)	7.7 (6.6—8.7)	0.5 (—0.5—1.1)	199 (168—229)	3.6 (2.8—3.3)	0.0 (—0.1—0.2)
Increase during the 5th day of feeding	112 (62—156)	—1.2 (—3.0—1.0)	0.7 (—0.4—1.4)	137 (102—155)	0.0 (—0.1—0.2)	1.0 (0.9—1.1)
Value just before the 4th moulting	1240 (1178—1396)	13.7 (11.7—15.8)	6.3 (5.5—6.8)	1139 (1037—1294)	9.7 (9.4—9.9)	6.0 (5.6—6.3)
Value just after the 4th moulting	1040 (934—1130)	0.9 (0.78—1.25)	5.5 (5.3—5.6)	1060 (945—1125)	1.0 (0.9—1.2)	6.0 (5.6—6.3)
Increase during the 1st day of feeding	950 (890—1050)	11.8 (10.8—12.8)	5.6 (4.8—6.5)	560 (522—630)	8.5 (8.5—8.5)	2.7 (2.1—3.0)
Increase during the 2nd day of feeding	753 (722—780)	7.5 (5.9—10.8)	4.4 (3.3—5.2)	640 (561—692)	10.0 (8.7—11.8)	1.3 (1.3—1.6)
Increase during the 3rd day of feeding	740 (564—968)	5.5 (4.8—7.2)	6.2 (4.7—7.5)	757 (684—820)	3.5 (2.7—5.2)	7.0 (6.5—8.4)
Increase during the 4th day of feeding	730 (614—887)	7.2 (6.3—9.7)	3.5 (2.8—5.9)	811 (712—973)	4.5 (3.9—6.1)	6.5 (6.0—7.1)
Increase during the 5th day of feeding	782 (525—1080)	10.6 (7.1—14.1)	2.4 (—0.2—5.4)	938 (847—1132)	17.5 (16.7—18.1)	1.5 (0.2—3.4)
Increase during the 6th day of feeding	749 (398—1334)	24.7 (19.1—31.3)	0.0 (—1.5—2.4)	611 (461—704)	15.1 (13.4—18.6)	0.0 (—1.9—2.1)
Increase during the 7th day of feeding	—744 (—1021—487)	15.3 (5.8—27.3)	0.9 (—2.6—5.1)	—879 (—1143—638)	7.4 (2.2—10.8)	0.0 (—1.0—0.4)
Value just beforespinning	5000 (4723—5260)	83.5 (74.0—95.7)	28.5 (25.0—33.5)	4500 (42.40—4740)	67.5 (62.2—70.9)	25.0 (24.0—25.4)

Table IV

Glycogen and chitin in one individual during the 4th larval and the pupal moult of the silkworm. Mean value for 5 samples and the range

	State of development	Females		Males	
		Glycogen	Chitin	Glycogen	Chitin
		mg	mg	mg	mg
4th stage of growth	Feeding larva just before moulting	14.9 (13.7—15.8)	6.0 (5.5—6.3)	9.7 (9.4—9.9)	5.7 (5.4—6.0)
	Moulting larva, 10 hours of moult	13.7 (11.6—15.8)	6.3 (5.5—6.8)	9.7 (6.3—11.5)	5.7 (5.6—6.3)
	Moulting larva, 35 hours of moult	1.5 (1.43—1.56)	12.9 (11.5—14.0)	1.1 (0.7—1.6)	10.0 (8.7—11.3)
5th stage of growth	Larva, just after ecdysis	0.9 (0.78—1.25)	5.5 (5.3—5.6)	1,0 (0.9—1.2)	6.0 (5.6—6.3)
	(mean content in the exuviae)	—	(1.3)	—	(1.3)
	Feeding larva, 3 hours after ecdysis	1.9 (1.9—2.1)	6.8 (6.3—7.6)	1.9 (1.9—2.0)	6.9 (6.5—7.4)
	Feeding larva, 24 hours after ecdysis	12.7 (11.8—13.7)	11.1 (10.3—12.0)	9.5 (9.5—9.6)	8.7 (8.1—9.0)
	Feeding larva, 48 hours after ecdysis	20.2 (18.6—23.5)	15.5 (14.4—16.3)	19.5 (18.2—21.3)	10.0 (10.0—10.3)
Pre-meta-mor-phosis	Larva, at the end of spinning	53.0 (50.0—57.0)	31.5 (30.0—33.0)	44.0 (35.0—52.0)	20.0 (19.7—22.0)
	Prepupa	57.0 (55.0—58.3)	24.0 (23.2—24.5)	38.0 (37.3—40.0)	16.5 (16.2—17.1)
Meta-mor-phosis	Pupa, just after pupation	60.0 (57.9—60.9)	3,0 (2.7—3.1)	36.0 (37.3—40.0)	3.0 (2.6—3.3)
	(mean content in the exuviae)	—	(1.8)	—	(1.8)
	Pupa, 3 hours after pupation	60.0 (58.5—62.3)	7.0 (5.8—8.1)	36.0 (35.4—39.1)	6.0 (4.9—6.8)
	Pupa, 3 days after pupation	70.0 (68.0—73.0)	13.6 (12.4—14.8)	33.0 (32.6—33.8)	11.3 (9.0—13.6)

Table V

Glycogen and chitin in one individual during metamorphosis of the silkworm. Mean value for 5 samples and the range

State of development	Females					Males				
	Body weight mg	Glycogen		Chitin		Body weight mg	Glycogen		Chitin	
		mg	%	mg	%		mg	%	mg	%
Larva, just before spinning	5000 (4723—5260)	83.5 (74.0—95.7)	1.67	28.5 (25.0—33.0)	0.57	4500 (4240—4740)	67.5 (62.2—70.9)	1.5	25.0 (24.0—25.4)	0.56
Larva from cocoon	3260 (2880—3630)	53.0 (50.0—57.0)	1.63	31.5 (30.0—33.0)	0.97	2250 (1118—2380)	44.0 (35.0—52.0)	1.95	20.0 (19.7—22.0)	0.89
Prepupa	2400 (2280—2520)	57.0 (55.0—58.3)	2 37	24.0 (23.2—24.5)	1.00	1711 (1480—2011)	38.0 (37.3—40.0)	2.22	16.5 (16.2—17.1)	0.96
Pupa, just after pupation	2460 (2355—2665)	60.0 (57.9—60.9)	2.43	3.0 (2.7—3.1)	0.12	1651 (1595—1807)	36.0 (35.2—38.3)	2.18	3.0 (2.6—3.3)	0.18
Pupa, 3 days after pupation	2390 (2242—2541)	70.0 (68.0—73.0)	2.93	13.6 (12.4—14.8)	0.57	1680 (1670—1695)	33.0 (32.6—33.8)	1.96	11.3 (9.0—13.6)	0.67
Pupa, 6 days after pupation	2307 (1932—2565)	80.3 (69.6—91.3)	3.48	16.1 (13.2—21.5)	0.70	1581 (1474—1798)	31.6 (29.1—33.5)	2.00	11.8 (9.6—15.6)	0.75
Pupa, 9 days after pupation	2200 (1707—2500)	43.0 (43.0—43.1)	1.95	13.4 (11.0—16.3)	0.60	1607 (1342—1709)	16.5 (13.7—25.2)	1.03	12.5 (7.6—19.7)	0.78
Pupa, 12—13 days after pupation	1923 (1765—2017)	31.1 (28.3—33.9)	1.62	13.1 (11.2—15.6)	0.68	1460 (1290—1580)	13.5 (12.3—14.1)	0.92	12.4 (10.3—15.7)	0.85
Pupa, 16—17 days after pupation	1830 (1457—2250)	27.6 (26.1—29.1)	1.51	16.9 (10.4—22.2)	0.92	1339 (1187—1457)	10.4 (9.8—11.7)	0.78	15.2 (14.0—16.4)	1.13
Newly hatched imago (18 — 20 days after pupation)	1316 (1260—1390)	25.9 (25.4—26.1)	1.97	9.9 (8.7—10.6)	0.75	610 (568—740)	4.0 (3.6—5.1)	0.66	9.3 (8.8—9.6)	1.52
Imago 3 days after hatching	690 (570—840)	3.6 (3.1—5.2)	0.52	9.1 (8.5—9.8)	1.32	490 (450—520)	3.2 (2.7—3.4)	0.65	8.4 (8.1—8.7)	1.71

13 mg of glycogen. Larvae deprived of food during 72 hours (from the second day of the fifth instar), utilized only 3 to 4 mg of glycogen. The rate of glycogen catabolism in the moulting period is thus much higher than during starvation.

Period of metamorphosis

Table V shows the content of glycogen and chitin from the end of feeding (larvae just before spinning) and during metamorphosis.

Larvae after cessation of feeding contain on average: males about 66.5 mg and females about 83.5 mg of glycogen. During spinning both males and females utilize about 30 mg of glycogen. That is caused probably chiefly by the work of spinning. In the

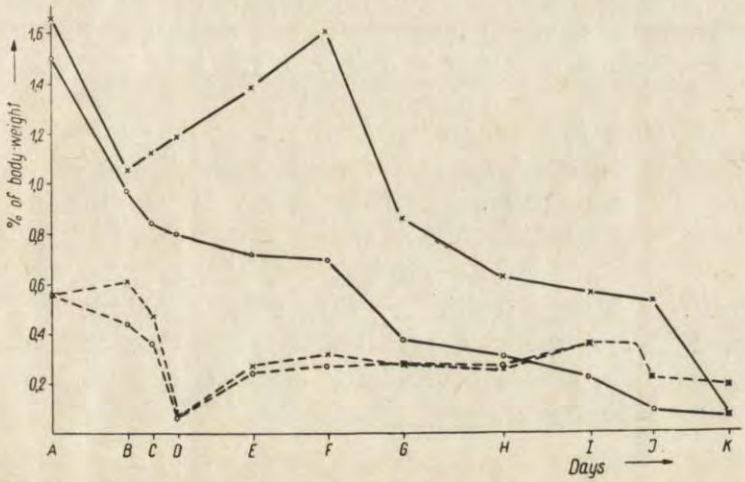

Fig. 2. Glycogen and chitin during metamorphosis in percent of larval body weight. (Mean value of larval body weight before spinning was: females 5000 mg, males 4500 mg

Glycogen: o—o—o—o males, x—x—x—x females
Chitin: o - - - o - - - o - - - o males, x - - - x - - - x - - - x females

A — larvae just before spinning; B — larvae from cocoon; C — prepupae; D — pupae just after pupation; E—F—G—H—I — pupae; J — newly hatched imago; K — adults

first days of metamorphosis males utilize small quantities of glycogen, whereas females accumulate about 30 mg of it.

In the middle of metamorphosis, between the 7th and 9th day of this period, both males and females consume great amount of glycogen. In the later days of metamorphosis the glycogen utilization is much smaller.

In the second half of metamorphosis the quantity of glycogen present in the female pupae greatly exceeds that found in the males. After laying eggs the female moth contains however as much glycogen as the male. This can be easily understood as the weight of eggs laid by one female is about 500 mg with an average content of glycogen up to 4%. The amount of glycogen eliminated from the female body with the eggs forms about 86% of the total quantity of glycogen present in the body of the newly hatched moth.

Important changes in chitin metabolism take place in the larval body during spinning of the cocoon. Both in males and females a large decrease of the amount of chitin is observed. The newly hatched pupa contains about 3 mg of chitin (Table IV, V) and the mean content of chitin in the exuviae is 1.8 mg (in another series of experiments in which larvae were examined 0 to 10 min. after pupation the amount of chitin found in the body was only 0.5 to 1 mg). After pupation a repeated synthesis of chitin is very intensive, and after three hours, about 7 mg of it is found in larval body. During particular days of metamorphosis the mean chitin content is 13.1 to 16.9 mg in females and 11.3 to 15.2 in males. Great individual variations (about 10.4 to 22.0 mg in females and 7.6 to 19.7 mg in males) could be observed. Newly hatched imago contains 5 to 7 mg of chitin less than pupae. Only about 1 mg of it was found in the skin cast off, therefore the rest of chitin must have been utilized during the last day, or even during the last hours of metamorphosis.

DISCUSSION

The results presented above show that the correlation between the changes of glycogen and chitin can be observed in some stages of silk worm development. During the prediapause, no chitin was found. The old experiments of Verson (1884) and Tichomiroff (1885) already showed that the shell of the silk worm egg does contains not chitin but keratin. According to the present investigation chitin appears only in postdiapause period, but even the total amount of glycogen utilized during this period is not sufficient to form the amount of chitin found in the developing egg. This shows indirectly that other substances take part in chitin formation, possibly lipids, as the amount of these compounds de-

creases considerably at the same time (cf. Tichomiroff 1885 Włodawer, Niemierko, and others (1957).

The results of the present investigation indicate that during larval growth, some interaction between glycogen and chitin metabolism takes place.

During feeding, on the days in which the increase of chitin is greater, the increase of glycogen is smaller in comparison whith other days of larval growth. The consumption of glycogen in the moulting period is much greater than during an even longer period of experimental starvation. The high rate of glycogen utilization during the moulting period can not be the result of intensive body activity as the larvae are quite motionless during many hours.

The first intensive movements due to casting off the skin begin (according to Wachter 1930) six hours before ecdysis, that in the time when, as our analyses show, the amounts of glycogen consumed are very small.

Consumption of glycogen, during moulting period, appears in the middle stage of "dormancy", when simultaneously a synthesis of chitin is observed. This shows that there is a possibility of a direct interrelation between these two processes. Seven to ten mg of glycogen utilized in excess of the needs of metabolism during starvation, could be sufficient to a complete synthesis of 4 to 6 mg of chitin formed simultaneously in the middle of moulting period.

It can be supposed that the considerable decrease of chitin in the final stage of the moulting "dormancy" can be a result of a decomposition of this compound in the old cuticle of the moulting larvae. A similar phenomenon during metamorphosis of *Platysamia cecropia* was shown in the experiments of Passoneau and Williams (1953). They have stated that in the imaginal moulting period, 80% of old cuticle is decomposed.

In the first hours after ecdysis the amount of chitin in the larval body increases rapidly. Passoneau and Williams stated that in the last two days before the hatching of cecropia imago the moulting fluid is absorbed by the integument. In hemolymph great amount of N-acetylglucosamine appears (1.2 mg in 1 ml of hemolymph). Similar results were obtained in the case of the silk worm by Jeuniaux and Amanieu (1955) and Zielińska and Laskowska (1958).

Passoneau and Williams injected into the moulting fluid glycine containing C^{14}, and estimated afterwards, the con-

tent of C^{14} in the proteins of imago. These experiments showed that the substances present in moulting fluid are resorbed by the organism and that they immediately participate in different processes of metabolism. This can explain why the amount of glycogen in the larval body increases more rapidly in the first day of feeding after ecdysis, than in the next two days. During these days great amounts of carbohydrates derived from food are probably utilized to form great amounts of chitin.

The data of K u w a n a (1933) concerning the content of chitin and thickness of exo- and endocuticle in silk worm larvae from the IVth moulting period, are in accordance with our results. They are as follows: 6 mg of chitin in a larva which casted off skin (during IVth moulting period): 13.7 mg of chitin in a larva feeding during two days after IVth moulting period; 7.6 mg of chitin in a larva starved during two days after IVth moulting period. In larvae after casting off of the skin the thickness of the exocuticle of the new skin is 5.3 μ, the thickness of the enducuticle — 4.24 μ. Two days later, the exocuticle of the feeding larvae is not thicker. The thickness of endocuticle increases to 10.55μ.

Comparing the data of K u w a n a and ours (shown in Table IV), it can be supposed that chitin produced during the moulting "dormancy" is the chitin of both exocuticle and endocuticle.

Endocuticle of the old skin is being destroyed (it contains about 80% of whole body's chitin) at the end of moulting "dormancy" period, while the old exocuticle (it contains about 20% of whole body's chitin) is cast away in exuviae. If after ecdysis feeding begins, the products of chitin decomposition are nearly completely utilized to form the new endocuticle. In the case when after moulting larvae do not receive food (the amount of glycogen in larval body after ecdysis is very small), the products of chitin breakdown are probably utilized to some degree for different other processes of metabolism.

The correlation between glycogen and chitin metabolism seen during larval growth is clearly visible in the moulting "dormancy" period, when the synthesis of chitin is accompanied by intensive catabolism of glycogen.

During metamorphosis, changes in the quantity of glycogen are different in males and females. Greater amounts of glycogen were found in females. Males on the other hand appear to contain more lipids (Fig. 2).

It is likely that the large amounts of glycogen formed during the first days of metamorphosis are synthesised chiefly from lipids. Such interrelation between lipids and carbohydrates during metamorphosis, was demonstrated by Haub (1941), Patton, Hichckoock, Haub (1941), Agrell (1953) and others in different insects.

Vaney and Maignon (1905) already confirmed the decrease of lipids in silk worm pupae. Recently Niemierko and Włodawer (1937) found that females metabolise more lipids than males.

As our experiments have shown, great amounts of chitin are being decomposed just before pupation (Table IV). The products of chitin decomposition are most probably resorbed with moulting fluid before casting off of the larval skin. Zielixska and Laskowska confirmed the presence of N-acetylglucosamine and glucosamine, both in moulting fluid during the fourth moulting and pupation. These results are in agreement with our supposition. Intensive synthesis of chitin observed in the first days of metamorphosis (Table V) is not accompanied by an increased glycogen catabolism. Chitin is formed probably by resynthesis from the products of its decomposition, resorbed previously in moulting fluid.

Concerning the mechanism of the synthesis of chitin which, as has been shown, takes place also during the last days of metamorphosis, nothing definite can be said so far.

SUMMARY

The amount of glycogen and chitin was examined during the whole period of development of the silk worm (*Bombyx mori* L.).

In the synthesis of chitin which is observed during the post-diapause stage of the development of egg, apart from glycogen, other substances, possibly lipids, takes part.

In the larval period an interrelation between glycogen and chitin metabolism seems to be apparent. This is especially noticeable during the moulting "dormancy" when the formation of chitin is accompanied by a sharp diminution of glycogen content. On the other hand, immediately after each ecdysis and during the first days after pupation chitin is formed probably chiefly

from the products of its decomposition resorbed previously in moulting fluid.

At the beginning of metamorphosis large amounts of glycogen are formed in females, possibly from lipids.

I wish to express my thanks to Prof. W. Niemierko and Dr. S. Niemierko for their helpful advice and stimulating discussion.

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