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THE PROPERTIES OF ALTERNATION OF CONDITIONED REFLEXES IN DOGS

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(Received September 13, 1963)

In the broadest sense of the word alternation refers to any experimental procedure in which one stimulus elicits different responses depending on the ordinal number of the trial. The simplest form of alternation, which may be called „simple alternation", is that in which a conditioned stimulus (CS) alternately signals either reinforcement or nonreinforcement in successive trials (i.e., + − type alternation). In more complex forms of alternation the schedule may be more complicated, as − − type of alter-

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Alternation was frequently used in classical conditioning many years ago in Pavlovian laboratories. It has been shown that in dogs not only is simple alternation possible, but also a more complex form in which three successive negative trials are followed by a positive trial (Usievič 1949).

With the application of instrumental conditioning the procedure of alternation may be greatly extended by alternating different positive motor responses, as is the case of the go left-go right schedule (Mishkin and Pribram 1955).

Since the alternation technique has been widely used in the study of the effects of prefrontal lesions in this laboratory (Brutkowski et al. 1956, Brutkowski 1957, 1959, Ławicka 1957, Szwejkowska, in preparation), it was decided to analyze the mechanism of this sort of conditioning by examining some of its properties. The present paper is concerned with this subject.

MATERIAL AND METHODS

Experiments were performed on normal dogs of various ages in the regular Pavlovian CR chamber. In the preliminary training, the animals were taught, under food reinforcement, to raise the right foreleg to an auditory CS and place it on the foodtray which was situated in front of the dog. When this task was mastered a different auditory stimulus was given instead, which, from the very beginning, was alternately reinforced and not reinforced by food. The duration of the CS in the negative trials was 5 sec. At the beginning of this training the animals perfomed the trained movement at every application of the CS (cf. Wyrwicka 1952), but afterwards, they gradually learned to restrain their performance of it in those trials in which food was not presented. In most instances, the intertrial intervals were 1 min. Eight positive and 8 negative trials were given in each experimental session.

When the alternation task was mastered at a criterion level of not more than 10 percent of errors in 10 successive sessions, a number of test experiments was performed aiming at the elucidation of the principle by which the alternation problem is solved by dogs.

RESULTS

1. *Alternation training.* The general course of the alternation training in 5 dogs is presented in Table I and Fig. 1. The following character-

Table I

Number of errors during the alternation training in individual dogs

Dog No.	Number of sessions till criterion	Number of positive trials	Number of errors in positive trials	Number of negative trials	Number of errors in negative trials
1	40	320	17	320	128
2	36	280	5	288	88
3	36	288	36	288	86
4	52	416	6	416	136
5	80	640	35	640	192

Table II

The performance in alternation in the last 10 sessions of training

Dog No.	Number of sessions	Number of positive trials	Number of errors in positive trials	Number of negative trials	Number of errors in negative trials
1	10	80	0	80	7
2	10	80	0	80	2
3	10	80	0	80	7
4	10	80	0	80	8
5	10	80	0	80	7

istics of this training may be observed. First, the rate of training was different in different dogs, the best animals reached criterion in 36 sessions, the poorest animal did so after 80 sessions. However, as seen in Fig. 1, if we take into account a less rigid criterion, all the animals including the poorest one mastered the task, in principle, after only about 24 sessions. Secondly, we see that there is only a negligible number of errors in positive trials, the chief bulk of errors occurring in negative trials. In other words, the task is mastered when the animal learns not

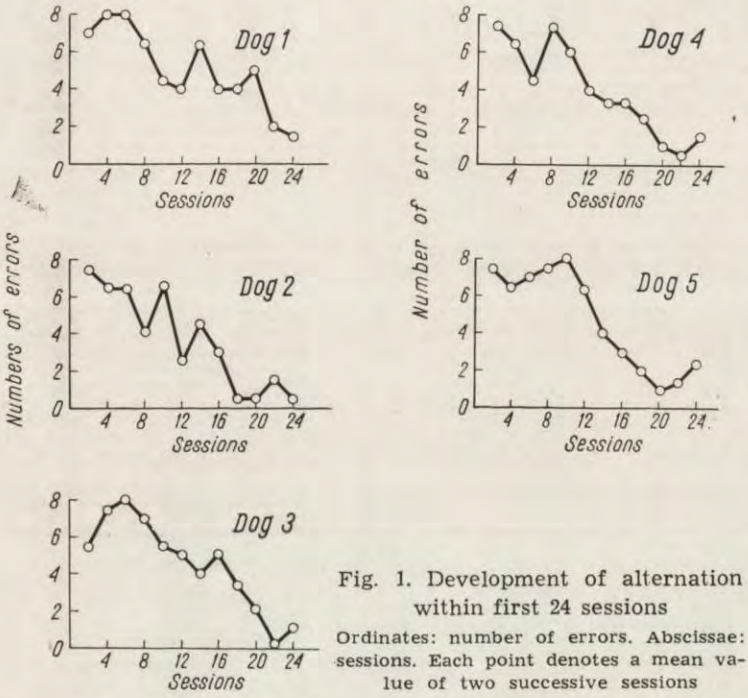

Fig. 1. Development of alternation within first 24 sessions

Ordinates: number of errors. Abscissae: sessions. Each point denotes a mean value of two successive sessions

to perform the trained movement in negative trials. As shown in Fig. 1 these responses fall down rather rapidly in the first 24 sessions, and, then, remain at a low level, only slowly lowering to criterion. The animals' performances in the last 10 sessions are presented in Table II. The typical record of an experimental sessions in a well trained animal is semi-schematically represented in Fig 2a.

2. *Omission of the CS in positive trials.* This test was repeatedly performed in all the animals. It consisted in presenting the food gratis in place of a normal positive trial. As seen in Fig. 2b, in the next trial the animals a l w a y s abstain from performing the trained movement, as if they did not notice that the food was presented without the concomitance

of the CS. This shows that the cue determining the lack of response in negative trials is the presentation of food in the preceding trial and not the application of the CS and/or the instrumental response elicited by this stimulus.

3. *Omissions of negative trials.* In all the dogs, from time to time the CS in the place of the negative trial was omitted, so that the interval between two applications of the CS was 2 min. In all such cases, the animals performed the trained movement to the CS which was given after

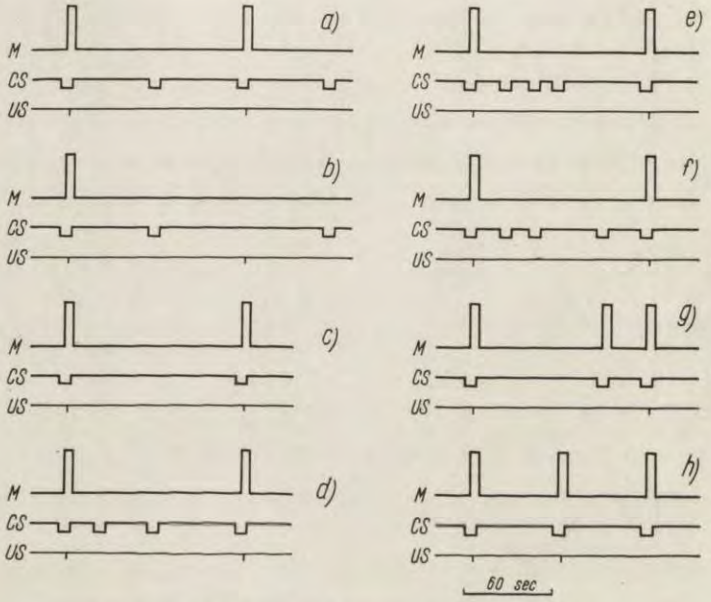

Fig. 2. Variations of alternation experiments

a) normal experiment; b) in third trial food is presented without CS in place of normal positive trial; c) intervals between trials are prolonged to 2 min.; d) additional negative CS is interspersed 25 sec. after the positive CS; e) two additional negative CSi are interspersed 25 and 45 sec. after the positive CS; f) g) negative trials are given 1.5 min. after the positive trials (in „f" each positive trial is followed by two supernumerary trials, in „g" no supernumerary trials are given); h) complex alternation in which a negative trial is given after two positive trials.

this prolonged interval, just as if they did not notice the lack of the CS in the negative trial (Fig. 2c). This result indicates that the chief factor determining the positive response to the CS is not the preceding negative trial, but the interval which elapsed after the last feeding.

It should be emphasized, however, that when the intervals between trials are permanently prolonged to 2 min. the animals are able to adapt to these new intervals and to learn alternation in this changed schedule.

4. *Introduction of CSi between the positive and the negative trials.* When the criterion in the alternation task had been reached, the experimental procedure was changed in such a way that 25 sec. after the positive CS, i.e. about 15 sec. after the animal had consumed the food, the additional negative trial was interspersed, so that a simple + − alternation was replaced by complex + − − alternation. From the very beginning of this training the animals did not perform, in principle, the trained movement either to the supernumerary negative CS or to the CS applied in normal negative trials (Fig. 2d). As seen in Table III, the number of errors in supernumerary trials was very small, and in normal negative trials it remained at the same level as in the preceding sessions. In two dogs a small impairment of positive responses was also noticed.

Table III

The number of errors after introducing one supernumerary trial

Dog. No.	Number of sessions	Number of positive trials	Number of errors in positive trials	Number of interspersed trials	Number of errors in interspersed trials	Number of normal negative trials	Number of errors in normal negative triais
1	10	80	0	80	0	80	1
2	10	80	0	80	3	80	9
3	10	80	7	80	0	80	8
4	10	80	0	80	1	80	12
5	10	80	8	80	2	80	11

Thereafter, in the next series of experiments *two* trials were interspersed between each positive and negative trials, one, 25 seconds and another, 45 seconds after the positive trial (Fig. 2e). In this way, the alternation took the form + − − −.

Table IV

The number of errors after introducing two supernumerary trials

Dog No.	Number of sessions	Number of positive trials	Number of errors in positive trials	Number of interspersed trials	Number of errors in first interspersed trials	Number of errors in second interspersed trials	Number of normal negative trials	Number of errors in normal negative trials
1	10	80	0	80	1	0	80	3
2	10	80	4	80	0	0	80	4
3	10	80	0	80	0	1	80	8
4	10	80	2	80	0	1	80	2
5	10	80	16	80	0	1	80	15

As seen from Table IV this change also did not produce any significant effect on the animals' performance. In both supernumerary trials

a negligible number of positive responses was observed, and the number of errors in normal negative trials was again below criterion, except in one dog in which it was slightly higher. Here also, a slight impairment of responses in positive trials was seen, especially in one dog.

These results show that the introduction of supernumerary trials between the positive and negative trials does not disturb the normal stereotype of animals' performance, nor does it even affect the lack of response in the normal negative trials. This again shows that the main factor determining the presence or absence of the response is the lapse of time between the positive and each successive trials.

5. Postponement of the negative trial for 30 sec. In this test, the negative trials were given not one min. but 1.5 min. after the positive trials, that is 0.5 min. before the next positive trial. In one series of experiments, each positive trial was followed by two supernumerary trials and in another series no supernumerary trials were given.

Table V

The responses to CSi applied 1.5 min. after a positive trial

Experiments	Dogs									
	After two supernumerary trials					Without supernumerary trials				
	1	2	3	4	5	1	2	3	4	5
1	+	−	+	−	−	+	+	+	+	−
2	−	−	+	−	−	+	+	+	+	+
3	−	−	−	+	−	+	+	+	−	+
4	−	−	−	−	−	+	+	+	−	−
5	+	−	−	−	−	+	−	−	+	−
6	−	−	−	−	−	+	+	+	+	+
7	−	−	+	−	−	−	+	+	+	−
8	−	−	−	−	−	+	+	+	+	+
9	−	−	−	−	−	+	+	+	+	+
10	+	−	−	−	−	−	+	−	+	−

\+ positive response
− negative response

The results of these experiments are presented in Table V and in Fig. 2f, g. As seen in the table, when the test trial followed two supernumerary trials the response to the CS was nearly always negative. On the other hand, when the test trial was given 1.5 min. after the positive trial without any interspersed trial, then the effect was nearly always positive. These results show that in those cases in which the trial has an ambiguous character, (i. e., where it is given next to the positive trial) the supernumerary trials suppress the conditioned response.

6. Alternation with two positive and one negative trial. In one dog (not belonging to the above group), instead of single alternation (+ −) or alternation with multiple negative trials (+ − − −), two positive trials were followed by one negative trial (+ + −). The intertrial intervals were 1 min.

In spite of the prolonged training comprising more than 300 trials no slightest indication of the establishment of alternation was noticed (Fig. 2h). To all the CSi the animal displayed the positive responses. The only effect of this training was that in the second reinforced trial the latent period was slightly prolonged, thus indicating that some tendency to single alternation had developed. In fact, when the task was simplified and the single alternation introduced, the animal mastered the task very promptly, after 40 trials.

DISCUSSION

The question posed at the beginning of this paper was what is the mechanism by which the dog solves the alternation task in our experimental procedure. It seems that our experiments give an answer to this question.

When the dog is confronted with the alternation problem, in the first period of experiments he performs the instrumental motor act at every presentation of the CS. Only gradually does he learn to suppress the motor responses in the unreinforced trials, and eventually he does so at every second presentation of the CS. From this course of events it may be concluded that the animal solves the alternation problem by developing *inhibitory* CRs to every second presentation of the CS. And so, the problem of identifying the cue which allows the dog to display the inhibitory and not the excitatory response in all the unreinforced trials arises.

The experiments described in Section 2 show that when the positive trial is replaced simply by presentation of food, the next stimulus possesses inhibitory properties. This indicates that the presentation of food is the main factor determining the character of the next CS. However, as shown in Sections 3 and 4 another decisive factor determining the character of the stimulus is the length of the interval which has elapsed after the last feeding. When this interval is 1 min. (or, more precisely, 45 — 50 sec. after the termination of the act of eating), the next CS is negative. However, when the CS follows the act of eating after 2 min., the CS is again positive. The time factor, as a cue determining the character of the response, is farther substantiated by the fact that introducing one or even

two supernumerary trials between the positive and the negative trial does not change the stereotype of the animal's behaviour. The dog reacts negatively in all the trials included in the period of 1 min., although by doing so he rather solves not the problem of simple, but that of complex alternation (+ − −, or + − −− − type of alternation).

The above considerations lead to the conclusion that although our experimental procedure was based on the alternation principle, the animals did not solve it as a *true* alternation, but rather as a *pseudoalternation*. They did not learn to react negatively in every trial following the positive one, and to react positively in every trial following the negative trial, but they learned something quite different: this was *to rect negatively in any trial following the act of eating within a definite period of time*. In other words, we can say that it is easier for a dog to use as a cue the act of eating, and as a factor determining the next response, the time interval, than to remember whether the last CS had a positive or negative character.

Returning to our analysis of the physiological mechanism of animals' responses we can describe it in the following way. The termination of each act of eating starts to elicit a very strong inhibitory CR which remains for some time and then gradually desappears. Each CS acting against the background of this inhibitory phase is negative. Since the intensity of inhibition gradually decreases, the earlier the CS is given, during this phase, the greater is the probability that the response will be negative. This is why fewer errors are made in the supernumerary trials interjected between the positive and negative trials, than in the normal negative trials themselves (cf. Table III and IV). Of course, the duration of the inhibitory phase is mainly determined by the experimental schedule, that is by the intertrial intervals used in the given series.

The question arises whether the duration of the intertrial interval is the only factor determining the character of the responses. In this respect, the results reported in Section 5 are most instructive. Here the negative trial was postponed so that the animal could consider it as either a negative or positive trial. As shown in Table V the results were most significant and apparently paradoxical: when the CS was simply given 1.5 min. after the positive trial, the animal nearly always reacted positively showing that in that moment the inhibitory phase had already been terminated. However, when two supernumerary trials were interspersed before that trial the response was nearly always negative. This fact clearly indicates that the inhibitory trial is not only determined by the phase against which it is given, but that it also plays an active role

prolonging that very phase. This result also shows how remote the animal's performance is from the alternation principle. If that principle were in operation, the inhibitory trial would rather cut short the inhibitory phase and not enhance it, as found in our experiments.

In connection with this another fact may be easily understood, namely that after introduction of one or two supernumerary trials between the positive and the negative trials, the conditioned responses in the normal positive trials were sometimes inhibited, something which had never occurred in the last period of training (cf. Tables II, III and IV). This shows again that the crowding of inhibitory trials leaves behind a prolonged inhibitory aftereffect overshadowing the normal positive trial.

Our results make it also clear why, as shown in Section 6, the complex alternation consisting of two positive and one negative trial could not be solved by the animal. It is easy to see that the schedule of this alternation is such that the act of eating is followed either by positive trials or by negative trial, in consequence, the animal could not develop either an inhibitory phase or excitatory phase after it. The prolongation of the latent period of responses in the second positive trial shows that after the first positive trial the animal developed the incomplete inhibitory phase, since he did not "know" whether the trial would be positive or negative.

It should be emphasized that according to our experimental schedule the intertrial intervals during entire period of training were constant and lasted 1 min. In this condition the animals learned not to alternate, but to react in accordance with the time factor. This result, however, does not exclude the possibility of solving the alternation task as such without the animals' resorting to the time factor. In order to test this possibility another experimental schedule should be used which would prevent the animal from utilizing the time factor. This problem will be dealt with in the next paper.

SUMMARY

1. The mechanism of the simple alternation instrumental CR was studied under the condition of fixed intertrial intervals of 1 min.

2. This alternation task is solved by developing the inhibitory responses to the CSi applied in negative trials.

3. After replacing the positive trial by simple presentation of food the response to the next CS is always negative.

4. Omitting the negative trial and presenting the CS 2 min. after the trial always evoke the positive response.

5. Interspersing supernumerary trials between the positive and the

negative trial always gives the negative effect and, as a rule, does not disturb the negative response in the "normal" negative trial.

6. When a trial is given 1.5 min. after the positive trial, its effect depends on whether supernumerary trials were, or were not interjected. In the first case the response in the test trial is negative, in the second case it is positive.

7. The complex alternation consisting of two positive and one negative trial cannot be solved by the dog.

8. It is suggested that in our experimental condition the animal solves the task with which he is confronted not by true alternation, but by developing an inhibitory phase of a definite duration after each act of eating.

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THE FORMATION OF DEFENSIVE CONDITIONED REFLEXES BY DIRECT STIMULATION OF THE HYPOTHALAMIC "FLIGHT-POINTS" IN CATS

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It is a well-known fact that both food and defensive conditioned reflexes can be established by stimulating cortical structures. Loucks (1935), and Konorski and Lubińska (1939) were the first to find that if the flexion of a dog's limb, provoked by stimulating the sensorimotor cortex, was reinforced by food presentation, then, after some period of such training, the motor response was actively performed by the animal. Similar results have recently been obtained in cats (Tarnecki 1962).

Lately, the role of subcortical structures in forming and executing conditioned reflexes has also been investigated by many authors. Now, there is ample evidence, indicating the formation of conditioned reflexes by direct stimulation of subcortical structures. Electric current has been used as a conditioned or unconditioned stimulus. Defensive conditioned reflexes (CRs) of the avoiding and escaping type were obtained by Delgado, Roberts and Miller (1954), as well as by Delgado (1955) who stimulated the gyrus hippocampus, the thalamus and the tectal area by means of electrodes, chronically implanted in cats. Delgado, Rosvold and Looney (1956) showed that defensive conditioned reflexes (CRs) could be formed in monkeys by stimulating the central gray. Cohen, Brown and Brown (1957) and Nakao (1958) produced in cats a conditioned avoidance response to a sound by reinforcing the response with a direct stimulation of the hypothalamus.

The present study was undertaken to compare the formation rate of conditioned avoidance response (CAR), obtained by using the stimulation of the hypothalamus as an unconditioned stimulus (US) with the formation rate of these reflexes when using an exteroceptive stimulus as a reinforcement. It was also aimed at studying the course of the avoidance response as a function of stimulating points, located in different parts of the hypothalamus.

MATERIAL AND METHOD

Experiments were carried out on 14 adult cats, males and females, 3 to 3.5 kg in weight, divided into two groups: Group I, 7 cats with chronically implanted electrodes, and, Group II, 10 control cats.

Procedure

Group I. In 6 cats, electrodes were implanted in the medial hypothalamus. The operation was made under Nembutal narcosis (40 mg/kg, injected intraperitoneously), local anesthesia (2 per cent Polocaine) and semiaseptic conditions. Each cat was placed in Horsley-Clarke stereotactic apparatus. The skin was cut along the midline and holes were drilled in the skull through which unipolar electrodes were introduced according to the Delgado technique (1955). Electrodes were distributed according to the following stereotactic coordinates of the Jasper and Ajmone-Marsan (1954) atlas: Fr. = 10.0 to 13.0, L = 1.0, H = − 2.0 to − 4.5. Each cat was implanted with three electrodes in each hemisphere. Stainless steel electrodes, 0.15 mm in diameter and Teflon coated over their entire length except for a 1.5 mm tip were used. After introducing the electrodes to the brain, the holes in the skull were cemented with Duracryl (SPOFA — United Pharmaceutical Works, Prague, Czechoslovakia) which thus fixed the electrodes in place. Opposed ends of electrodes, protruding over the skull surface, were soldered to miniature sockets which in turn were fastened, also with Duracryl, to the skull surface. The indifferent silver wire electrode was attached with one end to the crista of the occipital bone and, with another, to the socket. Then, the skin was sutured in such a manner as to leave the socket slightly protruding over the skin surface.

Following a recovery period of about 10 to 15 days, the experiments were started in a 100 × 100 × 100 cm. testing cage. During experiments, the stimulator cables were connected with the socket by means of a plug. These cables, passing through a system of pulleys, hung from the cage ceiling thus leaving the animals a full freedom of movements within the cage during experiments.

The first experiment consisted of a successive stimulation of brain points (a) to establish the threshold of excitability, (b) to select optimum stimulation conditions and (c) to determine the type of the response. An unipolar stimulation with rectangular impulses (Type 5) 60 stimulator, Laboratory of Electromedical Equipment, Warsaw Engineering College) of a frequency of 50 cy/sec., a duration of 10 msec and an amplitude of 0.5 to 2.0 mA was used. Impulsing was monitored on the screen of a cathodic oscillograph. Of six points, one was selected which, stimulated, produced the most conspicuous flight response, expressed in a strong locomotive reaction, mewing, rising on the hind legs, passing urine and attempts to

get out of the cage. After selecting such point, the formation of a CAR was started. The sound of a buzzer, located on a side wall of the test cage was a CS, while stimulating the flight-points in the hypothalamus — an unconditioned stimulus (US). The CAR was formed by the buzzer sound which, after 5 seconds, was reinforced by the hypothalamus stimulation. The sound and the stimulation persisted until the animal began to rise on the hind legs. When this response was obtained, the action of both stimuli was stopped. Following a few testing sessions, this prancing response was elicited by the presentation of the buzzer sound alone, thereby avoiding the stimulation of the hypothalamic flight-points. Five seconds were given for the occurrence of the CAR, otherwise the hypothalamus was stimulated. Testing the animals was conducted every day and 10 trials at 1-minute intervals were carried out during each experimental session. All cats were trained until a criterion of 50 correct CARs was attained in 50 successive trials.

After completion of testing, the points were coagulated for 15 sec with a 3 mA D.C. current. After 20 days, the animals were killed and their brains perfused with a 10 per cent formalin. Sections 20 μ in thickness, stained according to the Nissl method, were made subsequently.

Group II. The experiments were started by performing sham operations in 8 cats. These operations consisted of cutting the skin along the midline and drilling six small holes in the skull. Then, the holes were filled out with wax and skin was sutured. The operations were made under semiaseptic conditions, Nembutal narcosis (40 mg/kg, intraperitoneously) and local anesthesia.

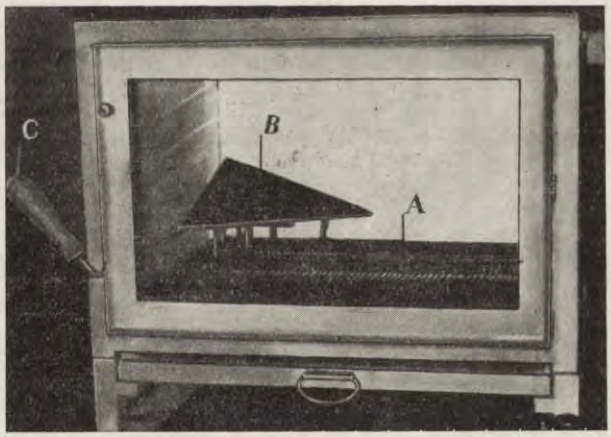

Fig. 1. Testing cage for cats of the control group (Group II)

A, wire mesh floor; B, bakelite platform; C, lever for tilting over the platform

After 10 to 15 days, the formation of defensive CARs was started. Experiments were carried out in the same testing cage as in Group I, except that the cage floor was made of a wire mesh through which the electric current could be transmitted. In the left back corner of the cage, 5 cm above the floor surface, a triangular, bakelite platform was horizontally placed. By means of a lever it could be tilted

over to a vertical position (Fig. 1). The buzzer sound was a CS, while the electric shock, applied to the animal's paws, served as a US. The voltage of the US was by 5 V higher than the threshold value and it varied in individual cats within limits of 10 and 30 V. Reflexes were formed by sounding the buzzer for 5 sec., and, subsequently, by delivering the shock to the paws. The action of the buzzer and the stimulation of the extremities were continued until a cat escaped on the bakelite platform. After some time of such a training, the buzzer sound alone was quite enough for cats to jump on the platform and thus to avoid the shock. Experiments were conducted every day and 10 trials at 1-minute intervals were used daily. All cats were trained until a criterion of 50 correct CRs in 50 successive trials was reached.

RESULTS

Group I (Cats Nos. C-31, C-32, C-37, C-39, C-42 and C-43). Points, the stimulation of which was used as an US, were located in the anterior, central and posterior subdivisions of the dorsal portion of the medial hypothalamus (Fig. 2). The CAR was established in all 6 animals, responding to the buzzer sound, reinforced by the stimulation of the hypothala-

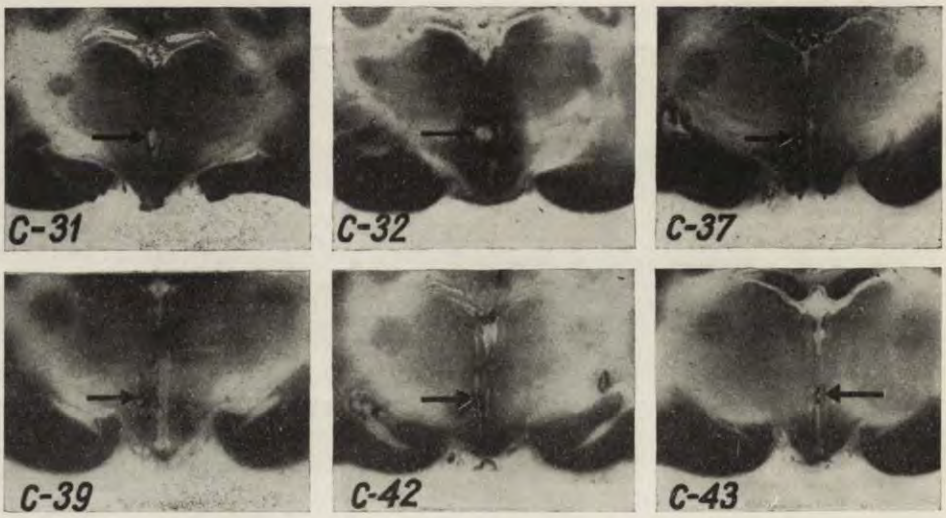

Fig. 2. Cat brain sections viewed in frontal plane
Arrows indicate the localization of the electrode tips, making up points from which the CAR was elicited

mus. The CR forming rate varied, however, from animal to animal (Fig. 3). In cats Nos. C-31, C-42 and C-43, the criterion level was reached within 4 to 7 days, while in the remaining animals (Nos. C-32, C-37 and C-39), this was not before 12 to 14 days of training. The interesting finding is that

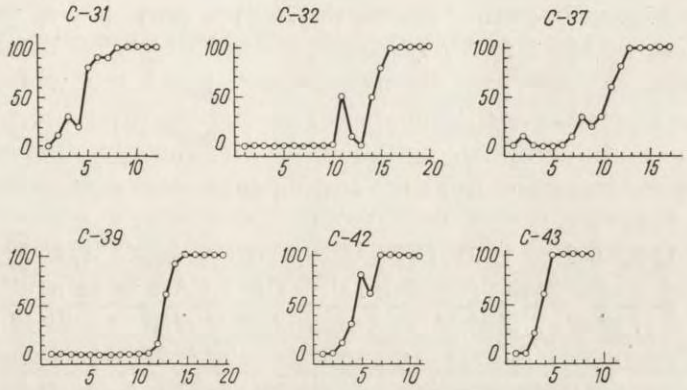

Fig. 3. The CAR course in Group I
Abscissa, testing days; ordinate, CAR percentage

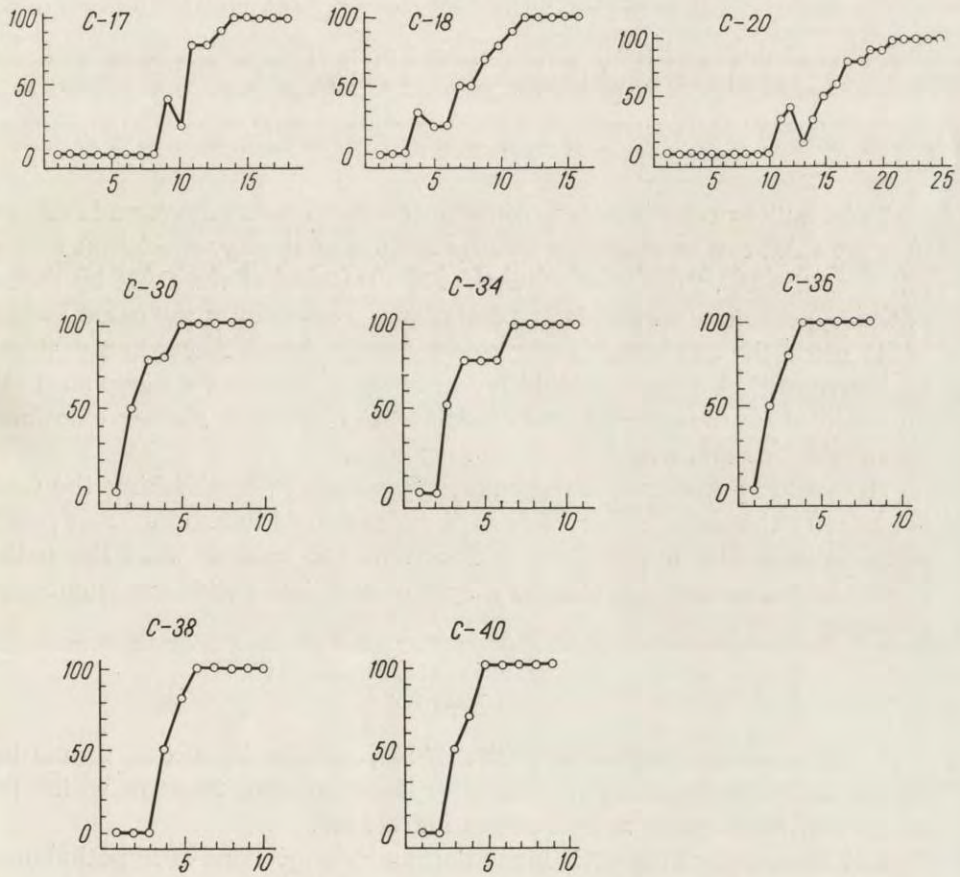

Fig. 4. The CAR course in Group II
Abscissa, testing days; ordinate, CAR percentage

once the CAR has been established, the 100 per cent level of performance was reached within 2 to 3 days. Also, the latent period became shorter with training. Initially, the latency amounted to 5 sec, while, after the reflex was formed, the CR occurred immediately after the presentation of the buzzer. A locomotive agitation, mewing, sporadic movements and even attempts to escape from the testing cage were observed during the intertrial intervals.

Group II (Cats Nos. C-17, C-18, C-20, C-30, C-34, C-36, C-38, and C-40). Both the course and the forming rate of the CAR were similar to those in Group I. Likewise, the CAR forming rate varied in individual animals (Fig. 4). In cats Nos. C-17, C-18 and C-20, the CAR was formed after 14 to 21 experimental days, while in cats Nos. C-30, C-34, C-36, C-38 and C-40, the training took only 3 to 7 days. Similarly, as the training was continued, a gradual shortening of the latent period was observed. Also, some more general behavior patterns during the intertrial intervals (a locomotive agitation, mewing, movements and attempts to get out of the cage) were reminiscent of those recorded in Group I.

DISCUSSION

The results presented fully confirm the previous findings and indicate that the CAR can be easily formed by the use of the hypothalamus stimulation as the US. They also show that the training of the CAR by means of the hypothalamus stimulation technique is essentially the same as that while using the „classical" technique (paw shocking). Accordingly, it can be accepted that a central stimulation is reminiscent of a peripheral stimulation since these two types of stimulation produce similar emotional manifestations and avoidance-type responses.

It should be also emphasized that the course of establishing the CAR is basically the same irrespective of the location of the stimulation points within the medial hypothalamus. This tends to indicate that the entire medial hypothalamus constitutes a system associated with the flight-type behavior.

SUMMARY

1. The rate and the course of the CAR formation by direct stimulating the medial hypothalamus are basically identical with those in which the peripheral nociceptive reinforcements are used.

2. The course of the CAR formation under conditions of hypothalamus stimulation is identical, irrespective of the localization of electrodes in the medial hypothalamus.

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THE EFFECT OF INTRA-MAZE VISUAL CUES ON RETURN REACTION IN RATS

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It has been shown that rats confronted with the task of going for food to some place and then returning to the starting place, are quite able to choose the correct return path (Łukaszewska 1961). This correct „return reaction" depends on the proprioception of sideturnings. When the proprioceptive cues were made less distinct by diminishing the angles of sideturnings the animals' perfomance became much poorer. On the other hand, even total elimination of vision did not impair the proper return reaction (Łukaszewska 1963).

It is well known that visual stimuli may play an important role in maze learning by providing additional cues which are used in determining the correct path. Therefore, the question arose as to what effect a combination of proprioceptive and visual cues would have on the return reaction. The experiments reported in this paper were done with the aim of answering this question.

MATERIAL AND METHODS

Three experiments were carried out on 44 naive male rats, divided into three groups. Each group consisted of 14 or 15 animals.

Two varieties of elevated T maze's were used: 1) a maze with ordinary wooden arms (normal maze), 2) a maze with visual differentiated arms in which the left arm was painted white, and the right arm was painted black (white-black maze). The basic scheme of a T-maze is presented in Fig. 1. Full description of the method has been given previously (Łukaszewska 1961). Briefly, the rat was required to go out of the cage which was placed on one of two starting platforms (S_1 or S_2),

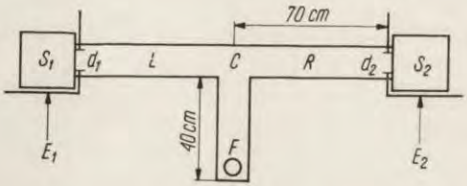

Fig. 1. The floor plane of T-maze.
S_1, S_2, starting platforms; E_1, E_2, wooden screens; d_1, d_2 two way doors in the screens; R, right path; L, left path; C, choice point of return route; F, bowl with food

grasp the food from the maze stem, and return along the same route to the cage where he was allowed to eat it. The intertrial intervals depending on how quickly the animal ate the food were from 30 sec. to 1 min. The animals were permitted to correct an error in the same trial.

Experiment I. Two groups of rats were employed in this experiment. Both groups experienced a series of 10 experimental sessions. Each session consisted of 3 trials in which the cage was located on the same starting platform. On alternate days the cage was located either on S_1 or on S_2. Group 1 was used in the experiment with a normal maze, and Group 2, with a white-black maze.

Experiment II was performed on the white-black maze using Group 3. The experimental series consisted of 20 sessions. The starting place was changed every two days, so that in two successive sessions the rats started from one platform, then, in two other sessions, from the second one, and so on. Each experimental session consisted of 3 trials.

Experiment III was carried out on the white-black maze employing one of the groups from Experiment I (Group 2). The 10 day series consisted of 5 preliminary and 5 test sessions. In the preliminary sessions the bowl with the food was placed at the choice point. The rats performed 3 runs from the cage to the bowl and back. On the next day, after each preliminary session the test session took place. In the test session, the rats did not start from the cage but were placed directly at the bowl (which now stood in its normal position on the maze stem) with the result that the animals only had to return to the cage.

RESULTS

Experiment I. The rats performed more poorly on the white-black maze only one rat ran without error, while some of the remaining ani- route on the normal maze in more than 90 per cent of cases, whereas on the white-black maze they chose the correct return route only 70 per cent of the time. The difference is statistically significant using χ^2 test ($p < 0{,}001$). Nine out of 15 animals responded without error in trial I on the normal maze, the remaining 6 rats comitted 1 to 2 errors in the 10 day series (Fig. 3). On the other hand, in the group on the white-black maze than on the normal one. In trial I, the rats chose the correct return

mals committed as many as 5 to 6 errors in the 10 day series. A comparison between the return reactions on the white and black arm (Table I) indicates that there were a few more errors performed on the white arm (right) than on the black (left) arm, but no permanent preference to one path was observed.

Table I

The comparison between correct and incorrect return reactions in trial I on white and black path

White path			Black path		
No. of session	+	−	No. of session	+	−
1	10	5	2	11	4
3	11	4	4	11	4
5	10	4	6	11	4
7	9	6	8	12	3
9	8	7	10	12	3
together	48	27	together	67	18
% %	64	36	% %	76	24

+ correct reaction,
− incorrect reaction.

In trials II and III, which was a repetition of the choice performed in trial I, the number of correct responses increased for both groups of rats (Fig. 2).

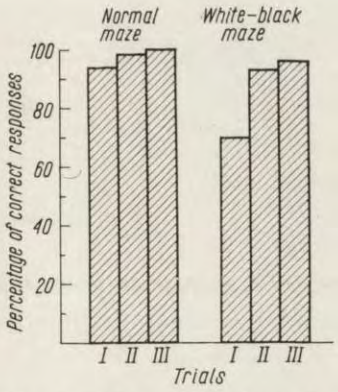

Fig. 2. The comparison between return reactions on normal and white-black maze

Each column represents the percentage of correct return reactions of all rats in the respective trial.

Experiment II. In this experiment the rats ran alternately on the white path for two days, and, then, on the black path for two days. The results are presented in Table II. The left half of this table concerns the return reactions of rats after changing the position of the cage. The re-

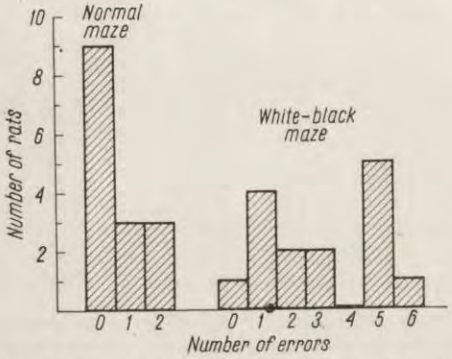

Fig. 3. The number of errors performed on normal and white-black maze in trial I by individual rats.

actions of the rats which returned by the same path as on the preceding day are given in the right half of the table. It is seen that with the unchanged path the rats committed fewer errors in almost every session. The correct responses in the whole series in the sessions with the changing path were at the 80 per cent level, whereas in the sessions without change, 91 per cent. This difference is statistically significant ($p < 0{,}01$).

These results suggest that under conditions of visually differentiated maze arms rats remember to some degree the correct return path of the preceding day and have some tendency to choose this path. Under conditions of an alternating starting place (from day to day) this tendency may affect the return reaction.

Experiment III. The aim of this experiment was to deprive the rats of any information concerning the return route by placing the animal directly at the bowl instead of in the cage. In this situation, the rat was required only to return with the food to the cage. Since he did not run to the bowl, he could respond by remembering the return path from the previous day. But, the rat could also remember that on the preceding day he performed a left turn, for instance, as well as that returned by the white path. However as the present study was concerned only with the memory of visual cues, in the preliminary session the bowl was placed at the choice point and not on the maze stem. In this situation, the rat could not remember the turn but only the visual cues of the path used during the preceding day.

Table II

The number of correct and incorrect return reactions in sessions with changed path and unchanged path (trial I)

Sessions with changed path				Sessions with unchanged path			
No. of session	path	reactions		No. of session	path	reactions	
		+	−			+	−
1	white	11	3	1 a	white	14	0
2	black	8	6	2 a	black	11	3
3	white	11	3	3 a	white	10	4
4	black	10	4	4 a	black	11	2
5	white	11	3	5 a	white	12	1
6	black	12	1	6 a	black	13	0
7	white	9	4	7 a	white	12	1
8	black	13	0	8 a	black	13	0
9	white	12	2	9 a	white	13	1
10	black	12	2	10 a	black	14	0
together		109	28	together		123	12

Table III

The number of correct and incorrect return reactions performed on the basis of memory of the return path from the preceding day

session	path	position of the bowl	direction of run	+	−
preliminary	white	choice-point	cage-bowl--cage		
test 1	„ „	maze stem	bowl-cage	9	5
preliminary	black	choice-point	cage-bowl--cage		
test 2	„ „	maze stem	bowl-cage	10	4
preliminary	white	choice-point	cage-bowl--cage		
test 3	„ „	maze stem	bowl-cage	5	9
preliminary	black	choice-point	cage-bowl--cage		
test 4	„ „	maze stem	bowl-cage	11	3
preliminary	white	choice-point	cage-bowl--cage		
test 5	„ „	maze stem	bowl-cage	7	7
			together	42	23
			% %	60	40

\+ return by this path which was not used in preliminary session
− return by this path was not used in preliminary session

The exact procedure and results of this experiment are given in Table III. In the preliminary sessions, all returns were correct because the choice of return path was not involved. In the test sessions, the number of „correct" return reactions (i.e., choices of the path used in the preliminary sessions) only slightly exceeded the „incorrect" reactions. Only 4 rats out of 14 made 4 correct choices out of 5; the remaining animals performed on the chance level.

DISCUSSION

The essence of a correct return reaction is that the animal remembers which way he travelled to grasp the food from the bowl, and, according to this information, he chooses the return route. It was shown that return reaction depends on the proprioception of the sideturnings (Łukaszewska 1963). It should be emphasized that as far as proprioceptive cues are concerned the return route is the mirror reflection of the route to the food, but as far as visual cues are concerned it is not quite the same. If one arm of the maze is black and the other one is white the animal has the same cue on the return route as he had on the route to the food. In consequence, it could be suspected that such visual cues as those used in our experiments would rather help the animal to choose the correct route when returning to the starting place. The results of the Experiment I clearly show that this is not the case. The performance of the animals on white-black maze was definitely poorer than on normal maze. How can this result be explained? According to the experimental schedule the starting place was changed alternately each day. Consequently, as shown in a previous paper (Łukaszewska 1961), the first run on a given day is handicaped by the return runs performed on the preceding day. The present series of experiments shows that this handicap is rather increased and not decreased by the visual cues provided by the coloured path. Thus, it may be concluded that visual cues help the animal to remember more clearly the return route utilized in previous trials, but that the animal is not guided by these cues in the actual return run.

The above conclusion is well substantiated by the data of Experiment II in which the pathways were changed in a double alternating sequence. Here the animals made significantly less errors on those days when the path was unchanged than on those days when the path was changed. According to this result, we could predict that the schedule used in Experiment III should help the animals to choose the proper pathway when running from the bowl. In fact, since in preliminary sessions the animal learned to run through a given path (white or black) it should choose the same path in the test trials. However, the results of

this experiment were not quite conclusive since the preponderance of the "correct" runs over "incorrect" ones was not great. There may be several factors which caused this ambiguous result. Perhaps the most important one was that the animals were compelled to make sideturnings only in the test sessions and not in the preliminary sessions. Consequently, it may be that the animals remembered the turn performed in the last session since the preliminary session did not interfere with this memory. As the test sessions were given in an alternating sequence this could handicap the animals to run according to the visual cues acquired in the last preliminary session.

SUMMARY

The ability to return to a starting place on an elevated T-maze with visually differentiated arms (white-black) was tested in rats and was found to be significantly smaller than on an uniform T-maze.

When the path designed for the return route was changed every other day the animals performed fewer errors. This suggests that visual differentiation of maze arms increases the memory of the return path from the preceding day and that it disturbs the correct responses. However, the performance of rats which were required to return solely on the basis of memory of visual cues connected with the return path from the preceding day was only slightly above chance level.

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TRANSURETERIC CYSTOMETRY IN UNANESTHETIZED DOGS: TECHNIQUE AND RESULTS

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Cystometry, a motor and sensory examination of the urinary bladder, is widely used in clinical and physiological investigations.

To perform cystometry a catheter has to be inserted into the bladder. Urethral catheterization of animals is rather a difficult and painful procedure. The animal gets excited and refuses to co-operate and that is why most investigators carry out catheterization and cystometry under general anesthesia.

There are many variables inherent in the cystometry itself which are capable of influencing and altering the normal bladder response to filling and stretching and, consequently, the cystometric findings. Under experimental conditions, urethral catheter and anesthesia are considered to be the chief responsible factors. The alterations of cystometrograms evoked by urethral catheter and/or anesthesia very often are so conspicuous that their proper evaluation and comparison is difficult or even impossible. Campbell (1940) states: "The method (cystometry) is valueless in the unco-operative individual or when any form of anesthesia is used".

Various solutions have been put forward to eliminate one or both of these undesirable factors.

Techniques of transurethral cystometry without anesthesia. Tang and Ruch (1955, 1960) catheterized cats under light ether anesthesia and kept them still by restraining in a woolen sack or in a plaster of Paris cast. Cystometry was carried out after a sufficient lapse of

time when the animals regained consciousness. Jacobson (1945) performed cystometric examination only in female dogs. Since the instrumentation of the female dog's urethra was rather easy and not so painful as that of the male, bladder readings could be made without anesthesia. Cieśliński (unpublished) performed cystometry in unanesthetized rabbits. In order to eliminate struggling, the animals were immobilized on the operating table in the supine position with extended legs. Kantorovič and Freydovič (1960) devised a perineal urethrostomy to facilitate the instrumentation of the male dog's urethra. The operative technique is simple and closely resembles that used in man.

Techniques of extraurethral cystometry under anesthesia. Remington and Alexander (1955) during operations on cats, catheterized the bladder through the ureters previously severed from the kidneys. One catheter was used to fill the bladder the other to record intravesical pressure. The animals were killed at the end of operation. Burghele et al. (1958) advocated somewhat similar procedure on dogs. They introduced a catheter unilaterally through a small incision in the ureteral wall.

Techniques of extraurethral cystometry without anesthesia. Sauvage (1960) described a technique of suprapubic transvesical cystometry. In dogs, the bladder which is situated intraperitoneally was exposed, partially extraperitonized and sutured to the abdominal muscles and fascia, the skin being closed over the vesical wall. Consequently, the bladder could be punctured with a trocar directly through the abdominal wall without any danger of peritoneal damage and without anesthesia. To perform cystometry a catheter was inserted through the trocar (Fig. 1).

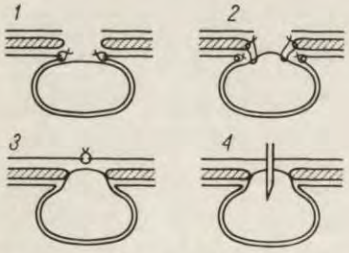

Fig. 1. Scheme of Sauvage's operation

Sauvage (1960) attempted also to approach the bladder for cystometric studies in unanesthetized dogs via the ureter implanted into the skin. Following the operation, however, stricture of the implanted ureter developed and catheterization through it was extremely painful and in most of the cases impossible. Therefore no cystometry could be done by

this route. Sauvage hoped, however, that transureteric technique of performing cystometry in unanesthetized dogs was promising and worthwhile working on. At the Meeting of the Belgian Urologic Society (1960) he stated: "..peut être que de chercheurs mieux outillés ou plus expérimentés reussiront cette technique (sur animal éveillé nous insistons) — nous pensons que les résultats obtenus apporteront des renseignements fort intéressant."

An attempt was made to reinvestigate this problem. The question was how to implant the ureter into the skin in order to avoid stenosis and subsequent difficulties in instrumentation. Techniques of ureterocutaneostomy used in man failed in Sauvage's investigations as well as in present attempts.

An original technique of implanting the ureter was devised by Kantorovič and Freydovič (1959). The lumbar part of ureter was led outside and enclosed in a pedunculated skin flap. Through a special indwelling cannula the ureter could be catheterized down to the bladder and up to the renal pelvis. This technique cannot be, however, applied to cystometric studies. It was then decided to implant proximal ureter together with renal pelvis cut away from kidney parenchyma (ureter-pelvis implantation). It was thought that the ureteral orifice situated in the middle of the pelvis mucosa would not have tendency to rapid stricture or complete closure, Pavlov, Orbeli, Balaknina and Obukhova, as quoted by Kantorovič and Freydovič (1959), and Dragstedt and Dragstedt (1928) successfully implanted into the skin the distal ureter with a cuff of bladder wall including the ureteral orifice uni- and bilaterally.

MATERIAL AND METHODS

Ten adult mongrel dogs weighing from 15 to 25 kg. were used. Only large animals were selected; they were moderately tempered and submissive and their ureters were wide enough to permit insertion of 8 to 12 Charr. catheters. They experienced no special training but they were submitted to a few weeks habituation to the experimental conditions. Before operation, all animals were anesthetized with 35 mg. per kg. nembutal which was administered intraperitoneally. All cystometric studies except those during the operation have been performed without any anesthesia.

Surgery

The dog was placed on a standard operating table over a cushion on the side opposite that to be operated upon. Lumbar approach was used to expose the kidney. The skin incision 8 to 10 cm in length started at costovertebral angle and extended

downward along the outer border of lumbar muscles. The incision was deepened through fascia and muscles. The ureter and kidney were localized and exposed. The peritoneum was dissected away from the abdominal wall by blunt and sharp dissection. In general, the laceration of peritoneum could not be avoided due to the lack of proper line of cleavage. The injured peritoneum was immediately sutured. The kidney was cut free and the renal pedicle after placing two or three silk ligatures was divided. This stage of operation offered no difficulties, for the dog's kidney though lying high was freely movable, perineal fat was scarce and the renal pedicle long. After the kidney had been removed the renal pelvis was cautiously excised from the renal parenchyma by sharp and blunt dissection to allow it to be implanted into the skin. After the pelvis had been freed and partially opened a size 10 to 12 Charr. plastic catheter was introduced into the bladder through the ureter. The place for a new ureteral orifice in the skin was chosen posterior to the operative wound. The ureter with renal pelvis was brought out through a small stab incision. After shaving away the epithelium the pelvis mucosa was stitched to the skin by interrupted sutures. The implanted ureter due to its lenght because of the high position of the dog's kidney was sometimes a little kinked or twisted in its lumbar part. In some cases, therefore, it seemed appropriate to anchor this part of the ureter with one or two catgut sutures to the muscles of the posterior abdominal wall. The kidney bed was drained with one or two rubber drains and the wound closed in layers.

Immediately after the operation when the animal was still asleep bladder readings were performed by means of a catheter previously inserted through the ureter into the bladder. Subsequently the catheter was withdrawn or left in place as a splint to maintain the ureter in good alignment for 24 hours.

A dry gauze dressing covering the implanted pelvis and the lumbar wound completed the operation.

Bladder Measurements

Intravesical pressures were measured in unanesthetized dogs with a simple water manometer (4 mm bore) connected by means of a glass T tube with the catheter inserted into the bladder via the implanted ureter. Interrupted recordings were made bacause it was felt that observing the level and variations of the fluid in the manometer and constantly watching the animal we could eliminate all accidental changes in intravesical pressure elicited by respiration or movements, and get a more delaited and better picture of bladder action than simply reviewing the continuous tracing on a chart.

Further details will be reported and considered later while discussing the results of transureteric cystometry.

RESULTS

Postoperative period and healing. In 9 out of 10 dogs (see case reports) operated on by the method described in this paper, the convalescence was rapid and uneventful. One dog died unexpectedly from an undetermined cause on the 4th postoperative day. In all dogs, the operation site healed *per primam union*. The operation was successful in 8 dogs in whom the

anastomotic site healed well and remained patent for a period varying from 4 weeks to 8 months (when the dogs were sacrificed or the catheterization discontinued). In the remaining one dog (No. 4), the ureterocutaneous opening closed spontaneously a few days after the operation. This failure was due to the extensive damage to the renal pelvis wall during the operation and to too early started instrumentation. Due to the fact that dog No. 7 died four days postoperatively, and in dog No. 2, the angulation of ureter a few centimetres under the skin impeded the catheterization, complete cystometric studies were carried out in 7 animals.

The healing of the implanted ureter-pelvis developed in two ways: 1) the renal pelvis mucosa dried forming a firm eschar covering the ureteral orifice. In a few days, the eschar after falling away, left a small, rounded protrusion ("ureteral caruncle") with ureteral meatus in the centre (type I healing); 2) the mucosa of renal pelvis turned oedematous and hypertrophied. In these cases, the "ureteral caruncle" was much more elevated and defined, about 2 to 3 cm in diameter. Its size reduced slowly from 3 to 6 weeks (type II healing).

A gradual shrinkage of the pelvis wall, observed in every case, was more rapid in type I than in type II. After complete healing independently of the type, a small opening was left usually covered by a delicate superficial eschar formed of dried ureteral secretion. This opening was often invisible at first sight. The instrumentation, however, was easy until the ureterocutaneostomy meatus atresia took place. Further details are included in the description of cases.

After the ureteral meatus had been definitively closed and cathaterization was no longer possible, attempts were made to reimplant the ureter. Under local anesthesia, the subcutaneously palpable portion of the ureter was grasped and after incision of the skin, partially freed. Then, the wall of the ureter was split longitudinally for 1 to 2 cm and the edges of this opening were sutured to skin. Such a procedure was employed in four dogs but only one favourable result was obtained. The failures in the remaining 3 dogs must be attributed to errors in surgical technique or to postoperative suppuration resulting in the dehiscence of the wound and ureteral opening atresia. The reimplanted ureteral orifice (dog No. 1) remained open for many months much longer than the originally implanted ureter-pelvis.

Catheterization. The instrumentation could be commenced at various time intervals after the operation depending on the type of healing at the implantation site. In the type II healing, catheterization might be started from the first postoperative day, but when the type I healing occurred,

some days or even a fortnight had to elapse before carrying out the instrumentation.

Catheterization was conducted under sterile precautions. The catheter was gently inserted into the meatus and passed down the ureter into the bladder. Instrumentation was easily performed and produced no pain or discomfort for the dog. It could be done in any position: standing, sitting or lying. The dogs, as a rule, did not watch the instrumentation; some of them did not pull out the inserted catheter even when left alone for many hours. In later periods, when the meatus was not clearly visible, the introduction of the catheter was facilitated by grasping the subcutaneous portion of the ureter and fixing it firmly. By this manoeuvre the catheter was more easily passed down the straightened ureter. In dog No. 5 at terminal stages of experimentation, just before the complete closure of the ureterocutaneostomy opening preliminary dilatations of the meatus were necessary. In dogs Nos. 3 and 6, an obstruction in the lower ureter was, sometimes, encountered which arrested the catheter and made it impossible to advance into the bladder. In such instances, no force was used and the catheterization discontinued. Sizes 8—12 Charr. plastic catheters were used. When the eye of the catheter penetrated into the bladder it was signaled by the escape of urine at the end of the instrument. The catheter was not passed too far into the viscus for its tip could have irritated the bladder wall. The dogs were regularly and repeatedly catheterized through the ureter over long period of time. Only in later stages of investigations low grade bladder infection of recurrent type was noted in some.

Cystometric studies. Cystometric studies were carried out at various intervals (daily, every other day, once a week) over varying periods of time before the closure of the ureteral opening in the skin or the death of the animal (4 weeks to 8 months). Sometimes two successive measurements at one sitting were performed. After insertion of the catheter, despite the fact that the procedure eliminates all pain and fear, sufficient time was allowed to pass for stabilization before the cystometric examination was started. The animals were permitted to walk and run in order to empty their bladders and bowels. All measurements were performed with unanesthesized dogs placed on the side opposite that of ureter-pelvis implantation. The dogs were kept lying comfortably and as still as possible on the floor. This was accomplished without particular difficulties as the procedure was not painful nor distressing. First bladder readings in dog No. 1 were performed in the sitting position. All dogs were co-operative except dog No. 3 which was somewhat restless and excited at the beginning. However, later on, he also became sub-

missive. A trained team consisting of two persons performed the examination with the highest possible precision and accuracy. One person was busy with bladder filling, the other with the sedation of the dog by proper handling as well as reading and recording of intravesical pressures and sensory points. The zero mark of the water manometer was adjusted to correspond with the level of the ureterocuta — neostomy opening. The bladder was filled with increments of 25 to 50 cc. of a mild antiseptic solution warmed to body temperature by means of a bladder syringe. Sometimes, a continuous filling was used. The readings were taken directly from the manometer after each increment when the flow was stopped and the animal was perfectly quiet and when the level of fluid in the manometer reached the lowest point. Usually, this was within 1 to 2 min. after fluid was admitted, rarely a longer wait was necessary. The dog's sensation was registered and correlated with the volume-pressure curve. All the time, while the bladder continued to be distended the animal was carefully watched. Having gained some experience both the desire to void and imminent micturition was easily recognized. These sensory points were most often manifested by slight nervous reaction or a yelp (dog No. 3 barked when his bladder became overdistended), or by slight elevation of intravesical pressure. Sometimes, however, when no evident signs were present the sensation could not be recognized. Performing the examination required in average 20 to 30 min. The examination was continued until there was evidence of distinct desire to void or when the limits of normal bladder capacity of the tested dog was reached. After termination of measurements the manometer was disconnected, the catheter removed and the dog allowed to micturate. The dog ran out hastily, and the act of micturition was prolonged and uninterrupted until the bladder emptied completely. In some examinations, the bladder was overdistended and micturition pressures have been estimated by having the dog micturate at the end of the test in the lying position.

Cystometrograms. A total of 226 cystometrograms were obtained and their normal appearance in repeated examinations estimated.

The tested animals are to be divided in following groups:

1. Successive volume-pressure curves are remarkably constant during prolonged observation as regards bladder capacity and intravesical pressure. The curves are long (500 to 600 cc. capacity) and flat (intravesical pressures being 5 to 15 cm of water throughout the entire period of filling). The intravesical pressure insignificantly ascends at the end of filling (20 to 30 cm of water). Periodically, however, even in those animals (dogs Nos. 1,3 and 6) some alterations of the cystometrograms are obser-

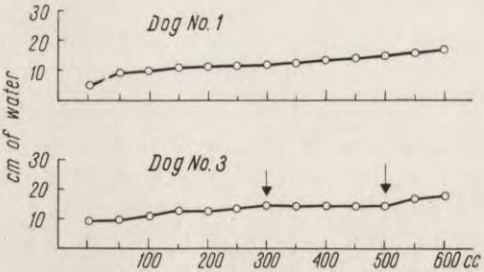

Fig. 2. Cystometrograms of the dogs Nos. 1 and 3.
Long, flat, slowly ascending volume-pressure curves.
Arrows indicate the desire to void.

ved. These alternations are probably due to surgical trauma (cystometrograms obtained in the early postoperative period, infection or other unknown humoral? factors. Fig. 2 and 3).

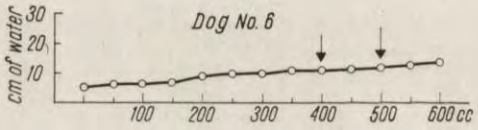

Fig. 3. Cystometrogram of the dog No. 6

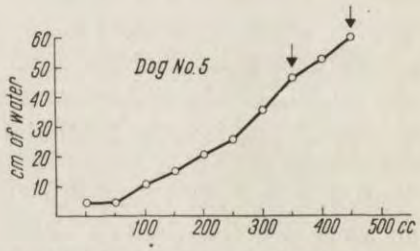

Fig. 4. Cystometrogram of the dog No. 5.
Hypertonic type of the volume-pressure curve.

2. On repeated examinations, constant, large bladder capacity is noted, the intravesical pressure being, however, high. The curves rise steeply (hypertonicity) after each increment attaining at final stages of filling 50 to 70 cm of water (dogs Nos. 5 and 9, Fig. 4).

3. Most of the curves are stabile but some differ more or less markedly from the normal pattern. Higher intravesical pressures are noted

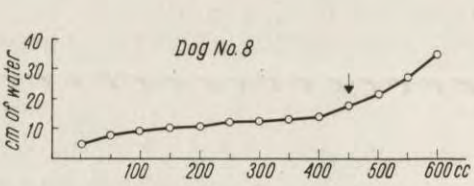

Fig. 5. Cystometrogram of the dog No. 8. More steep rise of the final limb of the volume-pressure curve.

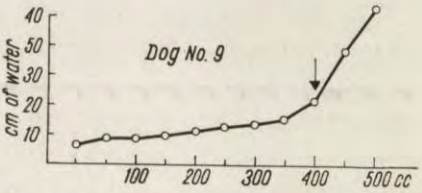

Fig. 6. Cystometrogram of the dog No. 9.

therefore, the final limb of the curves ascends somewhat more steeply than in the first group attaining 30 to 50 cm of water (dogs No. 8 and 9) — Figs. 5 and 6.

4. Successive recordings differ so markedly that no meaningful data can be obtained and no comparison can be drawn (dog No. 10).

Case reports

Dog No. 1, weight 17 kg. Subjected to operation Sept. 27, 1962 by the technique previously described. No splinting. Uneventful postoperative course. Type II healing process (Fig. 7). Catheterizations were begun from the 5th day and were carried out daily or every other day. A size 12 Charr. catheter was used. There were no difficulties in instrumentation and the dog showed no discomfort from the proce-

Fig. 7. Dog No. 1. Ureter-pelvis implantation healed by type II process.

dure. The consecutive urine examinations were negative. The first cystometric examination was done on the 19th day after the operation. Unexpectedly, on the 26th day all attempts to pass the catheter failed and in a few days the cutaneous ureteral meatus obliterated. Therefore only 6 cystometrograms were obtained. These primary investigations permitted to gather enough experience regarding the technique of catheterization, bladder capacity and behaviour of the animal during cystometry. Three months later, the same left ureter was reimplanted into the skin with good result. Consequently, 26 bladder readings were performed on days 2,5, 8, 11, 15, 18, 23, 25, 29, 32, 35, 39, 43, 50, 53, 57, 60, 64, 69, 75, 78, 81, 84, 95 and 104. The ureterocutaneous opening was patent when the dog was sacrificed on the 105th day.

The volume pressure curves (Fig. 2) obtained on repeated examinations were comparable, strikingly similar as regards the length (capacity) and height intravesical pressure. The vesical capacity was stable varying only in small limits and equalled 600 cc. in 22, 550 cc. in 3 and 500 cc. in one reading (from total 26 readings). Most of the curves were flat or gradually rising. Out of 26 examinations only in 6 was noted a slight hypertonicity of undetermined cause. In 14 readings, no sensory points were noted, sometimes, even when the tracing was slightly

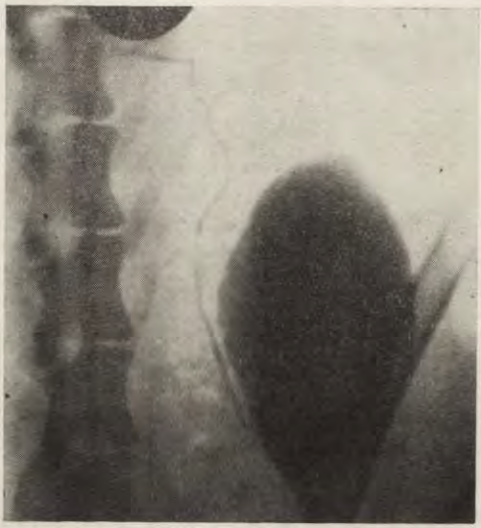

Fig. 8. Dog No. 2. Ureterography showing marked angulation of the implanted ureter impeding catheterization.

hypertonic. Out of remaining 12, the desire to void occurred at the end of filling in 5, and at the volume between 300 to 400 cc. in 7.

Dog No. 2., weight 15 kg. The ureter was implanted to the skin. The catheter was not inserted before implantation. While attempting to insert the catheter at the end of the operation an obstacle was met in the lumbar portion of the ureter and the passage of the cathater beyond this point was not possible. Type II healing process. Repeated attempts of catheterization in the post-operative period also failed. The ureterography performed on the 14 postoperative day showed an angulation in the form of a pigtail of the ureter a few cm. beneath the site of the implantation (Fig. 8). Four weeks later, an attempt of reimplantation of the ureter failed (the ureter was accidentally cut). Further attemps were discontinued.

Dog No. 3, weight 17.5 kg. After operation the catheter was left in place as a splint for 24 hours. Recovery was uneventful, the implanted ureter healed by type II process. The instrumentation which commenced from the 2nd postoperative day was easy and not painful for the animal. Sixty-three cystometric examinations were performed altogether on days 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 16, 18, 21, 25, 26, 28, 29, 31, 33, 34, 36, 38, 40, 42, 45, 47, 49, 51, 53, 55, 57, 59, 66, 70, 73, 77, 79, 83, 87, 95, 98, 105, 108, 112, 115, 120, 122, 126, 129, 136, 140, 147, 150, 154, 158, 162 and 167 (Fig. 9). The dog was killed for other reasons on the 173rd day after operation. The ureterocutaneous meatus was perfectly patent showing no signs of any constriction and could be still utilized. During the first tests, the sedation of this dog was somewhat difficult to achieve. The restlessness was, however, moderate and did not interfere with the readings and recordings. In later periods, having become accustomed to the procedure the dog was perfectly quiet as dog No. 1 who, sometimes, slept during the test. The urine was clear, sterile, devoid of leukocytes or red cells on successive examinations. The ureterography and cystography are depicted in Fig. 10. On autopsy, the bladder macroscopically appeared normal. Microscopic sections revealed no evidence of cystitis.

Fig. 9 Dog. No. 3 during cystometric examination. Implantation site healed by type II process.

Some variations were found among the cystometrograms obtained during the first month after operation. Some of them appeared normal, similar to that obtained in dog No. 1, the others were short indicating a decreased vesical capacity and micturition threshold. Commencing with the 31st day, evident stabilization of cystometrograms occurred (Fig. 2). Out of 41 readings, in 31 the vesical capacity was 500 cc.; in 7, 550 cc., and in 3, 600 cc. Intravesical pressures were low, the curves being flat or slightly ascending. A more steep rise of the terminal limb of the curve was seen only in 4 readings. In 4 readings (out of 41), no sensory points were registered. In other examinations, the desire to void occurred 14 times at a volume of 250 to 300 cc., 5 times at 350 cc., 9 times at 400 cc., 6 times at 450 cc., and 3 times at 500 cc.

Dog No. 4, weight 15.5 kg. The renal pelvis was partially torn during the operation. In addition, during the first attempts of instrumentation postoperatively (type I healing process) a false passage was made. An atresia of implanted ureteral opening was tought to be the consequence of these two errors. Also the reimplantation failed due to suppuration and breaking down of the post-operative wound. No cystometric studies were performed.

Dog No. 5, weight 15.5 kg. After ureterocutaneostomy smooth convalescence and healing by type I process. The instrumentation, started too early, resulted in a false passage. However, on the 11th postoperative day after the eschar had fallen away the catheter (size 10 Charr.) passed easily via the ureter into the bladder. While examined, the dog was perfectly quiet and often slept.

Twenty bladder pressure readings were obtained on days 11, 13, 14, 16, 18, 20, 22, 25, 27, 29, 31, 33, 35, 39, 43, 45, 49, 52, and 56. A few days after the last examination, the ureteral opening epithelialized and obliterated. A week prior to the complete ureteral constriction introduction of the catheter was possible only after a preliminary dilatation of ureteral meatus. The urine examinations made at intervals were negative. The reimplantation of the ureter failed due to delayed wound healing resulting in unpermeable stricture of the skin meatus.

Although the vesical capacity was maintained at relatively constant level (450 to 500 cc.) and the successive curves were similar or identical in lenght, the intravesical pressures were high, and a marked hypertonicity was noted (Fig. 4).

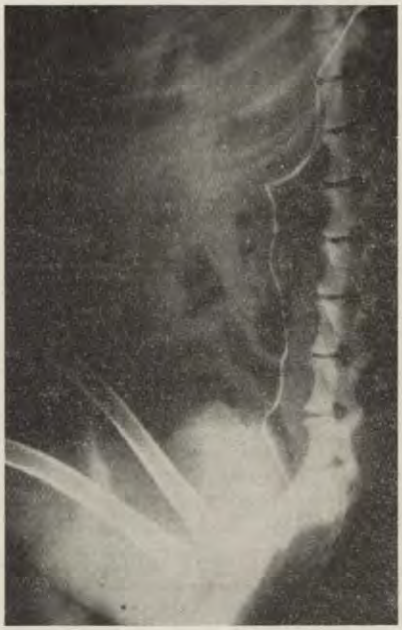

Fig. 10. Dog No. 3, ureterography and cystography.

It is to emphasize that both infection and emotional excitement, which could produce detrusor hyperactivity, were absent. Sometimes, the urine was retained even up to very high pressures. In spite of high intravesical pressure in most examinations, the desire to void was shown only in final stages of bladder filling when the threshold of capacity was reached.

Dog No. 6, weight 18 kg. After the operation, the animal remained in excellent condition. The implanted ureter healed by type I process. The eschar was removed early (4th day), permitting an easy catheterization with a size 10 Charr. catheter. Fifty-one cystometric stadies were performed on days 4, 5, 7, 8, 10, 12, 13, 15, 17, 19, 21, 24, 28, 36, 38, 45, 49, 52, 56, 58, 61, 65, 69, 73, 77, 84, 87, 91, 94, 99, 101, 104, 107, 125, 128, 132, 135, 139, 144, 151, 160, 164, 176, 182, 186, 190, and 196. The ureteral opening closed definitively on the 226th postoperative day, i.e., one month after the instrumentation was discontinued. In the final period, some narrowing of the subcutaneous portion of the ureter was noted smaller caliber catheters a size 8 Charr. had to be inserted than at the beginning. Occasionally, the catheter could not be advanced into the bladder due to an obscure obstacle (ureterography showed no organic stricture) in the lower part of the urater. Urine analyses taken at intervals postoperatively showed neither gross nor microscopic abnormalities, except in the last period when low grade infection occurred.

Cystometrograms were strikingly uniform and similar both in length and height throughout the entire period of investigation. (Fig. 3). Out of 30 bladder readings, the bladder capacity was 600 cc. in 22, 550 cc. in 6; and 500 cc. in 2. The pressure curves were of smooth ascent. Desire to void occurred when the bladder held between 300 to 600 cc. From the 101st postoperative day, among normal curves, cystometrograms showing hypertonicity and hypocapacity were obtained. This was possibly due to bouts of low grade bladder infection (urine examination showed 20 to 30 to 40 leucocytes per high powered field). It must be emphasized, however, that most of cystometrograms continued to show a normal structure and appearance.

Dog No. 7, weight 25 kg. After left ureterocutaneostomy the indwelling catheter was left in for 24 hours. Cystometric examinations were carried out the day after the operation and since the healing of the implanted ureter-pelvis was of type I process early instrumentation was impossible. The general condition of the dog suddenly worsened on the 4th day after operation and he died from unrelated causes.

Dog No. 8, weight 18 kg. Ureter pelvis implantation was performed with a favourable result. Since the healing process was type II instrumentations were com-

menced shortly after operation and they were easy and not painful. No difficulties were encoutered during intravesical pressure measurements, the animal was perfectly still while tested. The repeated urine examinations were negative. Twenty two bladder readings wer done altogether on days 4, 6, 15, 18, 21, 25, 28, 37, 43, 47, 49, 51, 53, 55, 57, 61, 74, 78 and 82.

The cystometrograms were not as constant as in dogs Nos. 1, 3, and 6. Bladder capacity varied from 350 to 600 cc. In most readings, however, the capacity was large (600 cc. in 9; 550 cc. in 5; and 500 cc. in 3). In 5 tests, the bladder held only 200 cc., 250 cc., 350 cc., 400 cc. and 450 cc. Only 4 curves were strictly comparable with these obtained in dogs Nos. 1, 3 and 6. In others, the intravesical pressure in the second stage of filling rose more rapidly which caused a distinct final sharp rise of ascending limb of the volume pressure curve (Fig. 5). Out of 17 stabilized cystometrograms (flat pressure curve, no evident signs of hypertonicity) the desire to void was manifested 6 times at 50 cc., 5 times at 100 cc., 3 times at 150 cc. and once at 200 cc. prior to the discontinuance of filling. In 3 examinations no sensory points were noted.

Dog No. 9, weight 19 kg. The recovery was uneventful after the operation. Ureterocutaneostomy healed well by type II process. Instrumentation was commenced from the day following operation. No discomfort was noted from catheterizing. Urine specimens showed no abnormalities. Twenty-one cystometric examinations were performed on days 7, 10, 13, 16, 20, 23, 31, 37, 41, 43, 45, 47, 49, 51, 55, 58, 62, 65, 72 and 76. The ureterocutaneous opening was wholly patent showing no sings of even the slightest constriction when the dog was sacrificed from other reasons on 77 day. The cystometrograms were slightly less stabile than those of dog No. 8. The vesical capacity was 600 cc. in 4, 550 cc. in 3, 500 cc. in 7, 450 cc. in 4, 350 cc. in 1, 300 cc. in 1 and 250 cc. in 1 determinations. Some of the first curves were similar to the previously obtained ones; they were long, flat or slowly ascending. In later stages variations in wide ranges were present. In final tests again a stabilization was evident again. As in dog No. 8 in most of cystometrograms some signs of hypertonicity in the second stage of filling were present (Fig. 6). In 7 readings, no sensory points were registered, the dog manifested no signs of bladder fullness. The desire to void occurred 4 times at 50 cc., 5 times at 100 cc., 3 times at 150 cc. and twice at 200 cc. before the discontinuance of filling.

Dog No. 10, weight 15 kg. Responded well postoperatively, implantation site healed perfectly by type II process. Later on the general condition gradually worsened and the loss of weight was noted. Instrumentation which commenced on the first post-operative day was easily performed and showed no discomforting reactions. Sedation during testing was easily achieved as the dog was perfectly still. Fifteen cystometric studies were carried out altogether on days 7, 10, 13, 16, 20, 23, 31, 37, 41, 45, and 47. The cystometric data showed a wide variation range and therefore the successive curves were difficult to compare and interpret. Most were short, rised steeply ending by the sudden occurrence of micturition. The microscopic examination of the brain after the dog had been sacrified disclosed meningoencephalitis which might be the cause of marked instability of bladder function and cystometrograms.

DISCUSSION

None of the methods of transurethral cystometry is accurate and precise enough to give reliable information on bladder function. Urethral catheterization often causes uncontrollable, reflex bladder spasm due

to local irritation of urethra thereby altering the bladder response to filling and strething.

Transurethral cystometry under anesthesia. Anesthesia may deeply influence the detrusor activity and cystometrograms.

a) In deep anesthesia, the micturition reflex is completely abolished or deeply depressed due to total or partial inhibition of spinal cord centers. The urination, if ever occurs late, the volume pressure curve being long and flat.

b) In light anesthesia, the spinal cord centers become released from inhibitory influences of the cerebral cortex. Micturition occurs with low bladder volume. The cystometrogram is short, segment III corresponding to the cerebral inhibition is lacking.

c) According to changes in the depth of anesthesia (at the beginning of the nembutal anesthesia the sleep is much deeper than that at the end), the vesical capacity and succesive cystometric determinations can vary considerably. As the sleep becomes lighter the capacity decreases, the volume pressure curves become shorter and vice versa.

Consequently, the cystometrograms obtained on repeated examinations may differ markedly not only from animal to animal but also in the same animal. The variations are sometimes, so conspicuous that it is very difficult to interpret and compare findings obtained in different subjects or in different experimental states of the same subject. In general, under anesthesia, in dogs the vesical capacity was found to be low (Veenema et al. 1952, up to 360 cc.), often markedly low (Nesbit et al. 1947, Rose and Deakin 1928, under 100 cc.) and by far differs from data obtained in unanesthetized animals (see below), the volume-pressure curve was one of smooth ascent, the intravesical pressures being low. Anesthesia limits the time and number of bladder readings and makes the study of bladder sensation impossible which is held to be even a more important part of cystometric examination than the intravesical pressure recording.

Transurethral cystometry in unanesthetized animals due to pain and excitement evoked by the presence of catheter is too often a disappointing procedure. Tang and Ruch (1955), only in a few from a large number of tested cats could obtain reliable and comparable data. In most animals, in spite of immobilization the struggling was violent resulting in wide variations of bladder capacity and length of cystometrograms (decreased micturition threshold or depressed voiding reflex) as well as in many movement artifacts. Nonphysiological conditions, short preliminary anesthesia and/or immobilization caused vivid emotional reaction and were thought to be the principal causes of unsatisfactory results.

Jacobson (1945), in unanesthetized female dogs, found by far larger vesical capacity than in other anesthetized animals. The data of successive bladder readings in the same individual, however, varied in wide limits too. Therefore their evaluation, interpretation and comparison was often dubious or impossible. Jacobso's technique of examination is much more physiological than the preceding one it necessitates, however, special training of the animals. Since the instrumentation of the female dog's urethra is, in general, easy and not painful, the test may be carried out in co-operation with the animal. The discrepancy between succesive bladder readings can be ascribed to the inevitable irritation of the urethra and/or external genitalia by repeated catheterizations and possible vesical infection due to pathogenic microorganisms which may be easily conveyed from the vagina into the bladder.

In unanesthetized rabbits, reliable and accurate cystometric data could not be obtained (Cieśliński, unpubl.). Though the instrumentation was easy (rabbit's urethra readily admitts even large catheters size 14 to 16 Charr.) and there was no general reaction on the part of the immobilized individual, in most readings marked hypertonicity of detrusor was recorded. Local urethral irritation was supposed to be the cause.

Perineal urethrostomy devised by Kantorovič and Freydovič (1961) makes repeated transurethal catheterization on the unanesthetized male dog possible due to shortening of the way to the bladder and due to by-passing a long portion of anterior urethra. This technique does not affect the bladder as a anatomophysiological entity and offers the possibility of performing cystometric studies without anesthesia. As far as is known to the author no such studies have been performed.

Extraurethal (transureteric) cystometry under anesthesia devised by Remington and Alexander (1955) and by Burgele et al. (1958) may be carried out only in acute experiments. The data are by far too influenced by anesthesia.

Extraurethral (transvesical suprapubic) cystometry in unanesthetized animals seems to give most favourable results. Sauvage (1960) reports that:

1) Vesical capacity (determined by the volume of fluid at which light excitement of the dog appears) is strikingly constant in the same animal even during prolonged testing.

2) Vesical capacity in different animals varies in wide limits equalling 100 to 650 cc. and is independent of the weight of examined dog.

3) The intravesical pressure is high (25 to 30 cc. of water-plateau) the final ascending limb of the curve may attain even 60 to 70 cm of

water. This hypertonicity was the consequence partly of the standing position in which the examination was performed and partly of some emotional reaction (Fig. 11).

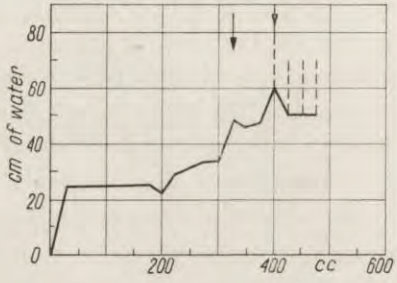

Fig. 11. Cystometrogram of the dog taken from Sauvage's paper.
Large bladder capacity. High vesical pressures during the entire period of filling.

4) The cystometrograms taken at intervals are comparable in the same animal and generally speaking are similar to those obtained in man.

Transvesical suprapubic technique, however, is not wholly satisfactory and has some distinct disadvantages:

1) Preliminary operation on the viscus which has to be explored is necessary. The fixation of the bladder to the anterior abdominal wall can reduce the bladder capacity thus altering bladder response to filling.

2) The vesical punctures, instrumentations and cystometric studies have to be spaced at intervals of 7 days. This time intervals are necessary to allow the postmanipulation reaction from puncture to subside. More frequent instrumentation is likely to produce trauma of vesical wall and complications.

3) The extravasation of urine around the suprapublically inserted catheter may lead to fatal abdominal wall phlegmon.

4) Pain and fear evoked by vesical puncture often results in psychic stimulation with consecutive hypertonicity.

Despite these shortcomings, transvesical suprapubic cystometry in view of the more adequate data it provides on bladder function no anesthesia no urethral catheter, though held brutal in man up to date it is considered superior to any other technique of cystometry in animal experiments.

Description of the operative technique and results of transureteric cystometry in unanesthesized dogs is included in this paper. It should be stated that:

1) The method necessitates a preliminary operation which however, it well stood does not affect in any way the general or urogical status of the animal.

2) The proposed method of ureterocutaneostomy (ureter-pelvis implantation) is simple.

3) The operation is successful in most cases provided the surgical technique is carried out minutely and the operation done delicately. The separation of the renal pelvis from the kidney parenchyma should be carried out gently to avoid tearing of the pelvis wall or mucosa. Any damage may prove fatal, resulting in impassable stricture at the implantation site. The catheter should be introduced into the bladder via the ureter after opening the pelvis but before it is totally excised from the renal parenchyma. Catheterization at this stage of the operation is easier to accomplish, the ureter can be even forcefully pulled straight and the danger of injuring the pelvis wall, while pulling, is minimized. The distance between two incisions (operative wound and stab incision at which the pelvis is implanted) should be as great as possible because of danger of suppuration and dehiscence of the operative lumbar wound which may interfere with primary union of the implanted ureter-pelvis. Angulation of the ureter should be prevented for it may make post-operative catheterization difficult or impossible. Better and more rapid healing is assured if no dressings are applied except for the first postoperative day. The splinting catheter, if inserted, should be left in place no longer than 24 hours. Prolonged splinting may lead to vesical infection and irritation with subsequent alterations of cystometrograms as well as to troubles in postoperative care. Too early or intempestive catheterization is contraindicated postoperatively. In dogs Nos. 2 and 4 where these precautions were not observed the ureterocutaneostomy failed.

4) The complete closure of the implanted ureter meatus occurs often many months following operation. This period of time seems to be sufficiently long enough to carry out even prolonged investigations of bladder function.

5) After constriction or obliteration of the meatus the ureter can be reimplanted. The reimplantation is an easy and simple procedure and if successful makes further cystometric studies via transureteric route possible. The reimplanted ureteral orifice may remain patent for many months (dog No. 1) and sometimes even longer than the originally implanted ureter-pelvis.

6) Animals, prior to operation and cystometric studies, need not be subjected to special training. Handling of the animals in the post-operative period should be delicate for any irritative stimuli resulting in pain, fear or excitation may significantly affect the cystometric findings.

7) Catheterization via the ureter is simple, not painful and can be started immediately or some days after the operation depending on the

type of healing. The catheter should be passed delicately and not too far into the viscus for its tip could irrate the vessical wall. If an obstacle is met (a rare occurrence in this series) no force should be used but the instrumentation discontinued. Too early attempts of introduction the catheter (as in type I healing process when the implanted pelvis and ureteral meatus are still covered entirely by an firm eschar) may resut in a false passage as the catheter is likely to penetrate between the edges of pelvis and skin. Since the false passage leads to stenosis or atresia of the ureterocutaneostomy opening and to failure of the operation, it is advisible to postpone the instrumentation till the eschar has fallen away. The size of transureterally inserted catheters corresponds with the size of urethral catheters used in dogs since the lumen of the dog's ureter is as wide as that of the male dog's urethra.

8) Transureteric technique allows the cystometric examination to be carried out under physiological conditions, without anesthesia, quietly and with the full cooperation of the animal. It eliminates fear and pain. All animals, placed in a comfortable position, were tame and quiet, and showed no signs of fear when measurements were made. The time required to accomplish the test is short taking into account the large amount of fluid introduced into the bladder. The examination can be repeated at will any time it is necessary and does not impair the general condition of the animal. Infrequently, in some animals, at later stages of investigations a low grade vesical infection develops.

9) During the examination not only intravesical pressures can be recorded but also vesical sensation can be studied. In addition, observation and recording of undisturbed micturition is possible, for the normal emptying possibilities of the bladder per vias naturales (no catheter in the urethra) are maintained.

10) The flat, smooth, slowly rising type of cystometrogram is recorded in most animals.

The majority of successive volume pressure curves in the same animal is similar often identical. Some differ only slightly in height and lenght. In some animals, however, on repeated recording a hypertonic type of curve is noted.

It is thought that only cystometrograms of the first group can be regarded as normal. The structure of the curve indicates that the examined dog is in a state of nervous and emotional stability and his bladder has a constant physiology. Findings in the second and third group indicate that some psychic instability exists or the examination causes some local or general irritation, despite all precautions taken.

In general it can be stated that:

a) the vesical capacity is large and constant during prolonged observation in the same animal, and it is by far larger than under anesthesia,

b) the vesical capacity equalling 500 to 600 cc. is comparable or the same in dogs of similar size and weight, the striking differences noticed in different animals as noted by Sauvage were not observed,

c) the intravesical pressures in emotionally stabile animals are low and gradually increase during the course of examination. Less or more marked hypertonicity is sometimes noted or in some animals,

d) further study on the micturition pressures is necessary to draw more detailed conclusions. Often the dog can hold introduced fluid in the bladder up to pressure greatly surpassing the normally found micturition pressures,

e) the sensory points vary in wide limits which is in sharp contrast with stability of vesical capacity and intravesical pressures as well as with data obtained in human,

f) during transureteric cystometry consecutive cystometrograms are similar and comparable, closely resembling those obtained in normal human subjects.

11) Transureteric cystometry in unanesthetized dogs permits not only obtaining comparable cystometrograms in successive determinations carried out in the same dog as well as in other dogs but also the selection of animals. After preliminary examinations for further study only animals with long, flat, stabilized „normal" cystometrograms can be chosen.

CONCLUSIONS

An ideal technique of performing cystometry in experimental animals is not available.

The less irritative the procedure of cystometry the better results obtained. The bladder, as emphasized by Rose (1961) has a constant physiology if not altered by psyche or other irritative stimuli.

A technique of transureteric cystometry described in this paper eliminates two principal factors responsible for alterations of detrusor activity, i.e., catheter in the urethra and anesthesia. The procedure eliminates fear and pain and leaves the bladder emptying routes free. Therefore the animal co-operation can be assured, intravesical pressures as well as bladder sensation and free micturition observed and recorded.

Transureteric cystometry gives better and more constant data, comparable on successive determinations. The normal cystometrogram of a large dog closely resembles that of the man, vesical capacity equalling

500 to 600 cc., and volume pressure curve rising slowly. Hypertonicity is evident only in emotionally unstable animals. For prolonged studies only suitable animals should be selected.

It is expected, therefore, that the technique of transureteric cystometry may be satisfactorily employed in experimental research, and help in developing physiologic urology of the lower urinary tract and contribute to our knowledge on bladder function.

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