BULLETIN INTERNATIONAL
DE L'ACADÉMIE POLONAISE
DES SCIENCES ET DES LETTRES

CLASSE DES SCIENCES MATHÉMATIQUES ET NATURELLES
SÉRIE *B*: SCIENCES NATURELLES (II)

Table des matières

Główne naczynia mózgu pstrąga tęczowego (Salmo iri-deus Gibb.) — The main vessels of the brain in Rainbow trout (Salmo irideus Gibb.)

Mémoire

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présenté le 11 juin 1945 par M. H. Hoyer m. t.

Problem, material, method

The blood vessels of the brain of the Teleost have been relatively seldom investigated, for the most part with reference to other systems e. g. very frequently with the skeleton. Therefore our knowledge of them is very inaccurate and fragmentary, without differentiating between adult or embryonic animals. On the other hand the same vessels in the Elasmobranchians or Amphibians, groups of animals of which the today existing species and individuals are much fewer, are fairly well known in many members of these systematical units.

There are at least two reasons for this. *Teleostei* raised but little interest in comparative anatomists because they lie out of the main evolutionary way of vertebrates. The knowledge of the structure of Teleosts did not contribute much to the elucidation

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I investigated the material collected in years 1938 and 1939 during the first two month of German occupation of Kraków. The first review of the results was written by me in november of the same year, while imprisoned in a single cell in Bresslau. The notes were happily saved during my sejour of three month in concentrating camp of Sachsenhausen. I was able to take up the work in september 1940. Manuscript was written in february 1941. Corrections and supplements were done in the spring 1943.

of the relationship between Fishes and Amphibians, because Teleosts do not furnish the intermediate links in the phylogeny of different organs.

The brain itself presented some difficulties to the comparative anatomists, when the homology of its different parts was being established. For a long time the *Mesencephalon* was taken for the cerebral hemispheres; just now after almost 80 years discussion all matters of controversy in this problem have been finally elucidated (1882 — Haller). It was impossible for the comparative anatomist to speak about the vessels of the brain before the accurate knowledge of its structure was established.

Technical difficulties frightened away some of the investigators. The vessels if not filled with dye do not show up against the cerebral tissue. The difficulties in the injecting of blood vessels are connected with the choice of the dye, syringe and point of introduction of the injecting needle. It is also comparatively difficult to get out the injected and fixed brain from the narrow brain-case. Dissection, unless we have at our disposal a large fish's head, requires plenty of pains and patiance, because the brain-case constitutes but small section of the skull.

The main vessels, which enter or leave the brain-case, have been relatively often seen and described. Beneath the base of the skull run confluently the two *Aa. carotides internae*. In the middle line of the skull they unite and as an unpaired trunk *(A. encephalica)* pierce the hole between the parasphenoid and prootica and enter the myodome (*Lopholatilus, Opsanus, Limanda* — Silvester, *Scorpaena, Belone, Esox, Salmo, Gadus* — Allis, *Amphipnous, Monopterus* — Hyrtl, *Labio* — Sen, *Spheroides* — Rosen, *Gobius* — Schötlle). From here they reach the brain in the region of the hypophysis. Rarely both arteries unite into an unpaired trunk within the brain-case proper (*Amiurus* — McKenzie, Allis, *Leptocephalus* — Silvester).

A. encephalica divides after a short course within the brain-case into two cerebral vessels, one of which turns backwards, the other forwards along the brain (Silvester, Allis). In *Lophius piscatorius*, according to Burne, *A. encephalica* divides when still in the myodome into two cerebral arteries. Each of them forms several loops above the anastomosis (*V. pituitaria* — Goodrich) between *Vv. cardinales anteriores* and each enters

separately the brain-case laterally to the hypophysis. Between the roots of nerves II, V and VII they form another accumulation of loops, from which fine vessels branch to different regions of the brain. The hypophysis and *Lobi inferiores* receive their vessels from the *A. optica (Ramus recurrens),* forming also abundant loops in the brain-case. Fundamentally they appear here similar to vessels in other Teleosts, but their course and several systems of highly complicated loops are a peculiar feature of the blood vessels in this extremely specialized fish.

The knowledge of veins is similar to that of the arteries and much more is known about the portions contiguous to the skull than about their ramifications on the brain. G e l d e r e n distinguishes three cerebral veins in Teleosts after having investigated an abundant embryonic material *(Belone, Blennius, Clupea, Gobius, Lophius, Muraena, Salmo, Silurus, Smarus). V. cerebri ant.* leaves the skull through different places in the alisphenoid in order to reach the orbita. *V. cerebri media spuria* accompanies *Nervus trigeminus* on its way out of the brain-case, resting upon its anterior margin. *V. cerebri post.* follows the course of *Nervus vagus.* All three cerebral veins merge into different section of *V. cardinalis ant. (V. jugularis, capitis media, infraocularis, orbitonasalis).* The investigations of G e l d e r e n confirm and bring into harmony the observations of previous authors (E m e r y, M c K e n z i e, A l l i s, S i l v e s t e r, S c h ö t t l e). The *Ophiodon* has not *V. cerebri ant.* (A l l e n). In literature no exact details about the course and distribution of the cerebral veins are to be found.

From this brief summary it is evident that our knowledge of brain vessels in the Teleosts is inaccurate; we know more about the arteries than the veins, but we miss a full picture of the blood vessels in at least one representative of this fish group. I have tried therefore to work out as accurately as possible the brain vessels in the trout (*Salmo irideus* G i b b). This fish was chosen as a member of the primitive not specialized family *Salmonidae* and as being accessible throughout the year.

Specimens 18—25 cm in length, supplied by the same fishery farm were deprived of blood by curtailment of the tail vessels. At first 2—3 cm of black Indian ink diluted with physiological salt solution were injected by means of the »Record« syringe

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through the caudal artery, later on a smaller quantity of shellac red dye in a similar way. The first dye filled the veins and capillaries, the second the arteries only. When the fish lost its blood completely the dye dispersed very easely within the blood vessels. Injected specimens were fixed with formaldehyde. The brains were dissected out with fine forceps, iredectomy knives and sharp pointed scissors under the binocular microscope. The exposed brains were investigated and further dissected in glass dishes with water or with oil of cedar, which facilitated the tracing of the course of blood vessels within the transparent tissues.

Disposition of blood vessels

Arteries.

A. carotis int. (Fig. 1. Aep, Al, Aci) arises below the base of the skull from *Aorta lateralis* at the point, where *A. epibranchialis* of the first gill reaches it. From the embryological point of view *A. carotis interna* constitutes a direct prolongation of the aorta, which in adults bends aside from it at an obtuse angle. *Aa. carotides internae* after having gone a little way, pierce the bony base of the skull and enter separately the myodome of its side of the head. Within the myodome they send out laterally *A. orbitonasalis* and turning themselves almost vertically unite with each other into an unpaired stem of *A. encephalica*.

A. encephalica (Fig. 1, 2, 3, Aen) passes through the membraneous base of the brain-case *(Dura mater)* and reaches the anterior surface of the pituitary body. Posterior to the optic nerve it divides into two vessels, which turn laterally and after a short distance each of them divides for the second time into two well known branches *A. cerebri ant.* and *post.* (Fig. 2, 3 A, Aca, Acp). The first supplies the anterior, the second the median and posterior part of the brain.

A. cerebri ant. (Fig. 2, 3 A, Aca) crosses the optic nerve, where gives off a branch of no interest to us here (*A. optica* — Fig. 1, 2, 3 A, Acr) and then passes along the lateral side of the nerve toward the base of the forebrain. Here it turns in a slight arc to the middle line of the brain and unites with its fellow from the opposite side in the groove between the hemispheres. In this groove runs, from the point of fusion down to the olfactory
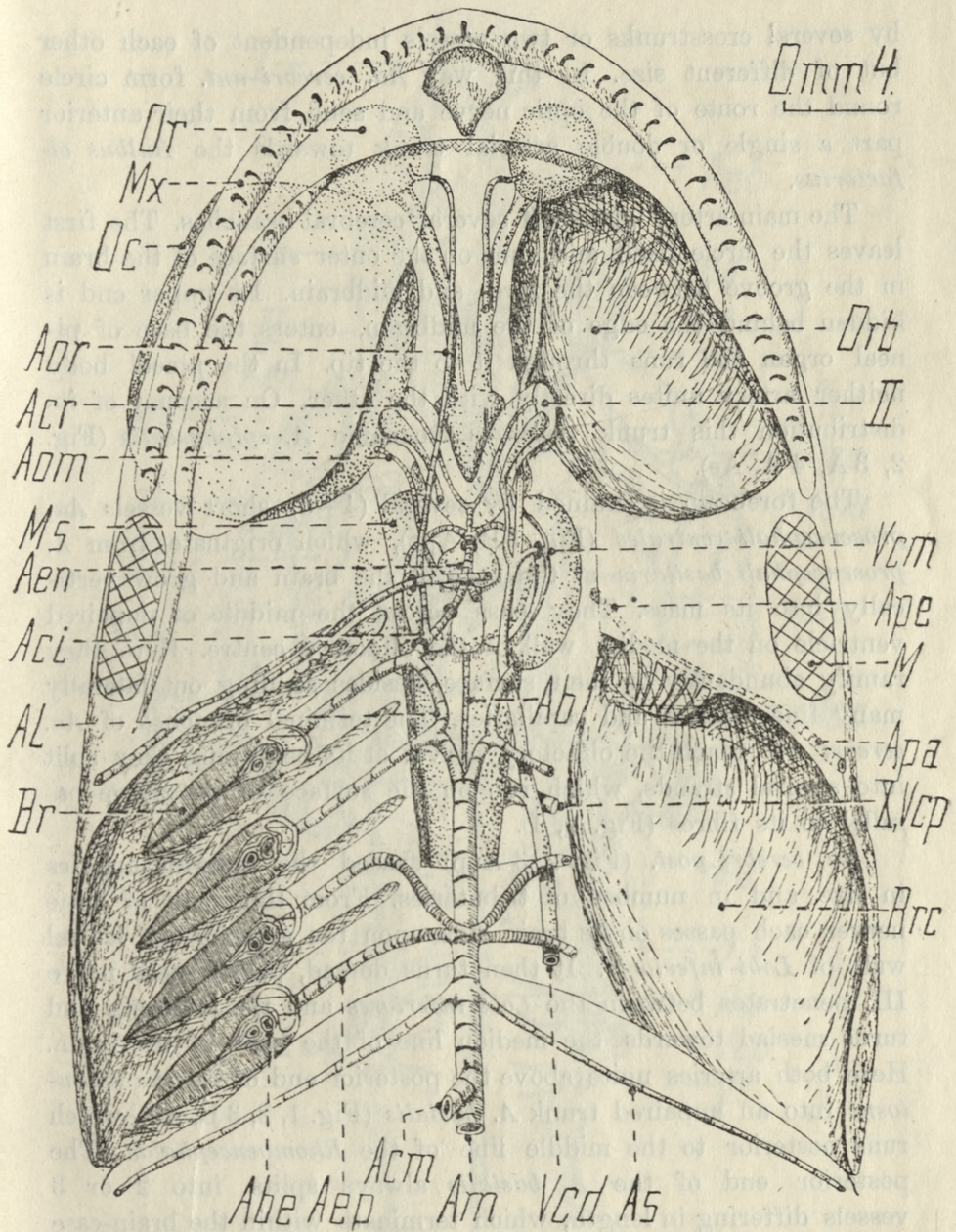
Fig. 1. Vessels of the base of the skull. By removal of bones, gills and eyeball the main vessells connected with the brain have been disclosed.

nerve, sometimes one arterial trunk, *A. prosencephali basilaris* (Fig. 2, 3 B, Apb), more frequently two parallel ones, connected

by several crosstrunks or two vessels independent of each other
but of different size. In this way *Aa. cerebri ant.* form circle
round the route of the optic nerve and send from their anterior
part a single or double arterial trunk towards the *Bulbus ol-
factorius.*

The main artery gives off several cerebral branches. The first
leaves the circle itself and runs on the outer surface of the brain
in the groove between the fore- and midbrain. Its upper end is
hidden behind the edge of the midbrain, enters the base of pi-
neal organ and runs through it to the tip. In the pineal body
neither artery unites directly with the other. On account of its
distribution this trunk deserves the name *A. epiphysealis* (Fig.
2, 3 A, 4 A, Ae).

The forebrain is drained by several (4—6) short vessels *Aa.
prosencephali centrales* (Fig. 3 B, Apc), which originate from *A.
prosencephali basilaris* at the base of the brain and grow verti-
cally into its mass. They pass across the middle of unpaired
ventricle on the medial wall of the olfactory centre. Here they
ramify abundantly on their surface, besides sending out laterally
many fine arteries and capillaries. The terminal branches of *Aa.
cerebri ant.* reach the olfactory nerve; at its beginning they split
into several strands, which run on the surface of the nerve pa-
rallel to its fibres (Fig. 2, I).

Aa. cerebri post. (Fig. 2, 3 Acp) exceed the anterior arteries
in size and in number of tributaries. From the roots of optic
nerves each passes on its brain side upon the anterior and lateral
wall of *Lobi inferiores.* It then turns dorsad, passes near nerve
III, penetrates between the *Lobi inferiores* and the midbrain and
turns mesiad towards the median line of the base of the brain.
Here both arteries unite above the posterior end of *Saccus vascu-
losus* into an unpaired trunk *A. basialis* (Fig. 1, 2, 3 B, Ab), which
runs posterior to the middle line of the *Rhombencephalon.* The
posterior end of the *A. basialis* always splits into 2 or 3
vessels differing in length, which terminate within the brain-case
and never continue as a spinal artery. The vessels under discus-
sion then form an arterial circle surrounding the attachments of
the *Saccus vasculosus, Lobi inferiores* and *Hypophysis. Aa. cerebri
post.* constitute a mirror-like image of *Aa. cerebri ant.*. Both pairs
of arteries form one arterial circle *(Circulus)* and one or more
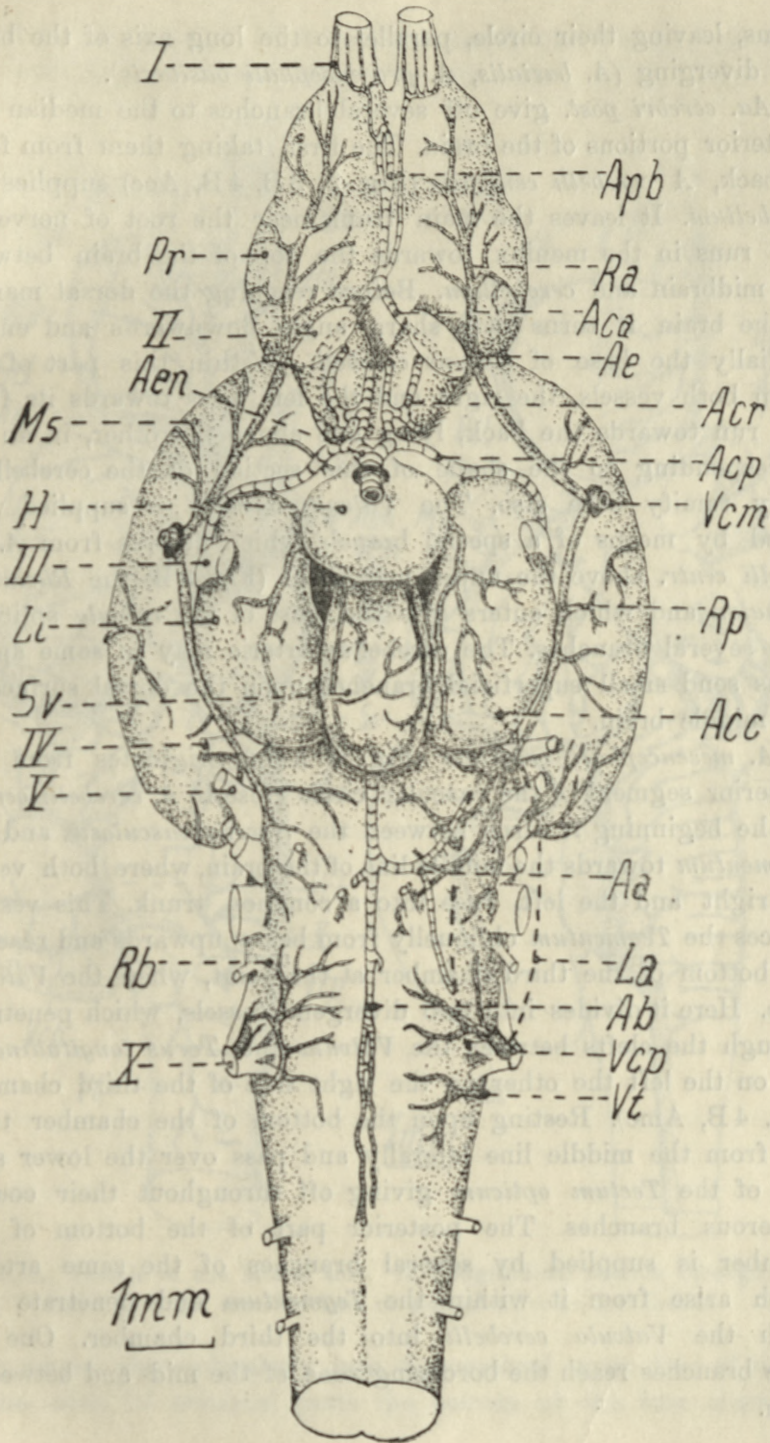
Fig. 2. Vessels of the base of the brain.

stems, leaving their circle, parallel to the long axis of the brain, but diverging *(A. basialis, A. prosencephali basilaris)*.

Aa. cerebri post. give off several branches to the median and posterior portions of the brain. The first, taking them from front to back, *A. cerebelli centralis* (Fig. 2, 3 B, 4 B, Acc) supplies the *cerebellum*. It leaves the main trunk near the root of nerve IV and runs in the meninx towards the top of the brain between the midbrain and *cerebellum*. Before reaching the dorsal margin of the brain it turns at a sharp angle downwards and enters medially the base of *Corpus cerebelli*. Within this part of the brain both vessels, the right and the left, pass towards its floor and run towards the back, lying one above the other, in an arc corresponding to the shape of this section of the cerebellum. They ramify here also. The *Valvula cerebelli* is supplied with blood by means of a special branch, which derives from *A. cerebelli centr.* above the upper margin of (Fig. 3 B) the *Rhombencephalon* and which enters into the mass of the *valvula* splitting into several branches. The cerebellar arterie may in some specimens send small superficial branches upon the dorsal surface of the middle brain.

A. mesencephali centralis (Fig. 3 Amc) originates from the posterior segment of the vascular circle close to *A. cerebelli centr.*. In the beginning it turns between the *Saccus vasculosus* and the *Tegmentum* towards the middle line of the brain, where both vessel the right and the left, fuse into a common trunk. This vessels pierces the *Tegmentum* obliquelly from below upwards and reaches the bottom of the third chamber at the point, where the *Valvula* ends. Here it divides into two divergent vessels, which penetrate through the clefts between the *Valvula* and *Torus longitudinalis*, one on the left the other on the right side of the third chamber (Fig. 4 B, Amc). Resting upon the bottom of the chamber they run from the middle line laterally and pass over the lower surface of the *Tectum opticum* giving off throughout their course numerous branches. The posterior part of the bottom of the chamber is supplied by several branches of the same artery, which arise from it within the *Tegmentum* and penetrate beneath the *Valvula cerebelli* into the third chamber. One or more branches reach the bordering zone of the mid- and betweenbrain.

Numerous branches of *A. basialis* supply the *Rhombencephalon* (*Aa. rhombencephali centrales*, Fig. 3 B, Arc), rising from the
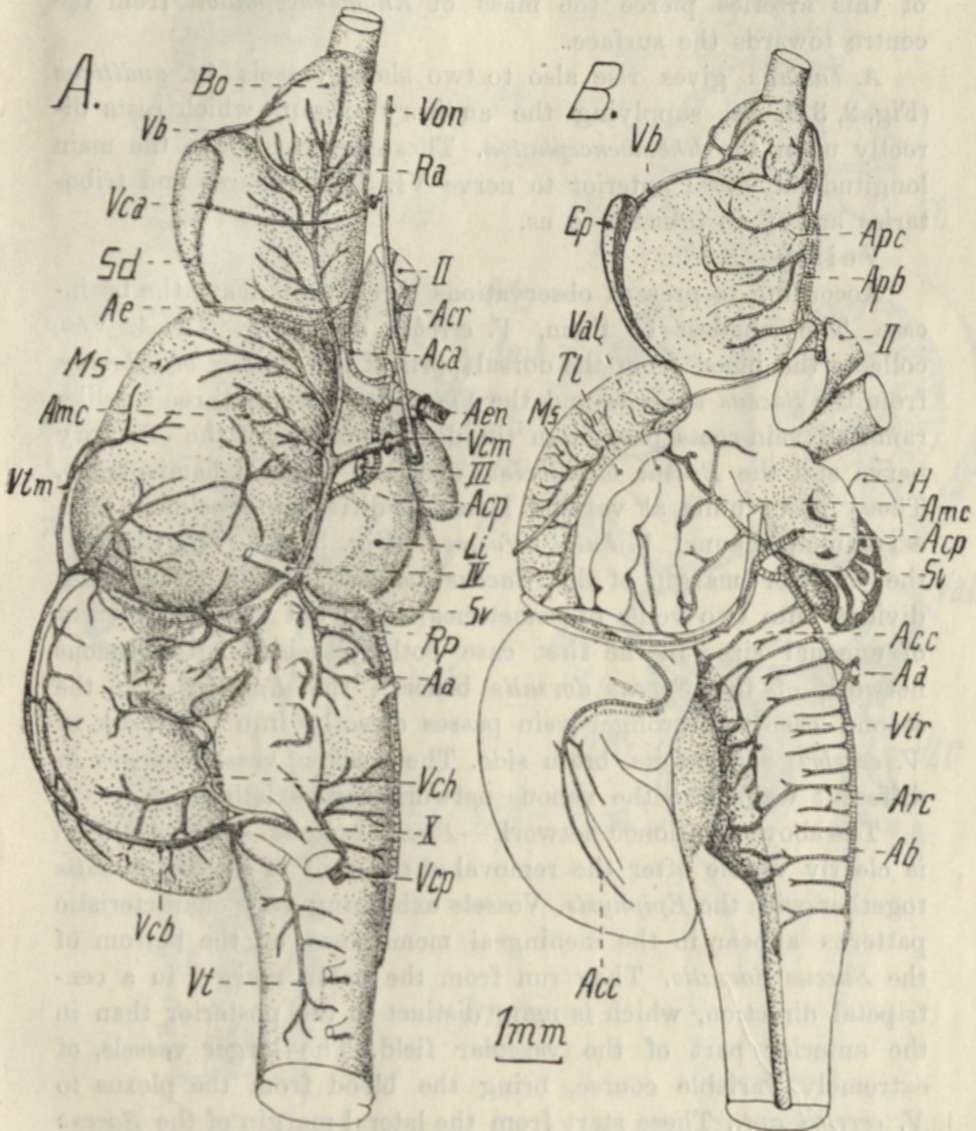
Fig. 3. A. Vessels of the lateral side. B. Longitudinal section through the brain. Internal ramifications of central arteries are shown.

main artery perpendicularly into the cerebral mass. One of them reache with its terminal parts the bottom of the four chamber

and form here the meshes of an irregular network, the others do not reach the bottom of the chamber. The lateral branches of this arteries pierce the mass of *Rhombencephalon* from the centre towards the surface.

A. basialis gives rise also to two short vessels *Aa. auditivae* (Fig, 2, 3 B, Aa) supplying the auditory organ, which rests directly upon the *Rhombencephalon*. These arteries leave the main longitudinal vessel anterior to nerve VI. Their course and tributaries are of no interest to us.

Veins.

According to present observations three veins leave the braincase. The smallest of them, *V. cerebri ant*. (Fig. 3A, 4, Vca) collects the blood from the dorsal parts of the *Bulbus olfactorius*, from the *Saccus dorsalis* and the *Epiphysis*. Two or three treelike ramified vein-roots pass from the dorsal portion of the olfactory nerve and the *Bulbus olfactorius* over the cerebral hemispheres. These roots unite at various levels in different specimens into a common channel *V. bulbi olfactorii* (Fig. 3A, 4, Vb). Close to the anterior margin of the *Saccus dorsalis* the unpaired trunk divides into two veins of sometimes equal but more frequently of unequal size. In the first case both vessels join the venous network of the *Saccus dorsalis* beneath the *Epiphysis*. In the second case the stronger vein passes directly into the trunk of *V. cerebri ant*. on its brain side. The weaker vessel merges in different ways into the venous network here existing.

The above mentioned network — *Plexus venosus* — (Fig. 4B, Plv) is clearly visible after the removal of the roof of *Saccus dorsalis* together with the *Epiphysis*. Vessels exhibiting very characteristic patterns appear in the meningeal membranes on the bottom of the *Saccus dorsalis*. They run from the outer margin in a centripetal direction, which is more distinct in the posterior than in the anterior part of the vascular field. The larger vessels, of extremely variable course, bring the blood from the plexus to *V. cerebri ant.*. These start from the lateral margin of the *Saccus dorsalis* and transverse the lateral surface of the brain almost perpendicularly to its long axis. Continuing they turn downwards and encircle the cerebral hemispheres separated from them by adipose intermeningeal tissue. Below the brain they pierce the orbital bones and join *V. orbito-nasalis* (Fig. 3A, Von).
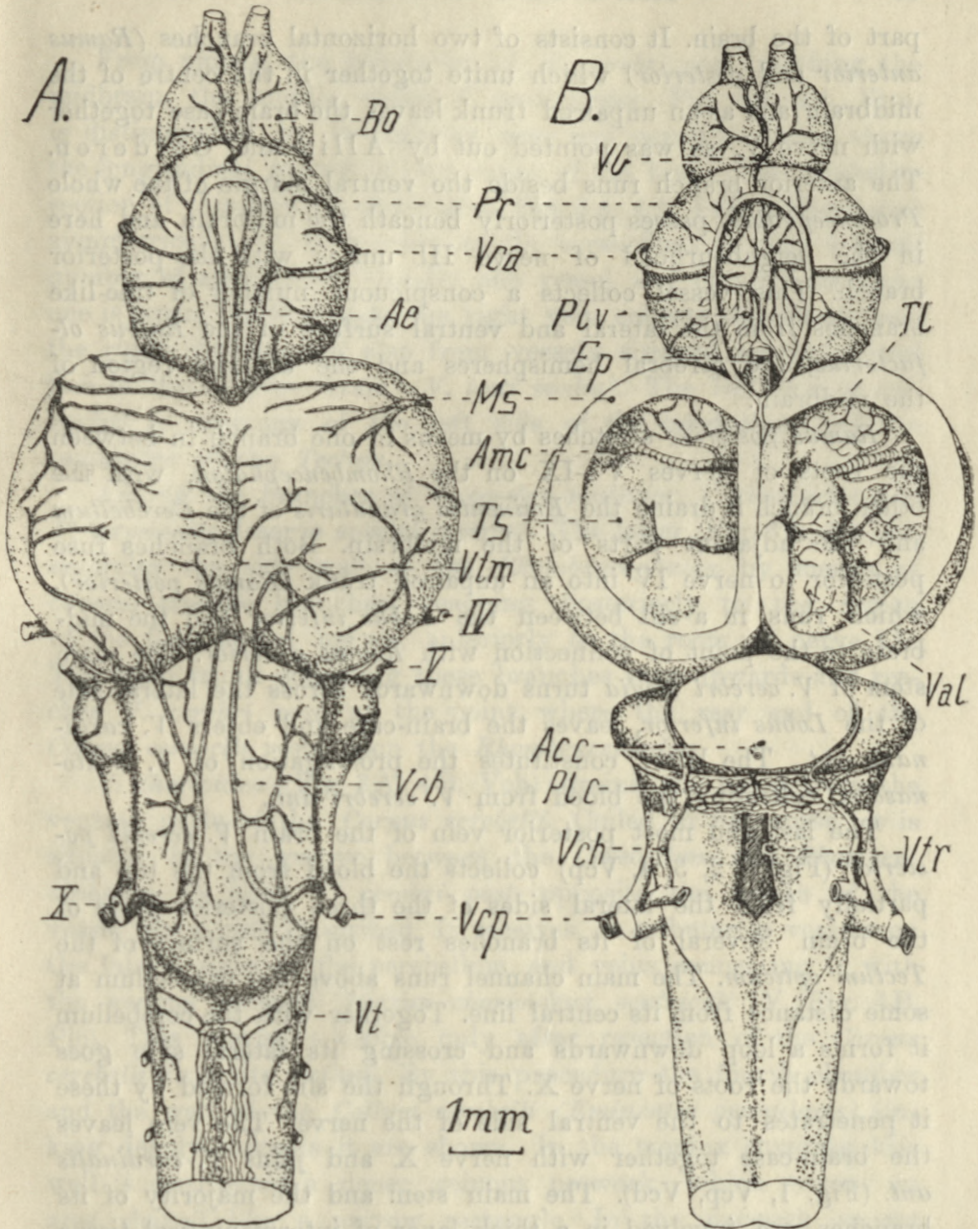
Fig. 4. Dorsal view of the brain. A. Superficial vessels. B. Deeper vessels revealed by removal of the pineal body together with the roof of the *Saccus dorsalis, Cerebellum* and *Tectum opticum.*

The second in turn, *V. cerebri media* (Fig. 1, 2, 3 A, Vcm Ra, Rp) collects the blood from the bottom and sides of a large

part of the brain. It consists of two horizontal branches *(Ramus anterior* and *posterior)* which unite together in the centre of the midbrain and as an unpaired trunk leaves the brain-case together with nerve V, as was pointed out by A l l i s and G e l d e r e n. The anterior branch runs beside the ventral margin of the whole *Prosencephalon*, passes posteriorly beneath the midbrain and here in the neighbourhood of nerve III unites with the posterior branch. This vessel collects a conspicuous number of tree-like branches from the lateral and ventral surface of the *Bulbus olfactorius*, the cerebral hemispheres and the anterior region of the midbrain.

Ramus posterior stretches by means of one branch in between the roots of nerves V—IX on the *Rhombencephalon*, with the other branch it drains the *Eminentia granularis* of the *Cerebellum* and the adjacent parts of the midbrain. Both branches fuse posterior to nerve IV into an unpaired trunk *(Ramus posterior)*, which runs in a slit between the *Lobus inferior* and the midbrain to the point of connection with *Ramus anterior*. The main stem of *V. cerebri media* turns downwards across the lateral side of the *Lobus inferior*, leaves the brain-case and enters *V. cardinalis ant.*. The latter constitutes the prolongation of *V. orbitonasalis*, collecting the blood from *V. cerebri ant.*.

The last and most posterior vein of the brain *V. cerebri posterior* (Fig. 1, 2, 3, 4, Vcp) collects the blood from the top and partially from the lateral sides of the three posterior parts of the brain. Several of its branches rest on the surface of the *Tectum opticum*. The main channel runs above the cerebellum at some distance from its central line. Together with the cerebellum it forms a loop downwards and crossing its lateral side goes towards the roots of nerve X. Through the slit formed by these it penetrates to the ventral side of the nerve. The vein leaves the brain-case together with nerve X and joins *V. cardinalis ant.* (Fig. 1, Vcp, Vcd). The main stem and the majority of its branches are involved in a thick layer of intermeningeal tissue directly touching neither the internal sheet of the *Endomeninx* nor the vaulted skull. The treelike endings of the vein rest directly upon the membranes covering the brain and unite with the vessels draining the substance of the brain.

From among the tributaries of *V. cerebri post.* reaching the midbrain, the middle vessel *V. tecti media* (Fig. 3 A, 4 A, Vtm) is distinguished by its greater size and constant course above the longitudinal groove of this region of the brain. The cerebellar section of this vein (*V. cerebelli* — Fig. 3 A, 4 A, Vcb) is developed symmetrically on both sides ot the cerebellum in only a small number of specimens. Usually one vessel and chiefly the right one is larger. In this case the right vein collects the blood from the right *Tectum* and also from vessels situated on the central line of the middle brain *(V. tecti media)*. The left vein is restricted to drainage of the left side of the cerebellum and the remainder of the *Tectum*.

Among the branches of *V. cerebri posterior*, *V. chorioidea* and *V. terminalis* deserve special interest. The latter (Fig. 3 A, 4 A, Vt) encircles the side wall of the *Rhombencephalon* by means of several treelike branches, reaching posteriorly to the region of the first spinal nerves, anteriorly to the roots of nerve IX. The main trunk collecting these branches runs upwards and reaches *V. cerebri post.* at the point where the rear end of the *Corpus cerebelli* rests upon the *Rhombencephalon*.

V. chorioidea (Fig 3 A, 4 B, Vch) drains the blood from the ventral parts of the *Corpus cerebelli*. Coiled in its course, it is situated in the groove between the *Cerebellum* and *Rhombencephalon*. It joins *V. cerebri post.* opposite the mouth of the vessel previously described. It receives as tributaries vessels on the lateral sides of the cerebellum and veins connecting it with the venous network *Plexus chorioideus ventriculi* IV (Fig. 4 B, Plc). This plexus is visible only after resection of the *Corpus cerebelli* from its *Collum*. By this procedure the fourth chamber and the wall of the *Collum cerebelli (Eminentia grunularis)* sinking deeply towards it are shown. In the meninx covering this wall is distributed a dense veinous network, which covers an area shaped like a narrow rectangle. In the network appear stronger stems, which originate in the middle of the vascular field and gradually gaining in dimensions pass laterally and fuse with *V. chorioidea*.

General futures of the main blood-vessels

Looking at preparations of the brain of the trout of which the arteries are filled with red dye and the veins with black, we remark the lack of small arterial branches upon the surface of the brain; the veins on the contrary project black everywhere. Strong arterial stems are visible on the ventral surface of the brain, where they carry the blood along the whole base. In the brain of other *Anamnia* e. g. in sharks (S t e r z i, H o f f m a n n, G r o d z i ń s k i) or in Amphibians (G a u p, R o o f e, S o c h a) arteries and veins are treelike distributed everywhere in the meninges nearly of each other.

From investigations upon the development of blood vessels (G r o d z i ń s k i) it is known that the real arterio-venous arrangement of blood vessels exists primarily on the surface of the brain in young trout embryos. Secondarily in older specimens the central arterial system develops and the superficial turns into a purely venous one. The cause of these complicated processes peculiar to trout lies probably in the way, the brain itself develops.

A relic of this primitive arterio-venous system is *A. epiphysealis*. There already exists in very young embryos a strong *A. tecti optici*, which passes from the front to the dorsal surface of the midbrain and supplies it abundantly. It gives off also a small branch to the rudiment of the pineal body. In proportion as the developing *A. mesencephali centralis* starts supplying the *Tectum* from inside, the branches of *A. tecti optici* draining it on the outer surface gradually disappear. Finally only the initial portion of the main stem and its epiphyseal branch persist as constituent parts of *A. epiphysealis* in adult fishes.

The cerebral arteries *(Aa. cerebri ant.* and *post.)* are arranged on the base of the brain in representatives of different vertebrates, among others in sharks and rays, in such a way that one turns forwards and the other backwards. Anterior to the optic chiasma both *Aa. cerebri ant.* may unite, also *Aa. cerebri post.* posterior to hypophysis. Owing to this unions a closed arterial circle — *Circulus Willisii* — originates on the base of the brain. In the trout the *Circulus Willisii* consists of two circles, the anterior smaller and the posterior bigger. From the latter runes backwards

the unpaired *A. basialis*, which terminates in trout within the *Rhombencephalon*. In the sharks it continues as ventral artery in the spinal cord.

The main vessels distributed on the base of the brain furnishe this organ with nutrient vessels. They can be found within the neural tissue only, but not on the surface of the brain as in other vertebrates. The arteries of the forebrain and rhomben-cephalon *(A. prosencephali centralis, A. rhombencephali centralis)* originate as unpaired vertical stems one after another in a line and after a short course break into branches within the neural tissue. The midbrain and cerebellar arteries *(A. mesencephali cen-tralis, A. cerebelli centralis)* arise from the posterior part of the *Circulus Willisii* as paired stems and remain paired in their course, with the exception of a short portion of. *A. mesencephali centralis*, which fuses within the *Tegmentum* into a common stem.

Because arteries enter the centre of the brain, they can only ramify towards the surface. There they meet venous branches collecting blood. The superficial distribution of the veins remains therefore in close relation with the central arrangement of the arteries.

The membranes investing the brain of the trout exert a great influence upon the distribution of veins. They form only one meninx *(Meninx primitiva)*, one membrane of which rests against the brain *(Endomeninx)* the other against the skull *(Ectomeninx)* In between these membranes the layer of intermeningeal tissue extends, especially thick on the top and sides of the brain, far thinner on its base. This tissue, jellylike in appearence, contains plenty of fat and numerous pigmented cells (Gelderen). The fatty tissue levels all grooves and depressions between the brain segments and at the same time closely fills the entire space between it and the brain-case. Only the anterior tip of the pi-neal body emerges and approaches the immediate neighbourhood of the vault of the skull. The majority of the smaller veins rest upon the internal membrane of the meninx *(Endomeninx)*, the main trunks *(V. cerebri anterior, media, posterior)* on the contrary are involved in the thick layer of fatty tissue between both membranes.

The bigger veins of the brain lie in many vertebrates more or less exactly in longitudinal grooves on the middle line of the

dorsal surface of the brain or in transversal ones, separating
different portions of the brain from one another. In the trout
no vein is situated in any transversal groove. Even *V. cerebri
ant.* runs involved in intermeningeal tissue laterally to the fore-
brain, in spite of the fact that in embryos it originates in the
groove between the anterior and median brain vesicles. Connected
with the vessels of the terminal portion of the pineal body it
migrates with this forwards on the side of the hemispheres
(Grodziński). This displacement is highly facilitated by the
intermeningeal tissue, which appears comparatively late in deve-
lopment and pushes away the existing *V. cerebri ant.* from the
surface of the brain. Similarly the same tissue pushes away
several other veins, such as *Ramus ant. & post.* of *V. cerebri
media, V. tecti media, V. bulbi* and *V. cerebelli.*

The veins of the brain in the trout and shark may serve as
an example of dependence between the pattern of vessels and
the structure and development of different portions of the brain.
V. cerebri ant. exists in sharks as a large vessel draining the
whole *Prosencephalon* and portions differing in size of the adja-
cent *Dien-* and *Mesencephalon* and *Cerebellum* (Grodziński).
In the trout it collects the blood from the pineal body, *Saccus
dorsalis,* and from a few vessels of the forebrain connected with
it only. Its tributaries and size are thus much smaller than in
the shark.

The cause of this condition is connected with the compara-
tively small size of the forebrain and especially in the manner
of brain development in the trout. In the early embryological
stages the midbrain and its vessels develop more quickly. *V. ce-
rebri ant.* connected with the rudiment of the pineal organ moves
together with it forwards along the surface of the hemispheres,
growing fairly late and slowly. This vein has no opportunity to
proliferate the tributaries upon the midbrain, because it is not
near them. At the same time, pushed away by intermeningeal,
tissue, it does not drain the base and sidewalls of the hemisphere.
In its place, the vein of the middle brain *(V. cerebri media)*, at
that moment well developed, penetrates with its branches to the
base of the hemispheres just in forming. In sharks the *prosen-
cephalon* develops somewhat faster than the *mesencephalon,* there-
fore the vein of the anterior vesicle *(V. cerebri ant.)* takes pos-

session of it by means of several branches and is able to invade the other neighbouring portions of the brain also.

V. cerebri media from the beginning is associated with the midbrain. It is therefore the largest vein of the brain and gradually reaches with its roots further and further forwards along the base and sidewall of the prosencephalon and at the same time backwards to similar portions of *Rhombencephalon*. This vein is entirely absent in sharks. During the development of their brain the midbrain has not an ascendancy in size over other vesicles. Hence the veins of the anterior part and posterior parts of the brain *(V. cerebri ant. & post.)* overlap the midbrain with their roots and divide into spheres of influence.

V. tecti media supplies the *Tectum opticum* in representatives of both fish groups. In sharks it opens into *V. cerebri ant.*, in the trout by means of *V. cerebelli* into *V. cerebri post.*. The rudiments of this vein arise in trout early, even before the protrusion of the cerebellum and remain in direct connection with *V. cerebri post.*. In proportion as the cerebellum protrudes into the space between the midbrain amd *Rhombencephalon, V. cerebri post.* is pushed away from the midbrain and its former course indicated by the new portion of bloodvessel — *V. cerebelli.* In sharks from the very beginning stages of development the cerebellum separates *V. cerebri post.* from the contact with the ·*Tectum.* The veins from this region join the course of *V. cerebri ant.* in the neighbourhood.

In the dorsal groove between the two hemispheres run, in most sharks, one or more longitudinal veins, which ramify abundantly and collect tributaries from the top of the forebrain. In trout, in the same place and depending on the size of the *Saccus dorsalis*, exists the *Plexus venosus,* which receives the blood from the pineal body and *Bulbi olfactorii* and conducts it into *V. cerebri ant.*. A similar but more fully developed organ called *Nodus vasculosus* appears in *Ambystoma* (H e r r i k, R o o f e). It is a net of sinusoid vessels, which drains the paraphysis and dorsal sac, and which collects the blood from almost the entire forebrain and *Plexus chorioideus* of the three anterior ventricles. Common to both organs is their situation and venous character. They differ in relation to some organs of the brain existing in this region. The *Plexus venosus* penetrates the *Saccus dorsalis*

and is connected with the pineal body. The *Nodus vasculosus* as mentioned above is connected with some other organs also. Because in the brain of trout the *Plexus chorioideus* of the three anterior chambers is absent, the dimensions of the *Plexus venosus* do not attain anything like these of the *Nodus*.

In the brain of vertebrates *Plexus chorioidei* appear in the form of thin brain walls, without fibers and concentrations of neural cells, closely connected with the adjacent meninges and abundantly drained with blood vessels. They reach the highest degree of development in the region of the forebrain and *diencephalon*, where they protrude as folds into the lumen of the chamber and in the region of the *Rhombencephalon*, roofing the fourth chamber. The trout lacks the two *Plexus chorioidei* in the chamber of the forebrain and here appears on its top only the *Plexus venosus*. The fourth chamber in trout appears to be a narrow and short cleft roofed by the cerebellum. Between the cerebellum and the lumen of the chamber the *Plexus chorioideus* is absent. The sole plexus in this region rests upon the *Eminentia granularis* of the *Cerebellum*. We consider this as a remnant of *Plexus chorioideus ventriculi* IV, which has remained in consequence of the excessive development of the cerebellum and lateral flattening of the lumen of the chamber. This plexus is purely venous in composition.

Summary

The main vessels of the brain in trout were described on the basis of preparations, the arteries and veins of which were filled with two different dyes.

The brain is reached by the unpaired *A. encephalica*, which arises by fusion of two *Aa. carotides int.*, being themselves prolongations of *Aortae laterales*.

Aa. cerebri ant. and *post.* form on the base of the brain a closed *Circulus Willisii*, subdivided into anterior and posterior circles.

From the arteries in the base of the brain penetrate into the neural tissue vertical arteries (*Aa. prosencephali centrales*, *mesencephali centrales*, *cerebelli centrales*, *rhombencephali centrales*), which dispatch nutrient branches from the centre of the brain towards its surface.

Veins distributed on the surface of the entire brain and collected into bigger branches leave the brain-case as three pairs of trunks *(V. cerebri ant., media, post.)*. They join different portions of *V. cardinalis ant.*.

The vessels of the brain in trout were compared with similar vessels of other vertebrates mainly with sharks and urodeles. On this occasion emphasis was put upon the different development and structure of the brain as well as upon the presence of adipose intermeningeal tissue *(Meninx primitiva)* in order to elucidate the difference in the pattern of blood vessels.

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Explanation of figures

The brains of the trout were drawn with the aid of Abbé's apparatus from preparations studied under the binocular microscope.

Arteries cross striped, veins dotted. Black rings on the vessels indicate places, where the blood vessels pierce the bones of the skull.

Each figure is provided with a scale in order to show the enlargement.

Abbreviations for all figures

Aa —	Arteria auditiva	Apa —	Arteria pseudobranchii aff.
Ab —	„ basialis	Apb —	„ prosencephali basil.
Abe —	„ branchialis effer.	Apc —	„ „ central.
Aca —	„ cerebri anter.	Ape —	„ pseudobranchii eff.
Acc —	„ cerebelli central.	Arc —	„ rhombencephali centr.
Aci —	„ carotis inter.	As —	„ subclavia
Acm —	„ coeliaco-mesent.	Bo —	Bulbus olfactorius
Acp —	„ cerebri post.	Br —	Gill
Acr —	„ optica [centr. retinae]	Brc —	„ chamber
Ae —	„ epiphysealis	Cb —	Cerebellum
Aen —	„ encephalica	Ep —	Epiphysis
Aep —	„ epibranchialis	H —	Hypophysis
Al —	Aorta lateralis	Li —	Lobus inferior
Am —	„ medialis	M —	Muscle
Amc —	Arteria mesencephali central.	Ms —	Mesencephalon
Aom —	„ ophthalmica magna	Mx —	Maxilla
Aor —	„ orbito-nasalis	Oc —	Eye

Or — Smell organ
Orb — Orbita
Plc — Plexus chorioid. ventr. IV.
Plv — „ venosus
Pr — Prosencepalon
Ra — Ramus ant. V. cerebri med.
Rb — Rhombencephalon
Rp — Ramus post. V. cerebri med.
Sd — Saccus dorsalis
Sv — „ vasculosus
Tl — Torus longitudinalis
Ts — „ semicircularis
Val — Valvula

Vb — Vena bulbalis
Vca — „ cerebri anter.
Vcb — „ cerebelli
Vcd — „ cardinalis anter.
Vch — „ chorioidea
Vcm — „ cerebri media
Vcp — „ „ poster.
Von — „ orbito-nasalis
Vt — „ terminalis
Vtm — „ tecti medialis
Vtr — Ventriculus IV
I—X — Cerebral nerves

Contents

Introduction

The blood-vessels of the ... head ... of the brain have been frequently investigated in the various representatives of Elasmobranch fishes. J. Müller (18...) was the first to mention the vessels supplying the brain of ... ... Hyrtl (18...) described and drew excellently the cranial arteries in fish. The cerebral arteries only were the subject of examination by ...

Naczynia krwionośne mózgu ryb spodoustych. — The blood-vessels in the brain of Elasmobranches.

Mémoire

de M. **Z. GRODZINSKI**,

présenté le 11 juin 1945 par M. H. Hoyer m. t.

Introduction

The blood-vessels of the head and of the brain have been
frequently investigated in the various representatives of Elasmo-
branch fishes. J. Müller (1839) was the first to mention the
vessels supplying the brain of *Centrophorus granulosus*. Hyrtl
(1858) described and drew excellently the cerebral arteries in *Raia*.
The cerebral arteries only were the subject of investigations by
Carazzi (1905 *Cetorhinus*), Craigie (1927 *Hydrolagus*), Cor-
rington (1930 *Galeus glaucus*), Ariëns Kappers (1933 *Lae-
margus, Rhina, Raja*) and Marples (1936 *Squatina*). Rex (1891)

represented accuratelly the venous systeme of the brain in *Scylliorhinus, Pristiurus, Squalus, Raja* and *Torpedo*.

P a r k e r first described (1887) both the arteries and the veins in *Mustelus*. H o f m a n n (1900, 1901) did the same in an excellent manner for *Squalus, Raja* and *Scylliorhinus*. S t e r z i (1909) completed and extended the observations of the aforementioned authors upon *Squalus, Scylliorhinus, Mustelus. Raja, Trygon, Myliobatis* and *Torpedo*. His important results were unfortunately hidden in a not easily accessible paper; and therefore have been overlooked by many subsequent investigators. The vessels of *Squalus* have been worked out by O'D o n o g h u e & A b b o t (1928), those of *Heptanchus* by D a n i e l (1928). The list of authors and of the investigated species looks quite imposing, but is still far from complete.

The main vessels, which carry the blood in or out of the braincase, were the subject of special investigations (H y r t l — 1858, 1872) or were taken into consideration occasionally when dealing with the structure of the skull orbit (T r e t j a k o f f 1926), nerves (G o o d r i c h 1930), sense organs or other organs of the head. A l l i s represented in a series of papers (1908, 1911, 1912, 1914) the arteries, G e l d e r e n (1924, 1933) the veins. Both had at their disposal an abundant material and extensively reviewed in their work the previous results known from the literature.

D a n i e l (1928) supplied in his manual a short comparative review of the blood-vessels in the brain of Elasmobranch fishes. G r o d z i ń s k i (1938) tried to do the same for all the fishes in the manual of B r o n n. The limited dimensions of the article about the blood-vessels of the fishes did not allow this problem to be extensively dealt with. Besides, the mere review of literature is not sufficient for the due estimation of the comparative anatomical value of the particular cerebral vessel. The vessels must be seen in preparations or at least in excellent drawings. Therefore I decided to extend my work on the material of Elasmobranch fishes collected during my sojourn in Roscoff (1927) and Naples (1937). which consisted of several specimens of *Scylliorhinus canicula* L., some of *Raja punctata* and *asterias* M. H. and one each of *Mustelus laevis* Risso and *Torpedo ocellata*. All specimens had the blood-vessels filled completely with Indian ink or Prussian blue. The brain remained until investigation »in situ« in the head;

just a small part of the roof of the skull was removed in order to facilitate the penetration of the formaldehyde to the neural tissue.

The brains of the four species lately enumerated differ markedly from each other. The brain of *Mustelus*, compact and squat takes an intermediate position between the slender one of *Scylliorhinus* and the dilated one of *Raja*. The highly specialized brain of *Torpedo* approaches in many respects the brain of *Raja*. The vessels always adjust themselves to their organs, therefore they differ significantly from each other in various brains. Having at our disposal the exact pictures of the blood-vessels from several brains, we have an opportunity of contrasting the differences in their distribution with the differences in general structure of the various segments of the brain. Knowing the distribution and appearance of the blood vessels, we may try to elucidate the manner in which the blood circulates in them. In the species investigated the circulation is performed in quite different ways.

General description of the blood-vessels

Scylliorhinus canicula L. Both *Aa. carotides internae* (Fig. 1 Aci), the right and the left, unite below the base of the skull in a continuous arc-like trunk, crossing transversally the long axis of the head. From the middle of this arc a short unpaired vessel perforates obliquely upwards the cartilaginous part of the skull and emerges in the brain-case beneath the caudal end of the hypophysis. Close to the bottom of the brain-case the vessels divide in two *Aa. encephalicae*, which being contiguous to *Lobi inferiores* turn forwards (Fig. 1, 3 Aen). They unite on their way with *A. pseudobranchialis efferens* (Fig. 1, Ape). In the space between the nerves II—III each of them divides into *A. cerebri anterior* and *posterior* (Fig. 4, Aca, Acp) and gives off besides that a slender vessel — *A. optica* — to the optic nerve.

A. cerebri ant. runs as a direct prolongation of the main vessel beneath the brain anteriorly and gives off a series of branches to the base of the brain and to the organ of smell. Among the more significant branches should be mentioned: *A. olfactoria* (Fig. 3, 4, Ao) directed straight towards the smell organ, *A. prosencephali basilaris*, *A. prosencephali dorsalis*, *A. prosencephali lateralis* and *A. bulbi olfactorii*.

A. prosencephali basilaris (Fig. 3, 4 Apb) branches from the main artery anteriorly to the optic nerve. It turns medially along the bottom of the betweenbrain. Here it meets the corresponding vessel from the other side, fuses with it into an unpaired trunk, continues forwards in the groove between the hemispheres and sends numerous branches to the lateral walls.
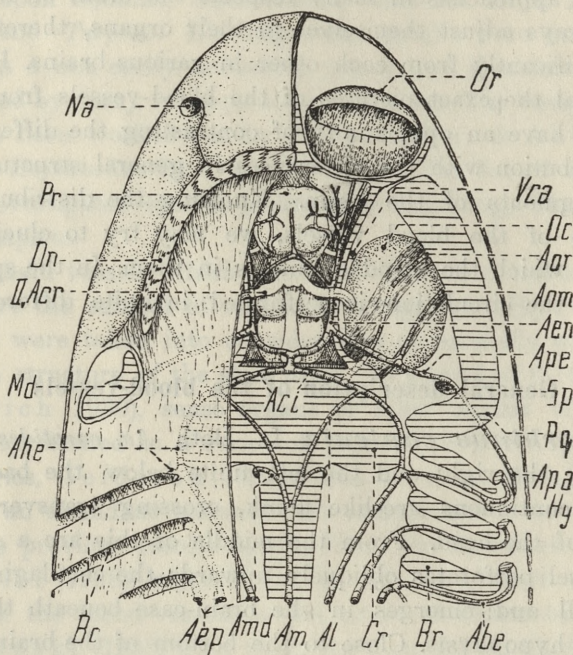
Fig. 1. *Scylliorhinus canicula* L. Bottom view of the head with removed mandible and dissected gullet. At the right side of the preparation the mucous membrane, the base of the skull and the muscles are removed. In this way the relations of the arteries to the brain and the skeletal parts are clearly shown.

A. prosencephali dorsalis (Fig. 2, 3 Apd) and *A. bulbi olfactorii* (Fig. 3 Abo) arise as a medial branch from one of the olfactory arteries. After a short common course *A. bulbi olf.* passes on to the base of *Bulbus olfactorius* and ramifies there. *A. prosencephali dors.* runs through the slit between *Bulbus* and the hemispheres sending throughout its course some tributaries to the base of the latter. Its main trunk penetrates within the above mentioned slit
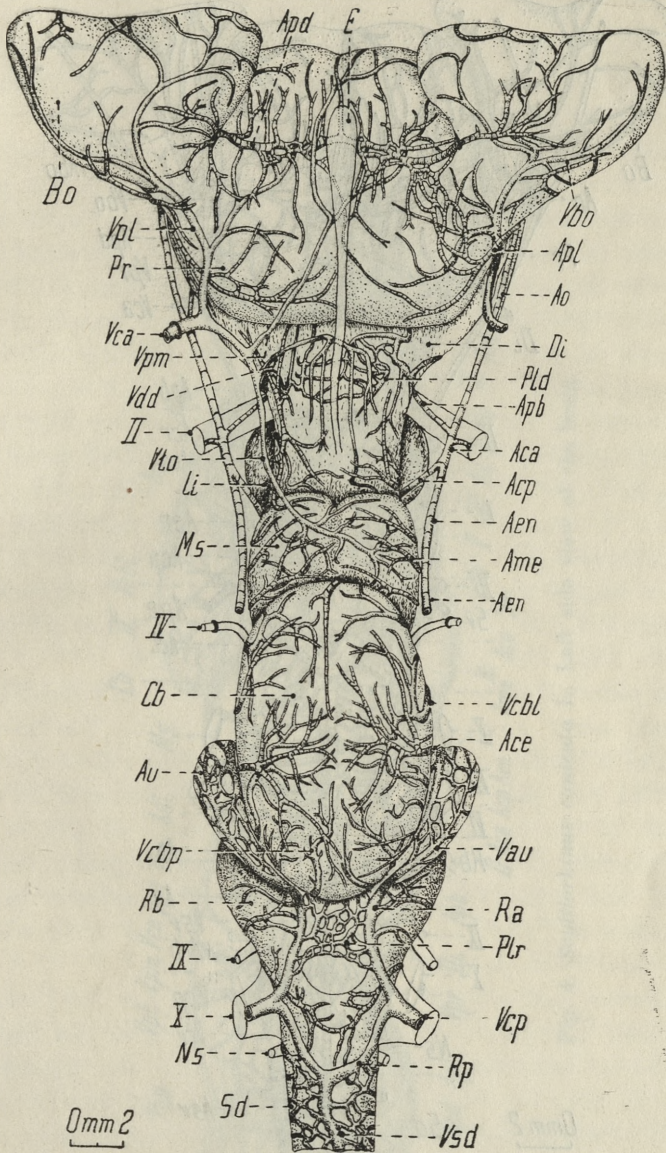
Fig. 2. *Scylliorhinus canicula* L. Top view of the brain.

on top of the brain and divides here into two diverging bran-
ches. One of them turns laterally on *Bulbus* the other medially
on the hemisphere. The latter crosses transversally its surface
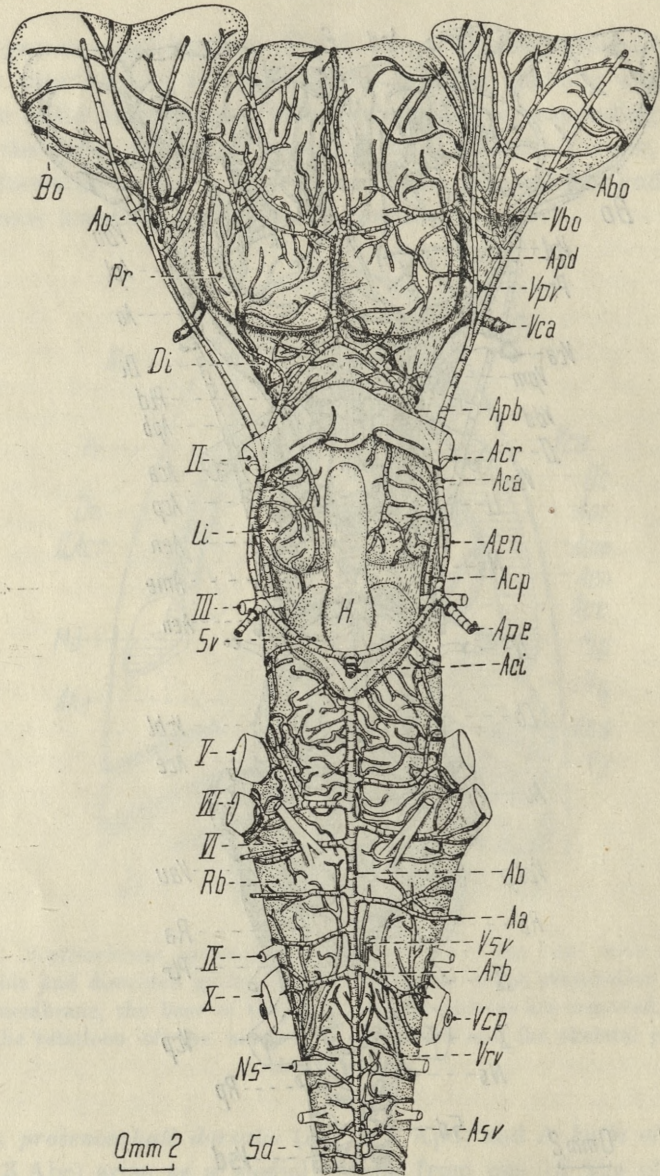
Fig. 3. *Scylliorhinus canicula* L. Bottom view of the brain.

and fuses with its fellow from the opposite side in a continuous tube. From this transverse channel arise more or less perpendicular vessels on the surface of the hemispheres.

Fig. 4. *Scylliorhinus canicula* L. Left side view of the brain.

A. prosencephali lateralis (Fig. 2, 4 Apl) drains the back and the side of the hemisphere. It branches from *A. olfactoria* close to the root of *A. prosencephali dors.*

A. cerebri posterior (Fig. 3, 4 Acp) turns at an acute angle from *A. encephalica* posteriorly and forces its way through the groove between the base of the brain and *Lobus inferior* with *Saccus vasculosus.* Describing medially an arc it meets above the caudal end of the *Saccus vasculosus* the corresponding branch from the other side. They join together and run as an unpaired trunk backwards (*A. basialis* Fig. 3, 4 Ab) along the middle line of the *Rhombencephalon.* Thinning gradually they continue as a similarly placed vessel in the spinal cord (*A. spinalis ventralis*, Fig. 3 Asv).

The longitudinal arteries give off branches to all parts of the brain e. g. to the betweenbrain (*A. diencephali* Fig. 4 Adi), to the midbrain (*A. mesencephali* Fig. 2, 4 Ame), to the cerebellum (*A. cerebelli lateralis* Fig. 2, 4 Ace), to the *Rhombencephalon* a series (5—6) of bigger and as many of smaller *A. rhombencephali laterales* (Fig. 3, 4 Arb). The distribution and ramification of these arteries are shown in the accompanying drawings.

Two pairs of veins, *V. cerebri posterior* and *anterior* (Fig. 2, 4 Vca, Vcp), carry away the blood from the brain. The midbrain constitutes a bordering zone, where all four veins collect the blood; the bottom and sides of this cerebral segment however lie within the reach of the posterior, the top within that of the anterior vein. *V. cerebri posterior* leaves the brain-case together with the nerve X and joins *V. cardinalis anterior.* The anterior cerebral vein pierces the skull in the anterior part of the orbit, where it merges into *Sinus orbitalis.*

Vv. cerebri ant. are asymmetrical, usually the left vessel is bigger than the right. The left vein arises from the fusion of two anterior branches with one posterior and one ventral, the right from one anterior and one ventral only. But sometimes the converse occurs. The posterior branch *V. tecti optici* (Fig. 2 Vto) arises from several roots in the top of the midbrain. It runs from here towards the left hemisphere beneath the vault of the skull not being contiguous with the betweenbrain. In its course it receives as a tributary an unpaired *V. chorioidea* (Fig. 2, 4 Vdd), implicated with its roots in the *Plexus chorioideus ventriculi* III. In the neighbourhood of the dorsal caudal border of the left hemi-

sphere *V. tecti optici* unites with two anterior branches into *V. cerebri anterior*. The vault of the betweenbrain, slender and membraneous in structure, contains an extensive vascular plexus. *Plexus chorioideus ventriculi* III consists of two venous meshworks situated one beneath the other. The superficial layer of this plexus exhibits slender vessels arranged in meshes stretched only on the longitudinal axis of the brain; the underlying layer has much thicker vessels and more irregular meshes. The plexus just described also collects the blood from the extensive plexuses of the lateral chambers. *V. chorioidea* arises from the centre of the plexus and ascending dorsally reaches *V. tecti optici*.

V. prosencephali media (Fig. 2 Vpm) originates with its furthest roots in the middle and front of both hemispheres. The main stem of this vein runs, applied to the vault of the skull, backwards above the left hemisphere. The second anterior branch *V. prosencephali lateralis* (Fig. 2, 4 Vpl) collects the blood from the side and top of the hemisphere and *Bulbus olfactorius*. The base of the hemispheres is drained by *Vv. prosencephali ventrales* (Fig. 3, 4 Vpv). With their terminal sections they turn dorsally and empty themselves into *V. prosencephali lat*, each on its side of the brain.

Vv. cerebri posteriores consist of two branches *Ramus anterior* and *posterior*. The posterior branches (Fig. 2 Rp) are short and constitute the prolongation of the unpaired vein of the spinal cord *V. spinalis dorsalis*. *Ramus anterior* extends from the midbrain to the nerve X; at the top of this nerve it unites with *Ramus posterior* into the unpaired *V. cerebri post*. Its extreme roots consist of a series of branches, which originate in the midbrain and adjacent parts of the *Rhombencephalon* and unite into a sinusoidal trunk beneath the roots of the nerve IV. From here it runs along the ventral border of the cerebellum, passing over the front of *Auricula cerebelli*, where it breaks into a network of broad vessels. From the caudal end of the *Auricula V. auricularis* (Fig. 2, 4 Vau) emerges and then passes upon the *Rhombencephalon* following the border of the fourth chamber till the nerve X. *Ramus anterior* receives a series of veins from the midbrain, larger vessels from the *Cerebellum* (*V. cerebelli lateralis* and *posterior* Fig. 2, 4 Vcbp, Vcbl), some vessels from the base and sides of the *Rhombencephalon* and from *Plexus chorioideus v.* IV. The ple-

xus itself drains the roof of the fourth chamber, but incompletely, leaving the posterior section unvascularised. The plexus consists of irregular meshes without distinct larger channels.

On the base of the *Rhombencephalon* a longitudinal venous trunk appears, which runs parallel with *A. basialis* and is partially covered by it, and which continues in the spinal cord as *V. spinalis ventralis* (Fig. 3 Vsv). Besides this vessel are here the longitudinal *Vv. rhombencephali ventrales* (Fig. 3 Vrv) collecting several lateral branches and discharging into *Ramus posterior*.

Carazzi (1905) described correctly the main arteries of the brain and also some of their branches. Sterzi (1909) supplemented his data by a series of well-observed details. Veins were represented accuratelly by *Rex* (1891) especially all *Plexus chorioideus*. Hofmann (1901), Sterzi (1909) and O'Donoghue (1914) confirmed his results. In my description the topography of vessels entering the brain case and the distribution of the superficial cerebral branches, especially those of the side walls and top of the brain, are worked out more exactly.

Mustelus laevis Risso. Both *Aa. carotides internae* describe an arc below the base of the skull, directed from the lateral border to the middle. While burrowing gradually into the cartilaginous wall they meet each other and united into a short unpaired trunk enter the brain-case beneath the posterior end of the hypophysis. From here, divided into two *Aa. encephalicae* (Fig. 6 Aen), they diverge again laterally and proceed along the brain forwards below nerve III and above nerve IV. In their course they take up the *A. pseudobranchialis efferens* and give off a branch to the optic nerve *A. optica*. In the neighbourhood of the optic nerve both *Aa. encephalicae* divide into two branches *A. cerebri anterior* and *posterior*, which diverge in opposite directions, the one forwards, the other backwards.

A. cerebri anterior (Fig. 6, 7 Aca) drains the forebrain, parts of the betweenbrain and the smell organ by means of several arteries, namely: *A. olfactoria, A. prosencephali basilaris, A. p. dorsalis, A. bulbi olfactorii.*

A. olfactoria (Fig. 6, 7 Ao) runs as a direct prolongation of the main trunk beneath the hemispheres and splits into four branches, which leave the brain-case and pass over the smell

organ. The manner of arterial division is not constant and differs on the right and left sides.

A. prosencephali basilaris (Fig. 6 Apb) of the specimen investigated appears to be an unpaired vessel, which is unusual in *Mustelus.* It starts from *A. encephalica* in the neighbourhood of *A. cerebri ant.*, turns towards the mid-ventral line of the forebrain and runs in the longitudinal groove between the hemispheres forwards, divided into two parallel stems. In its course it sends a bunch of vessels to the right and a few smaller ones to the left hemisphere. The greater part of the base of the left hemisphere is drained by a big branch of the left *A. cerebri ant.* This vessel corresponds to the missing left *A. prosencephali basil.*, which lost terminally the connection with the arteries running in the longitudinal groove between the hemispheres. According to Sterzi *Mustellus* possesses two *Aa. prosencephali basilares*, which arise from *A. encephalica* and run in the groove separating both hemispheres. For the whole of their length no connecting vessels are present.

A. prosencephali dorsalis (Fig. 5, 6, 7 Apd) originates from one of the *Aa. olfactoriae.* As a strong stem it finds its way into the slit between the *Bulbus olfactorius* and the hemisphere and passes from the bottom to the top of the brain without piercing the mass of neural tissue. Within the above-mentioned slit and in the dorsal mouth of it, the artery splits into a series of vessels, which spread fanlike over the dorsal surface of the hemispheres. Strong connecting stems between the branches of both arteries are absent in this region.

A. bulbi olfactorii (Fig. 6, 7 Abo) constitutes a lateral branch of one of the *Aa. olfactoriae.* It distributes branches mainly on the base of the *Bulbus*, but reaches its dorsal surface by terminal tributaries.

A. cerebri posterior (Fig. 6, 7 Acp) supplies the remaining parts of the brain. Its course is similar to that in *Scylliorhinus.* Both arteries turn below the base of the betweenbrain backwards. They surround the attachments of hypophysis, *Lobi inferiores* and *Saccus vasculosus.* Posterior to these organs they fuse into the unpaired *A. basialis* (Fig. 6, 7 Ab), which runs backwards in the mid-ventral line of the brain and passes without distinct limit into the similar vessel of the spinal cord (*A. spinalis ven-*

tralis). Both the paired and the single trunk of the posterior cerebral arteries distribute several branches upon the surface of
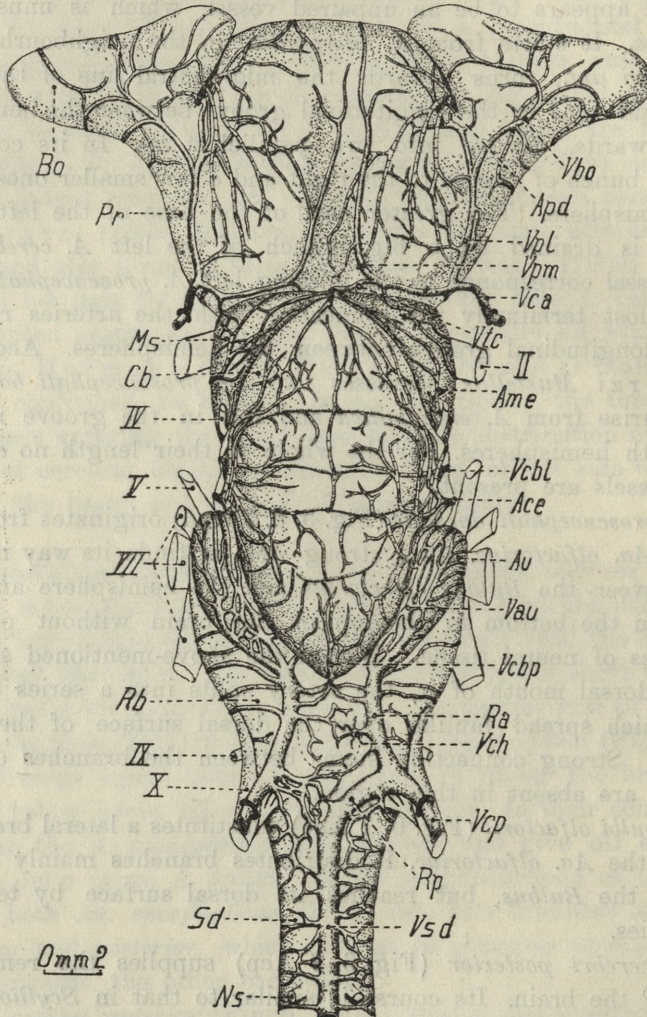
Fig. 5. *Mustelus laevis* Risso. Top view of the brain.

the neighbouring segments of the brain. The most important are: *A. mesencephali*, *A. cerebelli lateralis* and a series of *Aa. rhombencephali laterales*.

A. mesencephali (Fig. 5, 7 Ame) branches from the main artery anterior to nerve III and follows askew the lateral wall towards the top of *Tectum opticum*. It drains with its branches the ma-
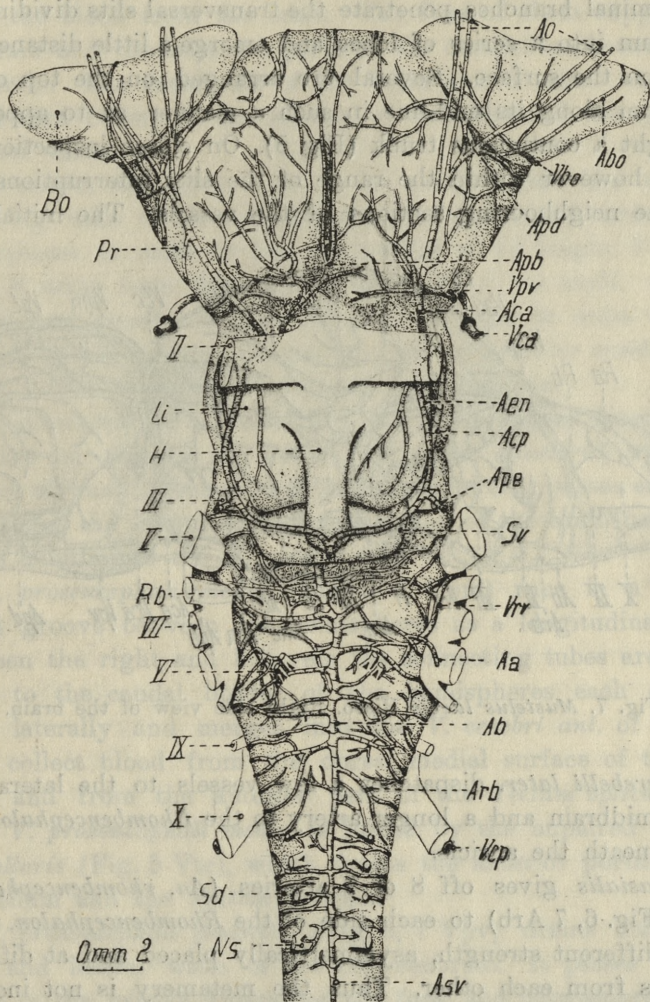
Fig. 6. *Mustelus laevis* Risso. Bottom view of the brain.

jority of the midbrain and those parts of the betweenbrain, not covered by the *Cerebellum*.

This artery is surpassed in size by *A. cerebelli lateralis* (Fig. 5, 7 Ace), which starts in *A. cerebri post.* posterior to nerve III,

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passes along the lateral side of the midbrain to the most ventral
section of the *Cerebellum* and here splits into several branches.
One of them turns forwards, others backwards, the rest dorsally.
The terminal branches penetrate the transversal slits dividing the
cerebellum into a series of lobes and emerge a little distance far-
ther upon the surface. Several are arranged on the top of the
cerebellum along its mid-line in such a manner as to appear at
first sight a continuous trunk (Fig. 5). On closer inspection one
can see however within the range of the slits interruptions bet-
ween the neighbouring sections of the vessels. The initial part
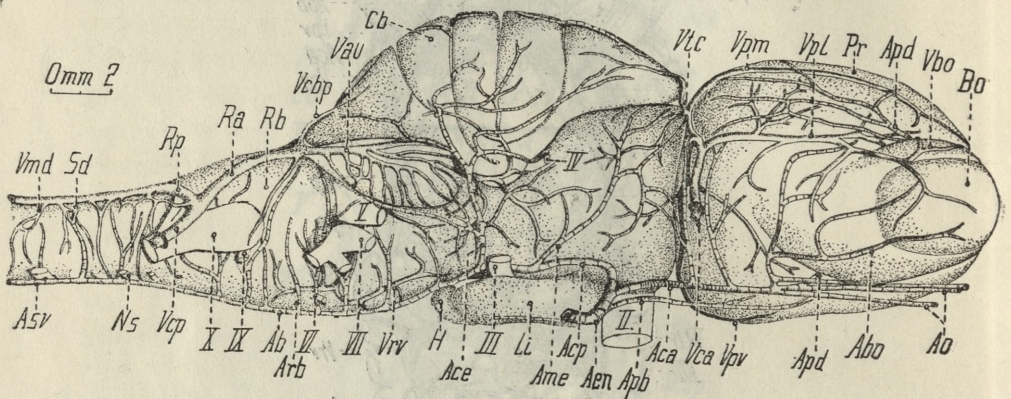
Fig. 7. *Mustelus laevis* Risso. Right side view of the brain.

of *A. cerebelli later.* dispatches a few vessels to the lateral wall
of the midbrain and a longer artery to the *Rhombencephalon* run-
ning beneath the auricle.

A. *basialis* gives off 8 or 9 arteries (*Aa. rhombencephali la-
terales* Fig. 6, 7 Arb) to each side of the *Rhombencephalon*, which
are of different strength, asymmetrically placed and at different
distances from each other. Thus the metamery is not included
at all in their distribution. These arteries reach dorsally far along
the lateral walls of the *Rhombencephalon*. In the region of ner-
ves V—VII and IX—X they have many ramifications surroun-
ding these nerves or piercing their roots. From A. *basialis* they
start at different angles. The anterior arteries turn slightly to-
ward the hypophysis, the middle ones are perpendicular to the

main artery and the posterior ones bend toward the spinal cord. The arteries of this section send also branches to the auditory organ (*A. auditiva* Fig. 6 Aa).

Two pairs of veins, *Vv. cerebri anteriores* and *posteriores* carry away the blood from the brain. The first of them supplies the forebrain and the adjacent parts of the cerebellum, between- and mid-brain. The remainder of the brain and a small section of spinal cord belong to the sphere of influence of the posterior cerebral veins.

V. cerebri ant. (Fig. 5, 6, 7 Vca) results from the union of three big veins in the neighbourhood of the caudal border of the hemispheres, at more or less the middle of its height. From this point it turns from the brain to the wall of the skull, which it pierces and penetrates into the orbit in order to unite with *Sinus orbitalis*. The three components of the anterior cerebral vein are *V. prosencephali media, lateralis* and *ventralis*.

V. prosencephali lateralis (Fig. 5, 7 Vpl) begins from a few branches on the top of *Bulbus* (*V. bulbi olfactorii*) and from a series of small tributaries on the hemisphere. It passes as a large stem along the lateral side of this region of the brain backwards to the mouth into the *V. cerebri ant.*.

V. prosencephali media (Fig. 5, 7 Vpm) runs in a shallow dorsal groove between the hemispheres as a longitudinal trunk. Between the right and left vein the connecting tubes are absent. Close to the caudal border of the hemispheres each of them turns laterally and merges into the *V. cerebri ant.* of its side. They collect blood from the dorsal medial surface of the fore-brain and from the anterior part of the *Tectum opticum*. The right *V. prosencephali media* is joined by the unpaired *V. tecto-cerebellaris* (Fig. 5 Vtc), which drains the anterior portion of the cerebellum and the *Tectum opticum*.

V. prosencephali ventralis (Fig. 6, 7 Vpv) drains chiefly the base and lateral walls of the betweenbrain. It passes as a big trunk in the groove separating the fore- and betweenbrain, from the base of the brain dorsalwards, where it unites with *V. prosencephali media*.

Vv. cerebri posteriores (Fig. 5, 7 Vcp) the second pair of vessels, which carry away the blood from the brain-case, consist of two branches *Ramus anterior* and *posterior*. Both branches

unite into an unpaired trunk on the upper surface of nerve X
and leave the skull together with it.

Ramus posterior (Fig. 5, 7 Rp), much shorter, constitutes the
prolongation of the spinal vein (*V. spinalis dorsalis*). The right
vessel in the specimen investigated belongs to the tubular type of
vessel, the left to the plexiform. It is not a constant feature, but
rather a result of some developmental disturbances, owing to
which the embryonic vascular network failed to transform itself
into a tubular vessel in the adult animal.

Ramus anterior (Fig. 5, 7 Ra) reaches with its farthest tri-
butaries the side and the top of the *Cerebellum* (*V. cerebelli la-
teralis* Fig. 5, 7 Vcbl). It collects also the small branches from
the adjacent areas of the midbrain. From here it turns towards
the anterior surface of the *Auricula cerebelli*, where it breaks
into a coarse venous network. From the opposite end of this
network arises *V. auriculi* (Fig. 5, 7 Vau), which constitutes the
terminal section of *Ramus anterior*, along the dorsal surface of
Rhombencephalon.

Ramus anterior collects in its terminal section vessels from
Plexus chorioideus ventr. IV, *V. cerebelli posterior* and *V. rhom-
bencephali ventralis*. Plexus penetrates the whole membrane, roo-
fing the fourth chamber as an irregular vascular network. Some
of its vessels attain bigger dimensions and join *Ramus anterior*.

V. cerebelli posterior (Fig. 5, 7 Vcbp), a small unpaired vein, des-
cends along the caudal end of the *Cerebellum* to the *Rhombence-
phalon* and here divides into some vessels joining the right and
left *Ramus anterior*. *V. rhombencephali ventralis* (Fig. 6 Vrv) con-
sits of a series of veins running at the base of *Rhombencephalon*,
which unite in the region of nerves V—VII and X into bigger
stems. The latter cross the lateral walls of this part of the brain
in order to reach the *Ramus anterior*.

Parker (1887) described and drew the main arteries and
veins of the brain. He did not analyse the superficial vessels
and failed to discover the part of *V. cerebri post.*, which leaves
the brain-case. According to Sterzi (1909) the arteries of *Mu-
stelus* closely resemble the arteries of *Squalus*. He did not in-
vestigate the veins accurately, but the lack of the terminal por-
tion of the *V. cerebri post.*, as pointed out by Parker, seemed
to him be suspicious. My investigations bring some supplemen-

tary knowledge of the distribution of the veins and also of the arteries.

Raja punctata. *Aa. carotides internae* (Fig. 8 Aci) meet together in the midventral line of the skull, where they merge into cartilaginous tissue, cross each other and rise within the brain-case beneath the hypophysis. The arteries turn in the brain-case to the opposite side of the body and go above the bottom of the brain-case as *Aa. encephalicae* (Fig. 8, 10 Aen) towards the root of nerve III. From here they follow the lateral border of *Lobus inferior* to nerve II. On their way they unite with *A. pseudobranchialis efferens* and give off *A. optica* to nerve II (Fig. 8, 10 Ape, Acr). On the borders of the between- and the mid-brain they divide into the two longitudinal vessels of the brain *A. cerebri anterior* and *posterior*.

A. cerebri anterior (Fig. 10 Aca) constitutes the anterior prolongation of *A. encephalica* and reaches with a series of smaller and larger branches the forebrain and the organ of smell. On the base of the hemispheres it sends *A. prosencephali basilaris* (Fig. 9, 10 Apb), which gains the brain from behind by means of several branches. One of them runs in the longitudinal groove between the hemispheres, and does not unite with its fellow from the opposite side.

A. prosencephali dorsalis (Fig. 9, 10 Apd) passes in the slit between the hemisphere and *Bulbus olfactorius* upon the anterior surface of the hemisphere and eventually upon its top. Here it divides into several branches. The medial unite with the corresponding branches of the opposite side. *A. prosencephali lateralis* (Fig. 9 Apl) ascends by means of a bunch of arteries from behind on the top and lateral wall of the hemispheres. Some of them join the branches of *A. prosencephali dorsalis*. *Aa. olfactoriae* (Fig. 10, 11 Ao) pass allong the base and lateral walls of *Bulbus* to the smell organ.

Aa. cerebri posteriores (Fig. 10, 11 Acp) extend along the ventral, lateral border of the brain from nerve II to the spinal cord, where both vessels fuse into the unpaired trunk *A. spinalis ventralis*. They do not constitute within the range of the *Rhombencephalon* an unpaired *A. basialis*, but persist as two stems situated far apart. Their lumen increases gradually from the begin-

ning backwards and attains its maximum size in *A. spinalis ventralis.*

Aa. cerebri posteriores give off along their whole length superficial vessels to the both sides of the brain. To the midbrain follow 2 to 3 arterial stems (*A. mesencephali* Fig. 11 Ame), that split after a short course into treelike branches. The *Cerebellum* is supplied by 1 to 2 *Aa. cerebelli laterales* (Fig. 11 Ace). Each
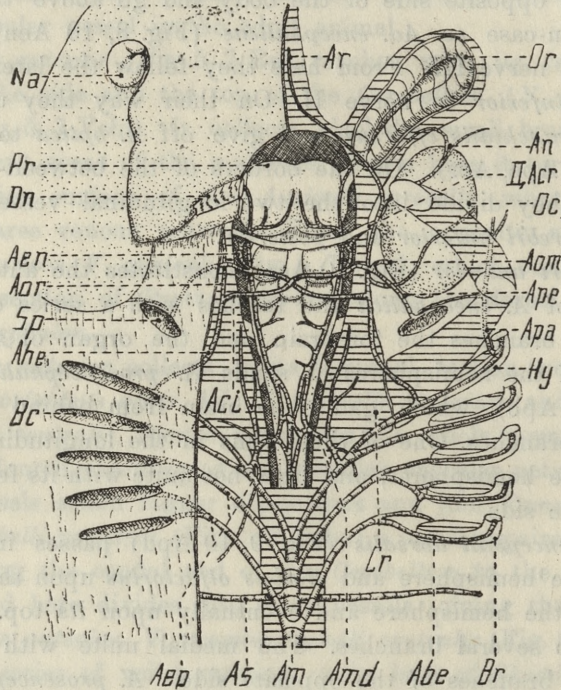
Fig. 8. *Raja asterias* M. H. Base of head after removal of jaw and gullet. At the right side of the preparation the mucous membrane, the base of the skull and the muscles are removed. In this way the relation between arteries, brain and skeleton is shown.

of the *Aa. cerebri post.* sends medially on the base of *Rhombencephalon* a series (8—10) of *Aa. rhombencephali ventrales* (Fig. 10 Arbv) and 4—6 *Aa. rhombencephali laterales* (Fig. 10, Arb) to its lateral sides. From among the latter some branches reach with their tips the region of *Auriculae cerebelli.*

In the spinal cord the pair of *Aa. spinales collaterales* follow parallel to the *A. spinalis ventralis* (Fig. 10, 11 Asv; Asc), connec-

ted with it by some transverse vessels. *Aa. spinales collaterales* adhere in their caudal portion to the lateral side of the spinal cord, anteriorly they pass beneath the cord and in the region of the first spinal nerves they run immersed within the cartilaginous channel of the skeleton. These arteries receive the blood
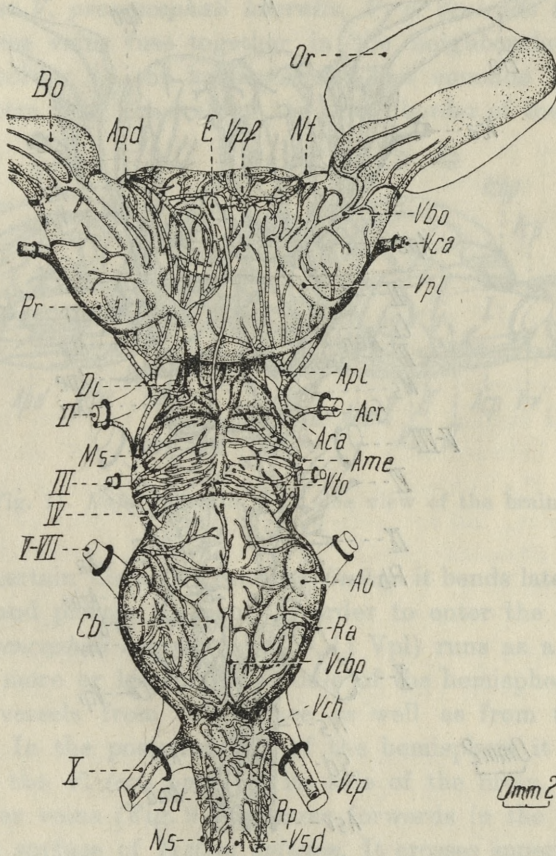
Fig. 9. *Raja punctata*. Top view of the brain.

from *A. epibranchialis* by means of *A. medullaris* (Fig. 8 Amd) of the 8—9 segment. It is difficult to settle the anatomical value of *A. medullaris*. It can be regarded as a branch of *A. segmentalis* or of *Aorta lateralis*. In the second case it remains to be elucidated embryologically if the vessel branching from *A. epibran-*

chialis is a remnant of the embryonic paired aorta, which has anteriorly lost connection with *A. carotis int.* and posteriorly moved its mouth from the dorsal aorta to *A. epibranchialis* (Fig. 8 Aci, Aep, Amd). This supposition gets some support in compa-
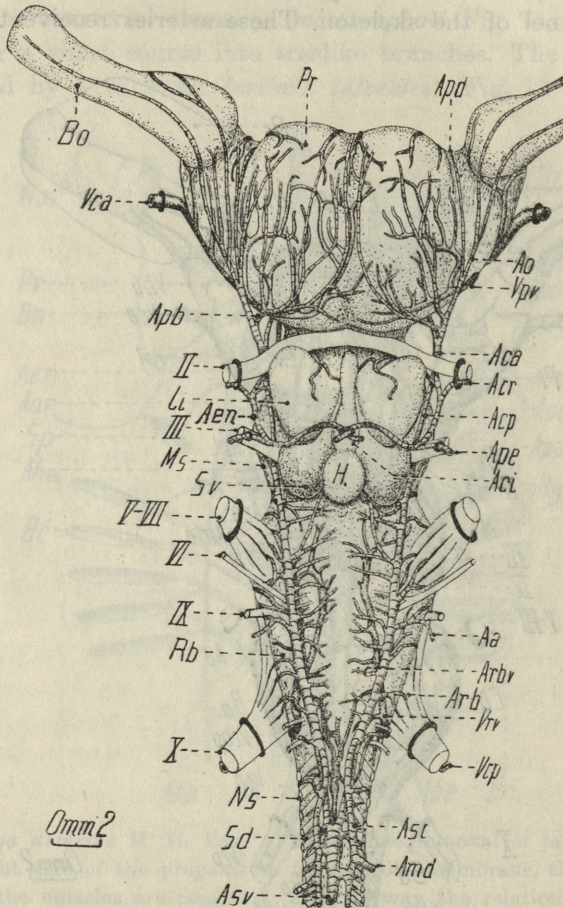
Fig. 10. *Raja punctata.* Bottom view of the brain.

rative anatomical observations. C a r a z z i observed in *Catulus catulus* the normal connection of *Aortae laterales* with *A. carotis int.* in all cases but one, in which it was absent. *Aortae laterales* in *Squatina vulgaris* branch constantly according to C a r a z z i from *A. epibranchialis* and not from *Aorta medialis.*

Two pairs of veins, *V. cerebri anterior* and *posterior*, carry away the blood from the brain. The latter leaves the brain-case together with nerve X adhering at its dorsal surface. The first pierces the skull within the range of the orbit, closer to the anterior border of the hemisphere than its middle.

V. cerebri anterior (Fig. 9, 10, 11 Vca) is formed by the union of three veins: *V. prosencephali lateralis*, *V. p. ventralis* and *V. tecti optici*. These veins fuse together in the neighbourhood of the posterior border of the hemispheres. The common stem turns forward from that place along the outer border of the hemisphe-
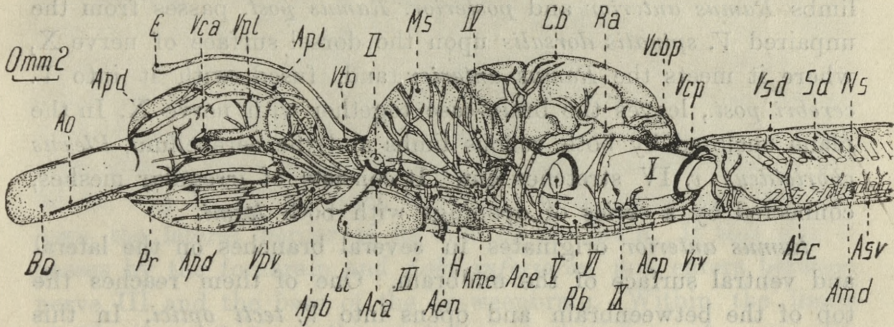
Fig. 11. *Raja punctata.* Left side view of the brain.

res. At a certain distance from the *Bulbus* it bends laterally from the brain and pierces the skull in order to enter the orbit.

V. prosencephali lateralis (Fig. 9, 11 Vpl) runs as a longitudinal vessel more or less in the middle of the hemisphere and collects the vessels from its surface as well as from the *Bulbus olfactorius*. In the posterior part of the hemisphere it meets and fuses with the *V. tecti optici* of its side of the brain. The right of the latter veins (Fig. 9 Vto) runs forwards in the mid-dorsal line of the surface of *Tectum opticum*. It crosses superficially the top of the betweenbrain and describing an arc on the outer border of the hemisphere joins the right *V. prosencephali lateralis*. The left *V. tecti optici* is much shorter. Its roots arise not from the surface of the midbrain but from its interior. The further course resembles that of the right one. *V. praefrontalis* (Fig. 9 Vpf), described first by H o f m a n n, passes from the vault of the brain-case on the middle of the forebrain and extends below

the pineal body to join the *V. prosencephali lateralis* of the left hemisphere.

V. prosencephali ventralis (Fig. 10, 11 Vpv) supplies the ventral and lateral parts of the hemispheres and merges into *V. cerebri ant.*, close to the place where the latter turns towards the anterior portion of the hemisphere. The poorly developed lateral chambers lack entirely the *Plexus chorioideus v. lateralis*. The insignificant *Plexus chorioideus ventr.* III is connected by means of a small vessel with one of the *V. tecti optici.*

V. cerebri posterior (Fig. 9, 11 Vcp, Ra, Rp) consists of two limbs *Ramus anterior* and *posterior*. *Ramus post.* passes from the unpaired *V. spinalis dorsalis* upon the dorsal surface of nerve X, where it meets the *Ramus anterior* and, fused with it into *V. cerebri post.*, leaves the brain-case together with nerve X. In the plane enclosed by both venous limbs and the cerebellum, *Plexus chorioideus v.* IV stretches out. It consists of irregular meshes, connected by a series of openings with both *Rami*.

Ramus anterior originates in several branches on the lateral and ventral surface of the midbrain. One of them reaches the top of the betweenbrain and opens into *V. tecti optici*. In this way arises the communication between the anterior and posterior cerebral veins. *Ramus anterior* (Fig. 11 Ra) runs as a big stem along the *Rhombencephalon* below the lower border of the *Cerebellum* to nerve X. It collects some small branches from the lateral surface of the *Cerebellum*, from the dorsal the bigger *V. cerebelli posterior* (Fig. 9 Vcbp). The latter while an unpaired trunk possesses however two openings into *Rami anter.* on the left and right.

On the ventral surface of the *Rhombencephalon* exist two elongated venous trunks: *Vv. rhombencephali ventrales* (Fig. 10, 11 Vrv). They originate in the neighbourhood of the *Saccus vasculosus*. In their course they accompany the medial border of *A. cerebri post.*. In the caudal section of the *Rhombencephalon* they thread their way sidewards beneath their arteries and passing by nerve X join *V. cerebri post.*.

Hyrtl (1858) admirably drew the arteries of the ventral side of the brain. Rex (1891) carefully described the veins. Hofmann (1900, 1901) and later Sterzi (1909) represented equally well arteries and veins, Kappers investigated the arteries of

the forebrain from the comparative point of view. The above-mentioned authors investigated large and very large specimens. Mine have been small ones. Hence result differences, especially in the distribution of the veins in the anterior part of the brain. These differences are discussed below (Page 75).

Torpedo ocellata. *Aa. carotides internae* fuse into a common trunk in the mid-ventral line of the base of the skull and after a short course pass through it into the brain-case. Here they emerge below the anterior end of the forebrain and divide into two diverging limbs *Aa. encephalicae* (Fig. 13 Aen). These vessels run on the bottom of the brain-case in the shallow cartilaginous groove side ways from the point of entry. After having crossed below the optic nerve each of them splits into two branches. One of them passes through the skull into the orbit (*A. orbitalis*, Fig. 12, 13, 14, Aor), the other turns backwards and follows the lateral border of its side of the brain. It this way it passes by the forebrain and *Lobi inferiores*, and enters between nerve III and the base of the betweenbrain. Within the limits of *Rhombencephalon* it lies medially to the roots of nerves V, VII, IX and X. In the spinal cord both vessels the right and the left unite into an unpaired *A. spinalis ventralis* (Fig. 13, 14 Asv).

A. spinalis ventralis possesses the largest diameter of all longitudinal arteries of the nervous system. Its paired prolongations on the brain are also wide tubes; they become gradually thinner from the back to the front. These vessels, laterally flattened, rest upon the cartilaginous base of the skull in the region of *Rhombencephalon*, and are immersed in the deep groove below nerve IX. In the region bordering the fore-and betweenbrain they attain their smallest diameter. From this extreme of narrowness the longitudinal cerebral arteries gain gradually in width. This increase continues as far as the point of entry of the *Aa. carotides int.* into the brain-case.

Knowing the vascular system of *Raia* we can easily homologize the paired arteries of *Torpedo* in the range between nerve II and *A. spinalis ventralis* as *A. cerebri posterior.*

A. cerebri anterior exists also (Fig. 13, 14 Aca) in *Torpedo* and consists of similar tributaries as in other Elasmobranchians. We find here *A. olfactoria* (Fig. 13, 14 Ao) gaining the organ of smell,

A. prosencephali dorsalis (Fig. 12, 14 Apd) supplying the front
and top of the forebrain, and *A. prosencephali lateralis* (Fig. 12,
14 Apl) distributing its branches on the back and side walls of
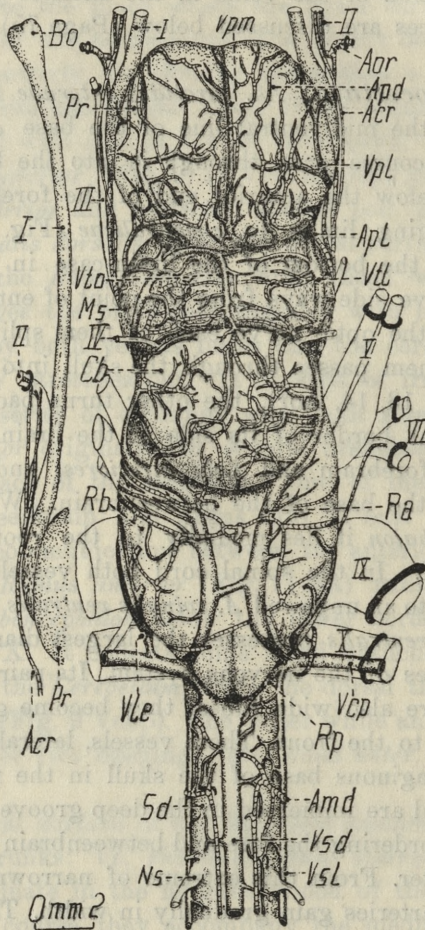
Fig. 12. *Torpedo ocellata*. Brain seen from above.

the forebrain. All these tributaries spring from a common trunk,
A. cerebri ant., which diverges in the region of nerve III from
A. crebri post. in the dorso-anterior direction.

The name *A. encephalica* (Fig. 13, 14 Aen) has to be given to
the pair of the longitudinal arteries which extend from the point of

entry at the base of the skull to the place where *A. cerebri ant.* and *posterior* come together. The lateral branches are *A. optica* and *A.*
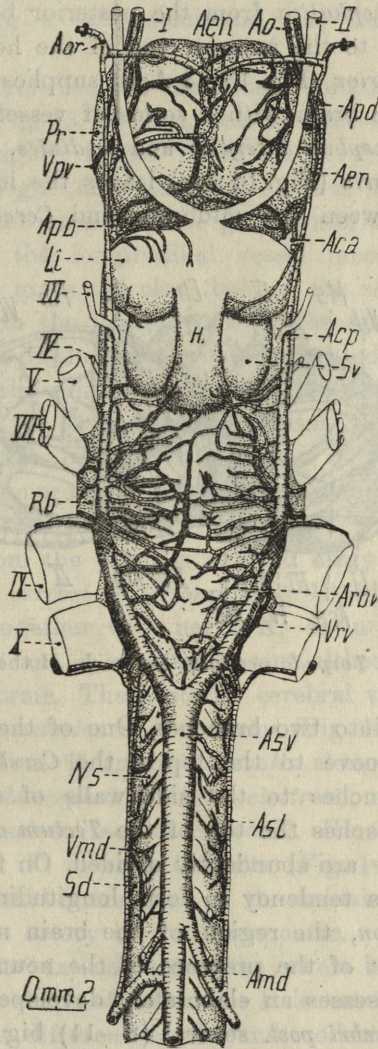
Fig. 13. *Torpedo ocellata.* Brain seen from below.

orbitalis. The latter originates within the brain-case, but in other species of Elasmobranchians it branches from the *A. carotides int.* outside the skull. In *Scylliorhinus* it pierces the cartilaginous

border of the brain-case but fails to enter it. The extraordinary
length of *A. encephalica* and its relation to *A. orbitalis* is pro-
bably connected with the significant displacement of the point
of entry of *A. encephalica* from the posterior border of the hy-
pophysis forwards to the anterior end of the hemispheres.

A. cerebri posterior (Fig. 13, 14 Acp) supplies the corresponding
ing sections of the brain with a series of vessels *A. tectocerebel-
laris*, *Aa. rhombencephali laterales* and *mediales*.

A. tectocerebellaris (Fig. 14 Atc) leaves the longitudinal trunk
in the groove between the midbrain and *Cerebellum*. Its large
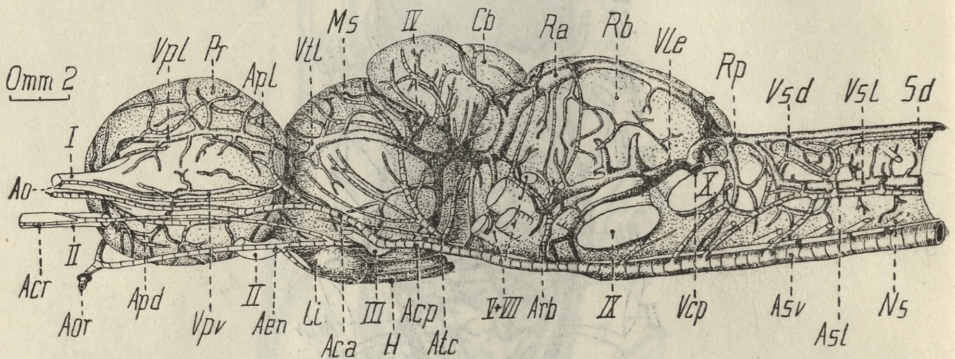
Fig. 14. *Torpedo ocellata.* Left side of the brain.

short stem splits into two branches. One of them ascends in the
aforementioned groove to the top of the *Cerebellum*. The other
sends several branches to the side walls of the mid- and be-
tweenbrain and reaches the top of the *Tectum opticum*. All mem-
bers of this artery are abundantly divided. On the top of the ce-
rebellum there is a tendency to form longitudinal median stems.

Rhombencephalon, the region of the brain most strongly de-
veloped on account of the presence of the neural centre of the
electric organ, possesses an elaborately developed arterial supply.
From both *Aa. cerebri post.* several (8—11) big branches of *Aa.
rhombencephali ventrales* (Fig. 13 Arbv) pass to the base of this
cerebral region distributed in the shape of a fan; this means that
the anterior turn forwards, the posterior backwards on the base
of *Rhombencephalon*. With their tips they rest upon the wall of
the brain close to the mid line and ramify here richly in the

mass of neural tissue. Besides these vessels there exist one big and two small *Aa. rhombencephali laterales* (Fig. 14 Arb), which supply the side and top of *Rhombencephalon*. The top of *Rhombencephalon* being a thin membrane, has few and slender arteries.

The mid-ventral line of the spinal cord is taken by *A. spinalis ventralis* (Fig. 13, 14 Asv), which receives in the region of the seventh to eighth spinal nerve the very big *Aa. medullares* (Fig. 13 Amd), right and left, which form the communication between it and the vessels of the gills. Anterior to these arteries the diameter of the longitudinal vessel increases significantly *A. spinalis ventr.* gives off close behind the skull a series of superficial vessels to the lateral walls of the spinal cord, at some distance from the brain dorsal vessels directed vertically into the neural mass. In the mid-lateral line of the spinal cord *A. spinalis lateralis* (Fig. 13, 14 Asl) extends from *A. cerebri post.* to the *A. medullaris* of the 7-th — 8-th segment of the body. This artery sends out numerous branches on the surface of the spinal cord.

The blood from the brain is drained away in *Torpedo* by one pair of veins *Vv. cerebri posteriores* (Fig. 12, 14 Vcp), which leave the brain-case together with nerve X, as in other Elasmobranchians. *V. cerebri ant.* is entirely absent although its tributaries exist in the forebrain. The posterior cerebral vein consists of two members, *Ramus anterior* and *posterior* (Fig. 12 Ra, Rp). The posterior extends from *V. spinalis dorsalis* along the posterior border of the *Rhombencephalon (Lobi electrici)*, partially concealed by it, to the dorsal surface of nerve X. Here it fuses with *Ramus anterior* and *V. spinalis lateralis* (Fig. 14 Vsl). The latter comes from the mid-lateral line of the spinal cord, where it follows dorsally the artery of the same name.

Ramus anterior extends along the whole brain conforming in its course to the shape of the brain. It is not therefore a straight tube, but runs at different levels and collects vessels from the entire surface. It starts in the forebrain as *V. prosencephali lateralis* (Fig. 12, 14 Vpl) and follows the external border of the hemispheres. *V. tecti lateralis* (Fig. 12, 14 Vtl) constitutes its prolongation on the midbrain, exhibiting a similar disposition. Further backwards it passes by the lower portion of the *Cerebellum*

and following its posterior border (contiguous to it or not) rises dorsally towards the mid-dorsal line of the *Rhombencephalon*. The vein attains here no fusion with its fellow from the opposite side, but turns with a gently curve laterally and ventrally, and passes on the dorsal surface of nerve X, where it unites with *Ramus post.* into *V. cerebri post.*

The main right longitudinal vein takes up from the dorsal surface of the hemisphere *V. prosencephali media* (Fig. 12 Vpm) and *V. prosencephali ventralis* from their base (Fig. 13, 14 Vpv). The left main vein receives only the latter. Some small branches from the *Tectum opticum* join both veins and a bigger one (*V. tecti media* Fig. 12 Vto) joins the left alone. The veins of *Rhombencephalon* are similar to the arteries. On the powerful base of the *Rhombencephalon* exist several transverse branches fusing predominantly into *V. rhombencephali ventralis* (Fig. 13 Vrv), which reaches *Ramus posterior*. On the membraneous top the veins are small and scarce; one connecting vessel between both *Rami anteriores* calls for attention here on account its strength.

V. lobi elctrici (Fig. 12, 14 Vle) runs on the lateral side of *Rhombencephalon* from nerve VII to nerve X, leaving nerve IX below and entering straight into *V. cerebri post.* Dorsally it collects some (2—3) characteristic short, thick branches which rise from the neural tissue at once as powerful tubes without superficial treelike ramifications.

R e x (1891) described in detail the venous patterns of the brain in *Torpedo*. S t e r z i (1909) finds arteries similar to those in *Myliobatis*, more thoroughly investigated by him. My results conform with those of my predecessors, and bring new details mainly in the arterial supply.

Comparative anatomy of the vessels in the brain

Numerous investigators, mostly mentioned in the preface to this paper, named the observed vessels of the head in a very arbitrary manner. They paid no attention to previous findings or, what happened more frequently, they applied different comparative anatomical criteria, while creating the names for the described vessels. This resulted in such confusion, that one and the same vessel possessed several names e. g. *A. orbitalis* — 11,

A. *pseudobranchialis af.* — 8, *Truncus arteriosus* — 9, *A. segmentalis* — 10 (C o r r i n g t o n). A l l i s brought some order into this hopeless state. He drew the schematic pictures of the blood-vessels of the anterior part of the body in all previously investigated fishes and by comparison of the corresponding parts of the drawings he was able to homologise many important vessels. He supplemented these theoretical studies based upon the knowledge of literature by a series of actual investigations. Due to that fact the initial conception, although in some respects altered, gained a sounder basis.

The mere knowledge of the topography is not always sufficient for a true estimation of the comparative anatomical value of blood-vessels. Very often the embryonic development helps solely to establish the homology or analogy between two vessels. The value of this kind of investigation was properly appreciated by D o h r n, G o o d r i c h, O' D o n o g h u e & A b b o t, A l l i s, C o r r i n g t o n and G e l d e r e n. In this review the results of previous anatomical and embryological investigations were taken into consideration. They are supplemented by experimente in the developmental mechanics of blood-vessels, which largely facilitates the understanding of some problems. The choice of name for a given vessel was influenced by the principle of priority and by the necessity of applying the term used in higher vertebrates. Mammals were studied first and then fishes; new observations have to be adjusted to the previous ones.

Comparing the blood-vessels in animals from two distantly related systemical units, we have to begin with a comparative anatomical estimate of organs or their parts. Homological regions of animals' bodies possess homological vessels. The latter unite closely with the supplied area from the very beginning of development. They grow together and are transformed always perfectly adjusted to its developmental level (S t r e e t e r, G r o d z i ń s k i). Relations between the vessels investigated and adjacent areas have little or no importance for comparative anatomical estimations. It holds especially true while comparing the cerebral vessels of Elasmobranchs and higher mammals. The brain of the former animals maintains predominantly the primitive linear arrangement of its parts and a fair balance between their size. The brain of higher mammals is more convoluted, its he-

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mispheres and cerebellum surpass many times the size of the other parts of the brain. Therefore the vessels are more complicated than those of fishes. An insignificant vessel not very constant in its course in sharks, may attain fairly great dimensions in a mammal. Conversly a significant stem in sharks may disappear or dwindle in a mammal. The dimension of vessels, their connections with other vessels, and the direction of their course, are not conclusive for homology, but solely the area supplied by them.

In young fish embryos (D o h r n, S c a m m o n, S h e a r e r, G r o d z i ń s k i) a pair of parallel arteries extends in the head portion from behind towards the developing cerebral vesicles. These are the paired aorta. Right in front each of them bends dorsally and turns backwards below the brain towards the spinal cord as an *A. cerebri posterior*. From the point of flexure they give off one branch to the *Prosencephalon, A. cerebri anterior*. The skull, which develops later, surrounds not only the brain but also part of the vessels which supply it.

From this process results the fact, that the anterior portion of the paired aorta comes to lie within the brain-case. It extends from the point of entry in the base of the skull to the place where the two cerebral arteries originate and diverge in opposite directions. This exactly defined section of the paired aorta deserves the name *A. encephalica* (Fig. 1, 3, 6, 8, 13 Aen).

Beyond the limits of the skull persists the remnant of the paired aorta and connected with it a series of the vessels of the gill arches. In the terrestrial vertebrates the vessels of the two first arches (mandibular and hyoid arches) disappear and the corresponding segment of aorta is given the name *A. carotis interna*. The vessels of these arches in fishes behave in various manners: they remain in entirety, disappear entirely in the first or second arch, or certain segments of the vessels persist in both arches. In spite of this partially different behaviour of the vessels in the two first gill arches, the portion of the paired aorta extending from (Fig. 1, 8 Ahe) *A. hyoidea eff.* (a branch of which forms the artery of the third gill arch *A. branchialis eff. anterior*) to the point of entry at the base of the skull is called also *A. carotis interna* (Fig. 1, 8 Aci). The remaining, more caudally placed section of the paired aorta bears the incontestable name *Aorta lateralis* (Fig. 1, Al).

H o f m a n n tried to settle the homology of the main cerebral vessels exclusively on an anatomical basis. He proceeded from the assumption, that the brain represents the properly modified anterior segment of the spinal cord. Consequently the brain ought to possess vessels similar to those of the spinal cord; he was thinking of the metameric *Aa. medullares* and the longitudinal *A. spinalis ventralis*. He was not however able to find in the brain a true well-developed *A. medullaris* connected with the cerebral nerve. He regards the arteries passing upon the nerves from the brain, especially in the region of the *Rhombencephalon*, as the remnants of segmental arteries. S t e r z i is of the same opinion. *A. encephalica*, although not connected with any nerve, is according to H o f m a n n the only real *A. medullaris*. This anatomical value has to result from the pattern of its ramification in the brain and meminges.

A. spinalis ventralis has, according to H o f m a n n, corresponding vessels in the brain, the *Aa. cerebrales anterior* and *posterior*. He supposes, that a pair of longitudinal arteries connected by several transverse vessels run primarily beneath the spinal cord and the brain. Gradually by the disappearance of one of the longitudinal trunks or by fusion of two resulted the longitudinal unpaired arteries. The blood-vessels in *Raja* exhibit the most primitive pattern. At the caudal end of the spinal cord of *Raja* exists an unpaired *A. spinalis ventralis*. *Plexus spinalis ventralis* constitutes its prolongation in the anterior portion of the spinal cord, which continues in the brain as the two longitudinal trunks *Aa. cerebri post.* and *ant.. Squalus, Acanthias* and representatives of other groups of vertebrates possess in the entire spinal cord an unpaired *A. spinalis ventralis* homologous with *A. basialis* of the *Rhombencephalon*. In the anterior part ot the brain persist only two longitudinal trunks.

The assumptions of H o f m a n n do not find support in embryological investigations. Neither the arteries of the nerves nor *A. encephalica* are metameric *Aa. medullares*. The first grow secondarily along the nerves from the longitudinal cerebral vessels. *A. encephalica* constitutes the intracranial portion of paired aorta. The longitudinal vessels of the spinal cord originate from the terminal branches of *Aa. medullares*, which form a vascular meshwork at the base of the spinal cord. *A. cerebri ant.* and *pos-*

terior are the prolongation of *A. encephalica. Aa. cerebri poste-riores* unites in the majority of species into the unpaired *A. ba-sialis. A. spinalis ventr.* and the longitudinal arteries of the brain are homodynamic but not homologous vessels. They perform the same functions in relation to the central nervous system, but are of different origin.

A. carotis interna exist in all Elasmobranchs with the excep-tion of *Hydrolagus* (P a r k e r, A l l i s, C r a i g i e) and is connec-ted by its terminal portion very often with *Aorta lateralis* and always with *A. hyoidea efferens.* At first sight it represents the proper prolongation of the latter and runs askew or perpendicu-larly to the long axis of the skull. *Aorta lateralis* reaches it at more or less right angle and often does not reach its diameter (*Scylliorhinus, Squalus, Heptanchus, Squatina, Cetorhinus*). *Raja* and *Torpedo* do not possess *Aorta lateralis*; theire are mere rem-mants which have lost the primitive connection with *Aa. caro-tides internae.* Whatever conditions occur, *A. carotis interna* cons-titutes from the anatomical point of view the prolongation of *Aorta lateralis,* but functionally is closer allied with *A. hyoidea efferens.*

The change in direction of the course of *A. carotis int.* may depend on the origin of the cartilaginous base of the skull. The *Trabeculae cranii* forming it, grow in width, and so narrow the *Foramen hypophyseos* and move medially the arteries, which pass through this hole in order to reach the brain. Other caudal seg-ments of the paired aorta avoid this pressure, because they lie on the surface of the cartilaginous base of the skull. The vessels of the gill arches reach them laterally and fix them in the pri-mitive position. Due to that fact only the short section of the paired aorta, situated behind the *Foramen hypophyseos,* bends from the primitive position conformed to the longitudinal axis of the head and ranges itself transversely to it. The behaviour of *A. encephalica* may be interpreted similarly. The commencing section of which is in consequence of the same process directed perpendicularly to the longitudinal axis of the brain.

Trabeculae cranii moved *Aa. carotides int.* towards the middle of the base of the skull, brought them in touch with each other and encircled them. That happened in the early embryonic sta-ges, when the arteries probably possessed the peculiarities of

a plastic vessel provided with thin walls. de Beer investigated exhaustively the closure of the *Foramen hypophyseos*. He re-marked, that when the cartilaginous borders of this hole are formed, the arteries passing through it are already crossed with each other. We may than assume, that the shifting of arteries took place earlier, in the stage, when condensations of mesenchyme appeare in the place of future cartilages. These condensations of tissues push the vessels toward the middle of the base of the cranium.

No wonder that, crowded · in a narrow space, they united with one another in very different manners. The most primitive conditions prevail in *Trygon pastinaca* (Sterzi) and *Chlamydose-lachus* (Allis). *Aa. carotides int.* pierce the base of the skull as independent vessels. Within the brain-case, beneatch the hypo-physis, a long transverse vessel connects them. In *Heptanchus* both arteries fuse in a short unpaired trunk just at the cartila-ginous base of the skull (Allis, Daniel). An ample vascular sinus (*Sinus cephalicus*) unites them within the cranial cartilage in *Galeus* (Corrington). On the outside of the skull they unite and as an unpaired trunk pierce the base of the brain-case in *Scylliorhinus* (Fig. 1 Aci) and *Mustelus* (Grodziński). Par-ker and Hyrtl assert that in this case the crossing of the ar-teries only occurs. Similar conditions to those in *Scylliorhinus* prevail in *Squatina* (Carazzi, Marples), but sometimes two arteries are connected by the transverse vessel within the brain-case (Daniel). Both arteries fuse in *Squalus* into one trunk beneath the base of the skull, or within its wal, and seldom pe-netrate into the brain-case not united (O'Donoghue & Abbot). Hofmann and Sterzi speak about the crossing of arteries within the hole of entry in this species. In *Torpedo* not only the united arteries lie beneath the base of the skull, but also the long section of the unpaired trunk (Hyrtl, Grodziński). Two in-dependent stems (Fig. 8 Aci) are crossed in the hole at the base of the skull in *Raja* (Hyrtl, Grodziński). Within the brain-case the left artery passes into the right *A. encephalica* and the other into the left. It is not necessary to imagine that the sec-tions of the paired aorta enclosed within the brain-case have changed their position in relation to the brain. Probably the cros-sing occurred within the narrowed *Foramen hypophyseos* only. The blood current decided which sections of the embryonic ves-

sels were to persist as final stems and what course they were to take. Similar processes can be easily followed during the formation of one venous trunk from two paired ones in the chick blastoderm (*V. vitellina anterior* — Grodziński).

Some authors assert, that *Aa. carotides int.* possess narrow clefts in the region of the crossing, through which the blood may pass from one vessel into the other. Hyrtl based this assumption on very flimsy ground. In one preparation of *Raja clavata* the dye filled intensively one *A. carotis.* In the other very little of the dye was present, probably according to Hyrtl it forced its way through »feine Spaltöffnung« from the well injected vessel. Hofmann and Sterzi mention these clefts also in *Squalus acanthias*, they do not explain however how they observed them.

I have tried to find this cleft in *Raja punctata*, magnified 30× with a binocular microscope, without success however. I failed also to prove indirectly its existence. In my preparations there were visible through the walls of the arteries small clots of Indian ink or Prussian blue suspended in the transparent fluid. Squeezing one artery by means of a prick of a needle I brought into motion all the particles but in the squeezed vessel only. In the other they remained motionless. Provided the fine cleft exists between the two vessels, the clots should oscillate everywhere. A series of similar attemps in two specimens furnished identical results. Therefore I am inclined to assume that no clefts between crossed vessels occur.

The points of entry for *Aa. carotides int.* may be found at the base of the skull beneath the caudal end of the hypophysis (*Scylliorhinus, Mustelus*), beneath its middle (young *Raja*), or in the region of the anterior border of the hemispheres (*Torpedo*). It moves then significantly forwards in relation to the brain. *A. pseudobranchialis eff.* constitutes in *Hydrolagus* the unique communication between the cerebral vessels and the vessels of the gill arches. This artery pierces the brain-case in the region of the roots of nerve II (Craigie). There occurs here also a displacement forwards of the point of entry. Both vessels entering the brain-case fail however to fuse into one stem. *Aa. carotides int.* reach the base of the cranium as fragmentary vessels, fail however to pierce it even in embryos 90 mm of length (de Beer).

The sections of the paired aorta — *Aa. encephalicae* — within the skull diverge from the point of entry into the brain-case laterally, running along the bottom of the skull, in some species (*Torpedo*) deeply sunk into the cartilaginous groove. Having attained the lateral border of the brain, they turn forwards in the direction of nerve II. They cross it from above and after a short distance split into two main cerebral arteries. On their way they take up the artery from the mandibular arch — *A. pseudobranchialis eff.*, provided this vessel exist, and give off a branch on nerve II to supply the eye — *A. optica. A. encephalica* communicates then with the vessels of both first gill arches, i. e. with *A. pseudobranchialis eff.* of the mandibular arch and by means of *A. carotis int.* with the vessel of the hyoid arch — *A. hyoidea efferens.* Exceptions from this rule are *Torpedo* which entirely lacks *A. pseudobranchialis eff.* and *Hydrolagus*, in which *A. pseudobranchialis eff.* constitutes the unique connection with the vessels of the gill arches. Corresponding to the situation of the point of entry into the brain-case *A. encephalica* passes in *Torpedo* and *Hydrolagus* not from the hypophysis to nerve II, because it comes in *Torpedo* from the forebrain backwards to nerve II and in *Hydrolagus* passes directly from the skull upon nerve II (Craigie).

Much simpler appear the main veins carying away the blood from the brain-case. Along the outer border of the brain-case *V. cardinalis anterior* runs on each side of the head. It originates in the region of the smell organ, passes the orbit and goes back above the gills to the heart. These veins, extraordinarly dilated, really form a row of sinuses communicating with each other. They take up among others the veins leaving the brain-case.

The longitudinal head vein lies in very young embryos medially to the cerebral nerves V—X and to the auditory capsule. During the further development the vein forms circles around the nerves. Then the medial portions of the circle atrophy and the vein moves laterally in relation to the nerves and auditory capsule. *V. cardinalis ant.* of the adult Elasmobranch fishes corresponds with *V. capitis lateralis* in embryos. *Mustelus* alone possesses in the region of nerve V the remnant of the *V. capitis media* (Gelderen, Goodrich).

The proper cerebral veins appear in all Elasmobranch embryos, two pairs in number (Gelderen), but the adult *Torpedo* is short

of the anterior cerebral vein. It is not known, when they disappear, de Beer observed them in embryos 24 mm of length, Köllberg in embryos 31 mm of length. Both pairs empty into *V. cardinalis ant.*, *Vv. cerebri ant.* pass through the cartilage into the orbit and merge into *Sinus orbitalis*. Both sinuses are connected by a straight vessel *V. pituitaria* (de Beer, Köllberg), which pierces the brain-case and runs beneath the hypophysis. The sinus appears as an extensive sac, divided internally into some chambers (*Mustelus, Squalus*) or as in *Raja* forms a spongy organ (Tretjakoff). *Vv. cerebri ant.* deflect from the brain at the level of the caudal border of the hemispheres, in rays within the range of hemispheres or anterior to them depending on the age of the specimen. *Vv. cerebri posteriores* leave the brain-case with nerve X, resting upon its dorsal surface, and join one of the sinuses of the cardinal vein (Parker, Rex, Hofmann, Sterzi, O'Donoghue, Gelderen, Daniel, Marples, Grodziński).

Without reference to the number of gill arches furnishing vessels to the brain-case, *A. encephalica* splits into two branches, *A. cerebri ant.* and *post.* These main longitudinal vessels diverge in opposite directions in the region between the roots of nerves II and III.

A. cerebri post. passes to the rear in the groove between the base of the midbrain and the hypophysis. In *Rajiformes (Raja, Torpedo, Trygon, Miliobatis)* it continues along the lateral border of the *Rhombencephalon* and just behind nerve X unites with its fellow from the opposite side into the unpaired trunk *A. spinalis ventralis*. In other representatives of Elasmobranchs (*Squatina* included) both *Aa. cerebri post.* fuse together already above the caudal border of the hypophysis and run as an unpaired trunk — *A. basialis* — in the mid-ventral line of the *Rhombencephalon*. This trunk may be double in some species (*Squalus* — Daniel, Sterzi, *Cetorhinus* — Carazzi); here run two trunks close together and form »islands« *(insula, circulus)*. In the spinal cord they pass without a distinct limit into *A. spinalis ventr.* (Hyrtl, Parker, Carazzi, Hofmann, Craigie, Daniel, O'Donoghue, Sterzi, Grodziński).

A. cerebri post. gives off a certain number of branches to the lateral sides of the brain, in *Rajiformes* also medial ones to the

base of the *Rhombencephalon*. Among the lateral branches we can distinguish *A. mesencephali*, *A. cerebelli later.* and *A. rhombencephali lateralis*. From these some branches *(A. auditiva)* reach the membranous labyrinth. *Aa. rhombencephali later.*, contrary to some opinions (Hofmann — *Squalus*), in none of the investigated species exhibit the metameric arrangement.

A. cerebri ant. turns towards the front of the head and supplies the forebrain, a part of the betweenbrain and the smell organ. After a short course it breaks into several branches. The original direction of the course is maintained even in *Torpedo* by *Aa. olfactoriae*, which finally leave the brain-case in order to reach the smell organ. *A. olfactoria* splits in *Cetorhinus* into a bunch of more or less parallel vessels, which follow *Tractus olfactorius* to the distant smell organ (Carazzi). From the constant branches of *A. cerebri ant.*, *A. prosencephali basilaris*, *dorsalis* and *lateralis* should be noticed.

A. prosencephali basilaris arises from the initial segment of *A. cerebri ant.* or directly from *A. encephalica (Mustelus)* and enters the longitudinal groove separating the bases of both hemispheres. In this groove these arteries run independently of each other *(Squalus, Raja)*, or they fuse into one common trunk *(Scylliorhinus, Torpedo)* or they are connected by transverse vessels *(Laemargus, Rhina, Hydrolagus, Heptanchus)*. The giant specimen of *Raja batis* investigated by Hyrtl possessed vessels which, collected in bunches, reached the walls of the brain and exhibited the character of unipolar *Rete mirabile*. In *Cetorhinus* a series of small branches passes upon the base of the hemispheres from *A. cerebri post.* and ramifies there like a net (Carazzi). A similar vascular network exists in *Galeus*, its arteries springing however from *A. cerebri ant.* (Corrington). Because in many Elasmobranch fishes already investigated connections occur between *A. prosencephali basilaris* and in all species connections between *Aa. cerebri post.*, we can speak about the presence of a closed *Circulus Willisii* in these fishes. Its anterior limit is fairly constant for all species, the posterior varies according to the presence or absence of *A. basialis*.

A. prosencephali dorsalis branches from *A. olfactoria* on the ventral side of the prosencephalon. It passes then in the split between the forebrain and the *Bulbus olfactorius* and reaches

the top of the brain, where it ramifies in different ways, accor-
ding to the shape of this cerebral region.

A. prosencephali lateralis constitutes a branch of *A. olfactoria*
or *A. cerebri ant.* itself. It passes on the caudal surface of the
top of the hemispheres, where it ramifies.

Besides the *A. cerebri ant.* and *post.* some authors distinguish
a third vessel, *A. cerebri media.* The argument for one name or
another is not an easy or simple task, because the value of
cerebral arteries has to be regarded from the anatomical and
embryological point of view. In the human brain three pairs of
cerebral arteries are recognized; to be precise, they are the
arteries of the forebrain. *A. cerebri ant.* passes round the roots
of nerve II, deepens between frontal lobes and both hemispheres
and turns back along the dorsal border of *Corpus callosum.*
A. cerebri media deviates at the level of nerve II upon the
lateral surface of the hemisphere, proceeds along it vertically
dorsad and supplies the neighbouring areas with several branches.
A. cerebri post. turns back from nerve II and adhering to the
base of the hemisphere suplies its caudal lobes. All three arteries
arise in the *Circulus Willisii* and their names *(anterior, media,
posterior)* determine the sequence, in which they ramify. The
expression »*cerebri*« is far from exact, it should rather be »*pro-
sencephali*« from the parts of the brain drained.

The cerebral arteries in man develop principally in the same
manner as in other vertebrates (*Salmo, Tropidonotus* — G r o d z i ń-
s k i, *Gallus* — Sabin, *Sus* — E v a n s, Sabin), at least in the initial
stages, when the differences in appearance of the brain in various
vertebrates are comparatively insignificant. *A. carotis int.* runs up
to nerve II. Here it puts forth a branch upon the lateral surface
of the anterior vesicle of the brain *(A. cerebri media)* and turns
back below the brain passing in the neck region into *A. verte-
bralis.* In older embryos the branch in the longitudinal groove
of the base of the hemispheres *(A cerebri ant.)* springs from it.
The caudal sections of the paired arteries fuse within the range
of the *Rhombencephalon* and *Pons* into the unpaired *A. basialis*
(E v a n s, S t r e e t e r). During the course of further development
lateral branches sprout out from the main arteries to all parts
of the brain, among others *A. cerebri post.*; their strength is pro-
portionate to the size of the areas supplied.

These conditions are reflected in the appearance of the main arteries, which can be especially well seen within the *Circulus Willisii*. At the beginning of the development the main arteries running below the base of the brain gradually diminish from front to back. In young embryos *A. carotis int.* markedly exceeds *A. vertebralis* in diameter. Later, when the hemispheres gain in size, their arteries drain the majority of the blood from the main vessel, i. e.: *A. cerebri post.* from the *A. basialis* and *A. cerebri media* from *A. carotis int..* As a result the connecting tube between *A. carotis int.* and *A. basialis* plays the part of the weak connecting vessel: *A. communicans post..*

The names of cerebral vessels in man are not based very exactly upon the anatomical data (»cerebri« instead of »prosen-cephali«), and in addition they are not influenced by the developmental relations. On account of this we cannot always apply the names from human anatomy to our purposes. The homology of the cerebral arteries in man and Elasmobranch fishes however should be settled, even though the names have to remain different.

The section of *A. carotis int.* lying within the brain-case is called in sharks *A. encephalica*, and in human anatomy bears the original unchanged name *A. carotis int.*. *A. cerebri post.* in sharks incontestably corresponds with *A. communicans post.* together with *A. basialis* in man.

A. cerebri post. in man may have its counterpart only among the arteries of the hemispheres and only among those supplying their base and back. *A. prosencephali lateralis* in *Scylliorhinus* corresponds to same extent with these requirements.

A. prosencephali basilaris in sharks can be regarded without serious opposition as the counterpart of *A. cerebri anterior* in man. Its course anterior to nerve II in the longitudinal groove between both hemispheres supports this assumption. It is of secondary importance whether the arteries fuse into a common stem, remain throughout independent, or are connected with each other by cross-vessels.

The homology of the human *A. cerebri media* remains very obscure. Its counterpart in sharks may be one of the branches of *A. cerebri ant.*, which supplies the lateral side of the hemispheres. We exclude *A. prosencephali lateralis* as suspected for affinity with another artery in the human brain. *A. prosencephali*

dorsalis also falls out because it reaches the top of the hemi-
spheres running mesial to the *Bulbus olfactorius. A. olfactoria*
need not be considered because it is not a strictly cerebral artery.
If then a counterpart of *A. cerebri media* in man exists in sharks,
it is certainly an insignificant branchlet connected with the tri-
butaries of *A. cerebri ant.*.

The homology of the cerebral arteries in sharks and higher
vertebrates has often been discussed. All authors are agreed in
using the name *A. cerebri posterior* for the same artery in Elas-
mobranchs and in conformity with human anatomy call its caudal
unpaired section *A. basialis.* They do not advance however any
opinions about *A. communicans post.* and *A. cerebri post.* in man.

The majority of authors (Hofmann, Daniel, Corrington,
Marples, Kappers) regard *A. prosencephali basilaris* in sharks
as a counterpart of *A. cerebri ant.* in man. According to Sterzi
it corresponds with *A. cerebri media* in man. Corrington,
Daniel and Marples regard *A. olfactoria* in Elasmobranchs
as a counterpart of *A. cerebri media* in man. Finally Kappers
advocates the opinion that *A. prosencephali dorsalis* in Elasmo-
branchs is the »primitive homologon« of *A. cerebri media* in man.
My point of view on this problem has been described above.

From two pairs of the cerebral veins *V. cerebri ant.* collects
the tributaries from the forebrain, betweenbrain and the top of
the midbrain. On the forebrain it has three main roots, *V. pro-
sencephali lateralis, media* and *ventralis.* The most constant of
them, *V. prosencephali lateralis,* occurs in all species, and runs
back along the lateral border of the hemisphere from *Bulbus
olfactorius.* It collects the blood from the lateral and dorsal walls
of the hemispheres. *V. prosencephali media,* unpaired in *Torpedo*
and *Scylliorhinus,* lies in the mid-dorsal groove between the two
hemispheres, in *Mustelus* and *Squalus* in the same position but
paired. This vein is completely undeveloped in *Raja.* In conse-
quence of the marked displacement of *V. prosencephali lateralis*
towards the mid-dorsal line, its medial branches drain the region
of *V. prosencephali media. V. prosencephali ventralis* includes by
means of its tributaries the ventral, caudal and lateral side of
the hemispheres.

V. tecti optici, as a caudal root of *V. cerebri ant.,* expands
over the top of the midbrain as an unpaired trunk, running in

the medial groove of this part of the brain *(Raja, Torpedo Squalus)*. Or it springs from *Tectum opticum* in a series of small branches converging into one asymetrical trunk *(Scylliorhinus, Heptanchus)*. When the cerebellum covers the *Tectum* completely, the vessels of the latter unite with the vessels of the anterior part of the cerebellum in a common unpaired trunk *V. tecto-cerebellaris (Mustelus)*.

Within the range of the anterior cerebral veins lies *Plexus chorioideus ventriculi* III and *lateralis*. Species with the betweenbrain uncovered and with large lateral chambers have all plexuses vell developed *(Scylliorhinus, Squalus)*. In *Raja* a small third chamber and a small corresponding plexus are present. Its lateral chambers are reduced, the plexuses are absent. When the midbrain covers the betweenbrain *Plexus chorioideus ventr.* III remains vestigial *(Torpedo, Mustelus)*. From the chorioid plexuses one *(Scylliorhinus, Raja)* or two *(Squalus)* vessels reach the veins of the forebrain.

All the veins discussed above, namely *V. prosencephali media, lateralis, ventralis* and *V. tecti optici* converge, with the exception of *Torpedo*, in *V. cerebri ant.* If one of these vessels happens to be an unpaired trunk, it may merge into the right or left cerebral vein; which is to be regarded as an individual variation. *V. tecti optici* in *Scylliorhinus* runs in the region of the elongated betweenbrain beneath the vault of the skull and takes up a comparatively long and big *V. chorioidea*. Although the *Torpedo* lacks *V. cerebrt ant.*, it has however the tributaries of this vein, which united into one stem constitute the most anterior roots of *V. cerebri post.*. A constant communication between *V. cerebri ant.* and *post.* exists in young specimens of *Raja*, which can be regarded as an indication of the relations in *Torpedo*.

V. cerebri post. consists of two branches, the long *Ramus anterior* and the short *Ramus posterior,* which constitutes in many species a prolongation of the spinal vein — *V. spinalis dorsalis.* In *Squalus* the latter vein is lacking (Sterzi, O'Donoghue & Abbot), but one or two feeble *Rami* run indepedently always from the spinal cord towards the posterior cerebral vein. *Ramus anterior* continues along the lateral border of the brain from the midbrain to nerve X, modelled in its course by the shape of the

corresponding sections of the brain. *Ramus ant.* is elongated in *Torpedo* by the branches of *V. cerebri ant.* As a connecting tube between the tributaries of the atrophied *V. cerebri ant.* and *Ramus anterior* proper the longitudinal trunk *V. tecti lateralis* appears on the lateral walls of the midbrain. It is developed probably in older embryos from this branch of *Ramus anterior*, which reached *V. cerebri ant.* and which gained in diameter corresponding to the reduction of this vein. This connecting branch might look like the vessel within the range of the midbrain in *Raja*, which units both main cerebral veins.

Ramus anterior is a continuous trunk in *Raja* and *Torpedo*. In forms possessing well-developed *Auricula cerebelli* it breaks into a dense network of blood vessels *(Scylliorhinus, Mustelus)*, on the surface of these organs. *Ramus anterior* collects branches from the lateral and ventral side of the midbrain, from the cerebellum and *Rhombencephalon*. The veins of the latter part of the brain consist in most cases of a few bigger branches. On the base of the *Rhombencephalon* a longitudinal vessel *V. rhombencephali ventralis* always appears, although differing in size. In *Torpedo* is also found a specific for this species *V. lobi electrici*. In the region of the cerebellum a distinct *V. cerebelli lateralis* appears and often *V. cerebelli posterior.*

In the vault of the fourth chamber in Elasmobranchs extends the *Plexus chorioideus ventriculi* IV, well developed in most species. The fourth chamber in *Torpedo* is filled with an accumulation of the cells of visceral motor nuclei, which form the *Lobi electrici*. Owing to this the lumen of the chamber takes the appearance of a narrow chink, and in *Telum* no vascular network develops, to cover it (Rex, Hofmann, Sterzi, O'Donoghue & Abbot).

A fundamental difference becomes evident when comparing the cerebral veins of Elasmobranchs with higher vertebrates e. g. with mammals. In mammals (man) exist cerebral veins and sinuses of the *Dura mater*. The first rest upon the surface of the brain or drain its interior, the latter are involved within the *Dura mater* and carry away beyond the skull the blood collected by the veins. All cerebral veins of Elasmobranchs rest on the brain and all drain its blood. Some of them can cross at least partially the free space between the skull and the brain without

touching the latter. Sinuses are entirely lacking, which has already been pointed out by Rex and Hofmann.

V. cerebri ant. and *post.* of Elasmobranchs possess their counterparts in mammalian embryos. In mammals during development these veins are transformed into various sinuses of the *Dura mater* (Markowski, Streeter, Gelderen) and after that they acquire an appearance far removed from their original similarity in both these groups of vertebrates. *V. prosencephali media (Torpedo)* may pass as a primitive homologon of *Sinus sagittalis superior* in man. Both run identically in the mid-dorsal line between the hemispheres, from the front backwards. They differ in their relation to the *Dura mater* and in the lateral branches, which in Elasmobranchs collect the blood from hemispheres.

Circulation of the blood in the cerebral vessels

Up to the present no actual blood circulation in the brain of adult Elasmobranchs has been observed. Some conception of this circulation can however be obtained from anatomical data. Following the course of the blood vessels from the heart to the brain we can distinguish, which vessels bring the blood to the brain and which carry it away. Within the arteries the blood passes from the vessels of wider to the vessels of narrower diameter; in the veins the converse holds true. The smaller arteries branch from the main trunk forming an acute or right angle, never an obtuse. Reversely we infer from the angle at which the arteries are branching off the direction of the current of the blood in the main trunk.

Based on these elementary foundations we assert, that *A. encephalica* brings the blood to the brain of Elasmobranchs. Further questions which arise are from where the blood enters the *A. encephalica* and whether the latter is the unique vessel supplying the brain with blood. From the general observations it is known that *A. encephalica* communicates with the vessels of two pairs of gills: *A. hyoidea efferens* and *A. pseudobranchialis efferens. Torpedo* lacks the first, *Hydrolagus* the second; in these forms *A. encephalica* receives the blood from only one pair of the vessels of the gills.

In living young embryos of Elasmobranch and Teleost fishes (*Scylliorhinus, Salmo, Fundulus*) it is easy to observe, that the blood passes from the vessels of the gill arches into the paired aorta and follows its branches all over the brain. It is generally expected and accepted that in these vessels of the adult specimens the blood current maintains the same direction. Solely H y r t l, an excellent technician in the field of injecting the blood vessels, and an ingenious observer, advocates the opposite view upon the circulation of the blood in the mandibular arch in *Raja*.

H y r t l observed in many excellent preparations of *Raja* three vessels connecting the eye with the arteries of the head, but none connecting it with the veins, in spite of the fact that the main venous trunk almost touched the eyeball. He assumed then, that one of these three vessels — *V. ophthalmica* (our *A. ophthalmica*) carries the venous blood into *A. pseudobranchialis eff.*. Hence the blood, exhibiting a mixed arterio-venous character, enters the pseudobranchial gills. These organs are well developed in *Raja* in contrast to the same gills in sharks, in which they are vestigial or entirely atrophied. The blood oxygenated within the pseudobranchial gills will reach by way of *A. pseudobranchialis afferens* the muscles of the jaw, but mainly flows to the gills of hyoid arch. If the current of the blood flows in the opposite direction, why are the pseudobranchial gills so perfectly adjusted to respiration? The thesis of H y r t l is supposed to be supported by the diameter of the vessels of this region.

In my preparations I missed also the ophthalmic vein, which in my opinion was due to the incomplete injection of the veins of the head. Yet I constantly found only two distinct vessels, *A. ophthalmica* (*V. ophathalmica* after H y r t l) and *A. optica* (*A. ophthalmica* after H y r t l), the third obserwed by H y r t l was always absent. The third vessel in the head of the gigantic specimen, which H y r t l had at his disposal, may have been a nutrient vessel of the nerve II. The drawings and description of H y r t l do not conform exactly, therefore it is difficult to settle the real course of this vessel. However, in my preparations these were just two arteries to the eyeball, as in other Elasmobranchs, one to the retina, the other to the walls of the eyeball. Neither of them was qualified to be a vein.

The diameter of the vessels and the angle at which they unite with each other in *Raja* require a detailed analysis. The terminal section of *A. pseudobranchialis eff.* [Fig. 8 Ape] adheres within the brain-case to the wall of *A. encephalica* for a considerable distance, and opens into it at a very acute angle. At the same time the diameter of *A. encephalica* significantly exceeds the diameter of the merging artery. According to this anatomical data we should expect the blood from *A. encephalica* to pass into *A. pseudobranchialis eff.*, which would be in accord with the opinion of Hyrtl. If this were true, the blood, or rather part of the blood would circulate in a closed circuit, omitting the heart, through the following vessels: *A. encephalica*, *A. pseudobranchialis eff.* and *aff.*, *A. hyoidea eff.*, *A. carotis int.* and again *A. encephalica*. An absurd circulation.

Allis remarks that *A. hyoidea eff.* in *Heptanchus* significantly exceeds in diameter *Aorta lateralis*, which is connected with it. He inferred from this, that the blood tends to flow from the thicker into the thinner vessel. Similar, but lesser, differences in diameter exist between *Aorta lateralis* opening into *A. carotis int.*, or between the gills section of *A. hyoidea eff.* and its palatinal section in *Scylliorhinus* (Fig. 1.). I cannot accept in this case that the blood flows from *A. carotis int.* into *Aorta lateralis* or returns into the hyoid arch from *A. epibranchialis*. Rather I believe that the blood enters from the thinner into the thicker vessel. I cannot imagine the moment of reversal of the blood current established in embryos within these vessels. I have observed the process of reversal of the blood current within the vascular field of the chicks blastoderm, but it was always connected with a total check of circulation for a certain period in the observed area and with complete morphological rebuilding of the vessels (1935).

The instances referred to do not overthrow the principles of the vascular branching quoted in this chapter, they limit them only to the vessels, which ramify in the form of a tree. Yet the vessels which possess from the very beginning a ladder-shaped arrangement, e. g. blood vessels of the gill arches, undergo in their arrangement and appearance not only the hydrodynamic influence of the blood current, but are also largely formed by

5*

the mechanical influences of the environment. In this process
a chief part is played by the skeleton of gill bars, that maintains
the vessels in a steady position and precludes all greater displace-
ments, which might occur under the influence of the blood cur-
rent. In the ladder-shaped arrangement of vessels the angle at
which two vessels unite with each other tells nothing about the
direction of the blood current, and the diameter of the vessels
proves only that they convey a greater or smaller amount
of blood.

On account of 1) the lack of the third vessel in the eye of
younger specimens of *Raja*, 2) the manner of circulation prevailing
in embryos, 3) a similar arrangement of blood vessels in this
region of the body in sharks, and 4) the specyfic properties of
the ladder-shaped system of vessels, it seems advisable to con-
sider the view of Hyrtl at least critically. A similar opinion
has been expressed by Corrington. The final resolution of
this problem can be only obtained by direct observation of the
circulation.

The blood then, reaches *A. encephalica* by means of *A. pseudo-
branchiatis eff.* and *A. carotis int.*, provided they exist in the
given species. *Aa. carotides int.* in *Raja* cross with each other
while passing the skull. Due to this fact the blood passes from
the right vessel to the left side of the brain and conversely.
When both carotid arteries form a short common stem *(Scyllio-
rhinus)*, the blood has an opportunity of mixing, but not to any
great degree. Two parallel blood currents diverge within the
brain-case in both vessels *(A. encephalica)*, each on its own side
of the brain. I have observed similar phenomena many times in
the blastoderm of the chick. Here two vessels unite into unpaired
V. vitellina ant.. The blood runs unmixed in this vein for the
whole of its lenght from the border of the vascular area to the
heart. Both streams show better in contrasted colour, if the blood
of the one constituent vein contains Indian ink (1935).

In *Galeus* both *Aa. carotides int.* unite in a trunk which
dilates into a sinus *(Sinus cephalicus)*; an eddy may occur in it
and favour the mixing of blood.

A. encephalica is the main artery entering the brain-case and
it distributes the cerebral vessels. *A. spinalis ventralis* passes from
the spinal cord upon the brain and at the base of the *Rhomben-*

cephalon unites with the branches of the main cerebral artery, i. e. with *A. cerebri post.* or *A. basialis.* It is then possible for *A. spinalis ventralis* to furnish a part of the blood to the brain, which is actually the case in varying degrees in different representatives of Elasmobranch fishes.

The pattern of blood vessels in *Mustelus* (Fig. 6) does not authorise the assumption that the blood washing the brain enters the skull by any other way than through *A. encephalica.* This artery supplies by means of its branches the entire brain. Its terminal prolongation, *A. basialis,* gradually diminishes, running back in the mid-ventral line of the *Rhombencephalon.*

The vessels of the base of the *Rhombencephalon* and spinal cord in *Scylliorhinus* (Fig. 3) are distributed differently from those in *Mustelus.* This difference lies in the lateral branches of *A. basialis* and *A. spinalis ventralis.* The lateral branches of the anterior section of *A. basialis,* following closely the hypophysis, turn backwards. In the region of nerve X and the first spinal nerves the lateral branches are directed forwards. The direction of these vessels demonstrates the direction of the blood passing into them from the longitudinal vessels. In the first case the blood moves from the hypophysis towards the caudal parts of the brain, in the second from the back to the front. Both currents meet in the neighbourhood of nerve X. In *Scylliorhinus* a short posterior section of the brain then receives the arterial blood from *A. spinalis ventralis.*

Quite different conditions appear in *Raja* (Fig. 10). The blood from *A. encephalica* certainly enters *A. cerebri ant.* and supplies the anterior part of the brain. *A. cerebri post.* exhibits its smallest diameter where it leaves *A. encephalica* and increases in size as it runs back. In the region of the spinal cord it finally passes into a big *A. spinalis ventr.* and two *Aa. spinales collat..* Its branches *(Aa. rhombencephali ventrales)* turn distinctly forwards, especially with their terminal segments. Both these features of the arteries in the posterior part of the brain indicate unquestionably, that *A. cerebri post.* receives the blood from behind from *A. spinalis ventr.,* but not from *A. encephalica.* In the spinal cord occur a pair of bigger *Aa. medullares* which bring the blood from the gills *(A. epibranchialis)* into *A. spinalis collateralis* and force it directly or through *A. spinalis ventr.* into the cerebral

arteries. It is however difficult to decide how far forwards the blood in *A. cerebri post.* reaches. It is not precluded that a part may passe into the *A. cerebri ant.*. This can be inferred from the diameter of *A. encephalica*, and from the direction of its course. *A. cerebri ant.* is, taking the direction into consideration, the prolongation of *A. cerebri post.* and not of *A. encephalica.*

The arterial cerebral pattern in *Torpedo* constitutes the ultimate development of the conditions in *Raja*. Here *A. spinalis ventr.* brings the blood to the entire brain. It is visible from the mighty dimensions of *A. spinalis ventr.* itself, which passes into the large *Aa. cerebri post.*, diminishing as they progress. The direct prolongation of this artery constitutes *A. cerebri ant.* In *Torpedo* occurs also *A. encephalica*, a comparatively small vessel pushed far forwards. After having branched an artery to the orbit it gets still smaller. From half the length of the hemispheres to the connection with *A. cerebri post.* it gains somewhat in diameter. In this section it gives off arteries to the eyeball and *Lobi inferiores.* The problem of blood circulation in the terminal section of *A. encephalica*, especially from *A. orbitalis* to the rear is not quite clear for the anatomist. I would be prone to assume, that two blood currents coming from the back and from the front meet with each other in the midway.

In connexion with the blood-supply of the brain from the spinal cord exist two big *Aa. medullares*, which furnish the blood from the vessels of the gills to *A. spinalis ventr.* In *Raja Aa. spinales laterales* act as intermediates to a great degree in the transmission of blood to *Aa. cerebri post.*, in *Torpedo* they supply almost exclusively the spinal cord itself.

Sterzi was the first to call attention to the significance of *A. spinalis ventr.* for the feeding of the brain in *Raja, Trygon, Myliobatis* and *Torpedo*. He observed that the diameter of these vessels exceeds in varying degrees the diameter of *A. encephalica.* Therefore *A. spinalis ventr.* furnishes the brain with a differing amount of blood depending on the species; in *Myliobatis* it attains the maximum.

It is not quite certain if *A. spinalis ventr.* in *Cetorhinus* does not play an important part in supplying the brain with blood. In Carazzi's drawings *A. carotis interna* and *A. pseudobranchialis eff.* on entering the brain-case are very small vessels. *A. ba-*

sialis is on the contrary a very strong artery and double for a great part of its course; it occupies thus an intermediate position between the unpaired *A. basialis* of sharks and the double (*Aa. cerebri post.*) of rays.

The main arteries of the brain, regardless of the direction of transmitted blood, extend as longitudinal vessels below the base of the brain. They send the blood to the lateral and dorsal walls of the particular section of the brain by means of the branches splitting treeshaped upon their surface. The blood enters from the surface into the neural tissue through capillaries. The number of capillaries in the neural mass taken as a whole gives some idea of the intensity of the blood circulation and of the metabolism depending on that circulation. This number changes within various species and within various regions of the brain. *Squalus* has fewer capillaries than *Hydrolagus*. The »vestibulo-latero cerebellar group of centres« is the region best supplied with capillaries, the hemispheres the worst especially the »general pallium« (C r a i g i e).

The blood drains the brain in Elasmobranchs in a different way from that in Teleost fishes, in which (*Salmo* — G r o d z i ń s ki) the branches of the longitudinal main arteries enter from beneath the neural tissue and from the center approach the surface of the brain by means of small branchlets and capillaries. Here the superficial veins collect the blood and carry it away beyond the brain-case.

Some of the superficial arteries often unite on the top of the brain with their fellows from the opposite side (*Prosencephalon — Torpedo, Raia, Scylliorhinus*). On account of this the blood of the left vessel may pass to the right side and the converse. It is difficult to assert from the anatomical observations whether it actually does passes. The top branches of *A. prosencephali dorsalis* in *Scylliorhinus* unite in a very characteristic manner and constitute a big trunk running across the hemispheres. Both blood currents, the right and the left; directed from the opposite ends of the arterial trunk, collide together and dilate sinus-shaped the walls of the vascular tube. The blood checked in its course and pressed from both ends of the arterial trunk escapes from it in the direction of the resultant acting forces, by means of branches more or less perpendicularly placed against the main vessel.

The veins collecting the blood from the brain spread over the surface, mostly below the arteries. The tree-shaped branches lead from the top and base of the brain to the sides, where they unite into bigger venal stems. The blood reaches the veins directly from capillaries distributed within the neural tissue. R e x however called attention to the fact, that in the forebrain of *Raja* the veins may possess deep roots (»tiefe Wurzeln«), which, like big trunks are deeply sunk within the pulp of the hemispheres. He connects this phenomenon with the lack of extensive chambers and with the thickness of the walls of the hemispheres. *V. lobi electrici* in *Torpedo* has even better-developed deep roots, probably on account of the great mass of neural tissue which is to be suppllied with blood. Thus in some cases in Elasmobranchs the blood enters the veins in deeper layers of tissue and not on the surface of the brain.

The superficial cerebral veins return the blood into two pairs of main veins, *V. cerebri ant.* and *post.*. In *Scylliorhinus* the blood from the forebrain, betweenbrain and the top of the midbrain and in *Mustelus* also from the anterior part of the cerebellum enters the *V. cerebri ant.* and reaches by this *Sinus orbitalis*. The remainder of the brain returns blood into *V. cerebri post.*

Direct communication between the branches of *V. cerebri ant.* and *post.* exists in young specimens of *Raja*. It is possible that part of the blood from the anterior regions of the brain actually flows to *V. cerebri post.* through this communicating branch. The probability of this assumption is favoured by the situation of the terminal section of *V. cerebri ant.*, which is not advantageous under the hydrodynamic aspect. The section mentioned carries away the blood from the skull and therefore should be placed in the middle of the area drained. Meanwhile in comparison with other Elasmobranchs it is considerably shifted forwards and is followed in this displacement by its tributaries. In older specimens the sections of *V. cerebri ant.* under consideration are situated in front of the hemispheres, which worsens still more the conditions for the flow of the blood. In these specimens, therefore, *V. cerebri post.* extends its tributaries upon the caudal surface of one of the hemispheres (R e x, H o f m a n n).

The adult specimens of *Torpedo* do not possess *Vv. cerebri ant.*, therefore all blood from the brain passes through the posterior cerebral vein.

The lumen of certain trunks has various diameters in various species. *V. prosencephali lateralis* in *Raja* is distinctly wider than in other species. Probably the blood flows more slowly in this vein in *Raja* than in other forms. *V. cerebri ant.* appears asymetrical in *Scylliorhinus*, the right usually exceeding the left in size. It is connected with the unpaired veins of this region of the brain, which reach either the left or the right *V. cerebri ant..* The bigger affluent of blood causes the differences in circulation in both veins.

Ramus ant. of the posterior cerebral vein passes along the lower edge of the *Cerebellum*. In forms provided with a poorly developed *Auricula cerebelli (Raja, Torpedo)* it remains a continuous trunk for the whole of its length. *Scylliorhinus, Mustelus* and *Squalus* have on the contrary a large *Auricula. Ramus anterior* passes from the front upon its meninges and breaks into a coarse venous network, from which the continuation of this vein rises at the opposite end, exhibiting again the appearance of a tubular trunk. The inclusion of the venous network in the course of the venous stem has a decisive influence upon the speed of the blood current. It is difficult however to say anything more accurate about the physiological significance of this arrangement of blood vessels.

V. spinalis dorsalis passes anteriorly into both *Ramus post.* of the posterior cerebral vein. *Squalus* lacks the spinal vein; one or two short veins extend however in this species from the spinal cord towards the brain and are engaged in the transmission of the blood in the same direction. Probably the blood from the anterior portion of *V. spinalis dorsalis* in other species flows also in that direction. In this area there are not sufficiently large metameric veins (*Vv. medullares*) to carry the blood away. On the other hand it seems to be highly improbable that *Ramus posterior* receives the blood leaving the brain-case along nerve X and directs it back on the spinal cord.

The topography of the brain and the distribution of the blood-vessels

Aa. encephalicae enter the brain-case in the region of the caudal border of the hypophysis in *Scylliorhinus* and *Mustelus*, beneath its middle in *Raja*, beneath the roots of nerve II in *Hydrolagus* and close to the anterior border of the hemispheres in *Torpedo*. The arterial apertures thus take various positions in the skull in relation to the corresponding sections of the brain and in a similar manner to other organs of the head. The spiracles of *Mustelus* and *Scylliorhinus* are situated at the level of the caudal border of the cerebellum, in *Raja* in the middle of its length, in *Torpedo* parallel with the midbrain. *Hydrolagus* lacks them entirely. The anterior border of the eyeball lies in *Scylliorhinus* within the region of the caudal part of the hemispheres, in *Raja* it reaches the anterior border of the hemispheres, in *Hydrolagus* and *Torpedo* it passes them by, significantly. The smell organ in *Scylliorhinus* and *Mustelus* rests upon the *Bulbi olfactorii* widely flattened and adherent to the hemispheres. In *Raja* it is deflected far to the side and in front of the hemispheres, in *Torpedo* and *Hydrolagus* an extensive olfactory nerve unites it with the hemispheres (Fig. 15, Oc, Sp, Or).

Scylliorhinus, Raia, Hydrolagus and *Torpedo* thus constitute a series of species in which the openings for the cerebral arteries, the spiracle, the eyeball and the smell organ are moved forwards in relation to the brain. It may be thought, that the brain has moved backwards in relation to the aforementioned organs. The changes in topography of these organs find some explanation in the powerful development of the neighbouring organs. The pectoral fins in *Raja* develop in the shape of a fan and together with the head they are able to move forwards the spiracle, the eyeball and the smell organ. The electric organ in *Torpedo* could do the same to a larger extent. The big eyes of *Hydrolagus* have probably influenced the piling up of the brain. Here the hemispheres lie at the bottom, and above them the midbrain, covered in its turn by the cerebellum. The eyeballs could also move the smell organ forwards. Provided the above suppositions are true, the topographical displacement of organs in *Hydrolagus* has resulted from other causes than the similar displacement in *Raja* and *Torpedo*.

The topographical relations between the brain and the organs alluded to above constitute a characteristic of the species. In many Elasmobranchs however, occur decided individual variations in the position of the brain, which render the whole problem more complicated. S t e r z i called attention to the fact that the brain in younger specimens tightly fills the cavity of the brain-case. In larger specimens the skull grows faster than the brain. The *Rhombencephalon* is held by the numerous nerves in its primitive position, consequently the anterior part of the brain has to move backwards. The *Bulbus olfactorius* remains however conjoined with the smell organ, and therefore a *Tractus olfactorius* of suitable length extends between the *Bulbus olfactorius* and the hemispheres. Nerves II, III, and IV, which in younger specimens run tranversely through the cerebral cavity, cross it obliquely from front to back (*Squalus acanthias* 35 and 100 cm in length — S t e r z i).

The disparity in size of the brain cavity and the brain, or what is practically the same, the displacement of the brain backwards, differs in various species. *Mustelus* exhibits in this regard almost no changes. In *Scylliorhinus* the displacement remains insignificant, in *Raja* and *Torpedo* it may attain remarkable dimensions in large specimens. During the great displacement the points of entry in the skull of nerves II, III and IV remain far in front, and *V. cerebri ant.* is also affected, which is clearly visible on comparing S t e r z i's drawing of *Raja clavata* (Fig. 73, specimen 62 cm in length) with mine of *Raja punctata* (Fig. 15 B, specimen 28 cm in length). *V. cerebri ant.* in the younger animal leaves the brain-case behind the anterior border of the hemispheres, in the larger animal far in front of the hemispheres in the neighbourhood of the *Bulbus olfactorius*. The point of entry in the skull has changed the position in relation to the hemispheres, retaining however its relation to the *Bulbus*.

Whether the point of entry of *A. encephalica* in *Raja* and *Torpedo* is influenced by the withdrawal of the brain or by the above discussed factors, can't be today decided.

The retraction of the brain backwards influences not only *V. cerebri ant.*, but also its tributaries upon the surface of the brain. In younger animals (*Raja*) *V. cerebri ant.* rests upon the caudal and lateral surface of the hemispheres. Here converge also its

various roots. *V. praefrontalis* (Fig. 9 Vpf) descends from the vault of the skull on to the centre of the top of the forebrain. Caudally the roots of both *Vv. cerebri ant.* attain the *Tectum opticum*, besides establishing distinct communication with the bran-
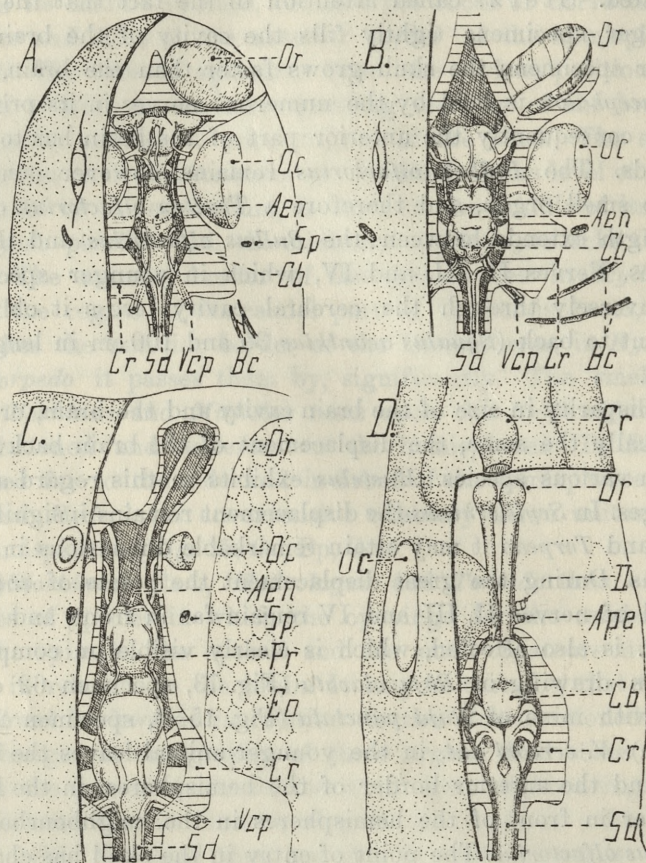

Fig. 15. Topography of some organs of the head. *A. Scylliorhinus, B. Raja, C. Torpedo, D. Hydrolagus*

ches of *V. cerebri post.* within the betweenbrain and midbrain. In older animals (Rex, Hofmann, Sterzi) the entire *V. cerebri ant.* is deflected from the hemispheres and lies in front of them. Anteriorly to the hemispheres converge all cerebral roots of this vein and also *V. praefrontalis.* The *Tectum opticum* is reached by only one vein, the remnant of the other drains the

hemispheres. *V. cerebri post.* in many specimens attains the hemispheres, predominantly the posterior and lateral sides of the left one. Here occurs a real migration of veins, which takes place in adult or adolescent animals, in any case long after the completion of the embryological development. The migration of *V. cerebri ant.* exhibits a passive character. The portion sticking to the skull at the point of entry remains unchanged. The brain moving backwards changes its relation to the superficial roots of this vein and loosens the junction with them. The further effect of this displacement of the brain is the loss of certain territories to the gain of *V. cerebri post.*.

General morphology ot the brain and distribution of the blood-vessels

The brains of the species of Elasmobranchs under consideration differ significantly from each other in general aspect and in the structure of their particular segments. The differences can be understood in numbers as shown in the accompanying table (p. 80). The absolute figures and the indices are of equal importance for our problems.

The great or small size of a given part of the brain influences the development of its own vessels and of the vessels of the neighbouring segments. The *Cerebellum* offers the stock example of this. The small *Cerebellum* of *Torpedo* lacks its own artery, arising directly from *A. cerebri post.*, and is supplied mainly by the branches of *A. mesencephali*. The *Cerebellum* of three other representatives of Elasmobranch fishes has its own *A. cerebelli lat.*, the dimensions of which increase according to the size of the *Cerebellum* from *Raja*, through *Scylliorhinus* to *Mustelus* (Fig. 4, 7, 11, 14).

In *Mustelus* the cerebellum significantly influences the vascular pattern of the adjacent parts of the brain. It covers the *Tectum opticum* and reaches the hemispheres with its anterior border. As a result of these conditions one of the veins of the forebrain reaches the top of the *Cerebellum* and a separate *V. tecti optici* is absent. When the *Cerebellum* is smaller (*Raja*), a powerful *V. tecti optici* develops; communication however with the veins of the forebrain does not exist.

The small *Cerebellum* in *Torpedo* leaves the extensive area of the *Rhombencephalon* uncovered. Here move the mighty *Vv. cerebri post.* close to the mid-dorsal line. The big *Cerebellum* of *Scylliorhinus* and *Mustelus* displaces these veins laterally and significantly shortens their free stem.

The *Rhombencephalon* in *Torpedo* exerts a similar or even greater influence upon the appearance of the blood-vessels. This segment of the brain, a centre among others of the electric organs, attains almost monstrous dimensions. It is supplied by the paired *Aa. cerebri post.* and its branches *Aa. rhombencephali ventr.*, which are entirely absent in *Scylliorhinus* and *Mustelus*, but which are found more weakly developed in *Raja*. Among its veins are *Vv. lobi electrici*, not occuring in other Elasmobranchs.

The *Rhombencephalon* of *Torpedo* decidedly exceeds in bulk any other remaining segments of the brain. These relations date from the earliest stages of development. K u p f e r draws a longitudinal section through the brain of *Torpedo ocellata* (Fig. 102), 18 mm in length. The *Rhombencephalon* is distinguished in relation to other segments of the brain by its large dimensions *(Lobi electrici)*. Beneath the base a very big longitudinal artery appears. From the beginning the blood circulation of the entire brain remains under the predominant influence of the mass of the *Rhombencephalon*. As a result, under the influence of some other factors, e. g. the displacement towards the front of the point of entry into the brain-case of *A. carotis int.*, *A. spinalis ventralis* carries the blood to the paired *Aa. cerebri post.* and *Aa. cerebri ant.. A. encephalica* probably does not supply the brain at all.

Similarly the dimensions of the *Rhombencephalon* of *Torpedo* affect the veins. In the embryos exist at the beginning two pairs of cerebral veins, the anterior and posterior (G e l d e r e n). In adults only the posterior remain. The latter develop better in relation to the predominant *Rhombencephalon*. It is enough to remember the existence of the *Vv. lobi electrici* and *Vv. rhombencephali ventrales*, which constitute their tributaries. *Vv. cerebri post.* in *Torpedo* are probably strong vessels from the very beginning and communicate directly with *Vv. cerebri ant.*, as is the case in young specimens of *Raja*. They carry the blood better and more easily than the anteriors, therefore they direct their

blood backwards, which results in the gradual disappearance of those parts of *V. cerebri ant.*, which leave the brain-case.

The dimensions of *Plexus chorioideus v.* III are correlated with the size of the *Diencephalon* and of the lateral chamber or rather the plexuses of the latter. *Scylliorhinus*, with a long, uncovered betweenbrain and with extensive lateral chambers possesses the best developed and the most complex vascular plexus. In *Raja* the plexus is far worse developed and it is absent in *Torpedo*, because the midbrain lies above and covers the betweenbrain and the lateral chambers are vestigial.

The indices for the particular segments of the brain are computed as a relation of their length to their width. The magnitude of the index approaches the unit, provided both dimensions differ slightly, otherwise the outline of a given segment of the brain resembles more or less a circle (*Torpedo* - forebrain 0·93, *Cerebellum* 0·91, *Raja* - *Cerebellum* 1·05). The index significantly higher than the unit indicates that the length exceeds the width (*Mustelus-Cerebellum* 1·35, *Scylliorhinus-Rhombencephalon* 1·58). The broad segments of the brain possess an index lower than the unit (*Raja*- forebrain 0·56). The index for the whole brain was calculated as the relation of the length of the entire brain to the width of its midbrain; this segment of the brain exhibits the smallest differences in the absolute width among the fishes under discussion. The brain of *Scylliorhinus* with the index 5·76 distinctly exceeds in slenderness the brains of other Elasmobranchs.

The vascular pattern is adjusted to the shape of a given segment of the brain; it has a different appearance in the elongated cerebellum of *Mustelus* and in the rounded of *Torpedo*. The vessels, both arteries and veins (Fig. 12), which are distributed upon the dorsal surface of the *Cerebellum* in *Torpedo* (0·91), acquire a more or less radial arrangement. A similar rounded outline occurs in the *Cerebellum* of *Raja* (1·05). Taking however into consideration that the *Auriculae cerebelli* possess a vascular supply independent of the *Corpus*, the latter is cuneiformly elongated caudalwards. Upon this wedge the longitudinal vein *V. cerebelli post.* appears (Fig. 9) with is mouth turned backwards. Elliptical in outline (1·14) the *Corpus cerebelli* in *Scylliorhinus* (Fig. 2) possesses, besides the radial vessels, distinct longitudinal trunks, in its anterior

portion the artery and in the posterior the vein. In the more slender spindle-shaped (1·35) *Corpus cerebelli* in *Mustelus* (Fig. 5) the longitudinal vessels in the mid-dorsal line acquire their most

Measurements and indices of the brain

sections of the brain	dimen- sions	Mustelus	Scyllio- rhinus	Raja	Torpedo
the entire brain [1])	length	28— mm	37·5 mm	26·5 mm	23— mm
	width	8·5 mm	6·5 mm	7·5 mm	7·5 mm
	index	3·29	5·76	3·53	3·06
forebrain [2])	length	10 mm	10 mm	8·5 mm	7·5 mm
	width	12 mm	14 mm	15— mm	8— mm
	index	0·83	0·71	0·56	0·93
betweenbrain	length		6·5 mm	2·5 mm	
	width		10— mm	5— mm	
	index		0·65	0·5	
midbrain	length	8— mm	7— mm	6— mm	5·5 mm
	width	8·5 mm	6·5 mm	7·5 mm	7·5 mm
	index	0·94	1·07	0·8	0·72
cerebellum	length	13·5 mm	12— mm	9— mm	5·5 mm
	width	10— mm	10·5 mm	8·5 mm	6— mm
	index	1·35	1·14	1·05	0·91
rhombencephalon	lenght	11 mm	13·5 mm	10·5 mm	10 mm
	width	9 mm	8·5 mm	8— mm	8 mm
	index	1·22	1·58	1·31	1·25

[1]) The entire brain. The length was measured from the anterior border of the hemispheres to the caudal border of the nerve X. Index: relation of the length of the brain to the width of the midbrain.

[2]) The particular segments of the brain. In the measurements of the forebrain the *Bulbi olfactorii* were omitted. Index: relation of the length to the width of the particular segment.

distinct appearance. There are two veins, one anterior and one posterior, and a series of short arterial branches which follow each other the whole length of the cerebellum and thereby give the illusion of a continuous trunk. The index of the *Cerebellum* in *Squalus acanthias* computed from Sterzi's drawing attains 1·67; it exceeds thus the index even of *Mustelus*. In relation with this both arteries and veins assume a longitudinal position in the mid-dorsal line of the *Cerebellum*.

The forebrain of *Scylliorhinus* (0·71) and *Raja* (0·56) is distinguished by its stricking width. In both cases *Aa. prosencephali dors.* distribute at least a part of their branches transversally to the long axis of the brain and unite with each other in the mid-dorsal line. In *Scylliorhinus* they even form a continuous trunk, crossing both hemispheres transversally.

The elongated *Rhombencephalon* with the index 1·22—1·58 is supplied with several longitudinal trunks. In all species occurs especially *A. basialis* or *Aa. cerebri post.* not united in one trunk. In addition *Vv. rhombencephali ventr.* exist in various stages of development, the weakest in *Mustelus* (1·22), having the lowest index. In the most slender *Rhombencephalon* in *Scylliorhinus* (1·58), *V. spinalis ventr.* is prolonged from the spinal cord to nerve VI. On the dorsal surface of that segment of the brain *V. cerebri post.* also forms longitudinal stems.

On the top of the midbrain having the index about the same as the unit or somewhat less (0·72—1·07) the terminal sections of arteries are arranged radially. The veins also converge radially into the centrally placed *V. tecti optici*, which conforms with the principle stated above. Only in *Mustelus* different conditions prevail, depending on the size of the *Cerebellum*, which covers the greater part of the midbrain from above.

The veins even in the widest forebrain do not form transverse stems, as occurs in the case of arteries, which can however be explained by some specific local conditions. *V. prosencephali lateralis* in *Raja* (0·56) gives off branches situated transversely to the length of the brain. They do not unite with their fellows from the opposite side, because *V. prosencephali lat.* itself occupies a position in the middle of the hemisphere and not on its lateral border, as do the arteries in *Scylliorhinus*. Second in the sequence of the width, the prosencephalon of *Scylliorhinus* (0.71)

exhibits the specific venous pattern on the top of the forebrain. The unpaired *V. prosencephali media* supplies the medial parts of the surface of the hemispheres and leaves no free space where a transverse venous trunk could be formed.

Summary

The arrangement of the arteries and veins in the brain of *Scylliorhinus*, *Mustelus*, *Raja* and *Torpedo* was investigated.

The distribution of vessels on the surface of the brain was described and graphically represented; previously existing observations were confirmed and in many cases supplemented.

The comparative anatomical value of the more important vessels in hitherto investigated species was discussed, taking into consideration the topographical, developmental and mechanico-developmental conditions.

An attempt was made to settle on the base of anatomical data the ways of supplying the brain with blood. Fundamental differences appeared in this field between *Rajiformes* and other Elasmobranchs.

Analysis of the topography of the organs in the head established that in the species discussed the brain in the brain-case takes a different position in relation to the point of entry of *A. encephalica* and *V. cerebri anterior*, of the spiracle, of the eyeball and of the smell organ. It depends partly on the species, partly on the age of the specimen. The distribution and appearance of the blood-vessels are adjusted to the shape and size of a particular segment of the brain. The segments rounded in shape or with the index close to the unit exhibit a more or less radial arrangement of vessels. In segments of an elongated shape, or with the index significantly exceeding the unit, appear distinct longitudinal arterial and venous stems. In broad segments, having the index lower than the unit, the arteries form more or less distinct transversal trunks.

The absolute volume of a given segment of the brain decides the size of the supplying vessels. The strong or weak development of a particular segment of the brain influences the vascular pattern in adjacent segments. In the first case it controls their circulation, in the second it favours the good development of existing or the formation of new vessels.

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Explanation of figures

The figures are drawn from preparations viewed under the binocular
microscope.

Arteries are cross striped, veins dotted.

Each figure is provided with the scale in order to show the enlarge-
ment.

Abbreviations for all figures

Aa	—Arteria	auditiva	Eo	— Electric organ	
Ab	— „	basialis	Fr	— Frontal clasper	
Abe	— „	branchialis eff.	H	— Hypophysis	
Abo	— „	bulbi olfactorii	Hy	—Hyoid bar	
Aca	— „	cerebri anterior	Li	— Lobus inferior	
Ace	— „	cerebelli lateralis	Md	— Mandible	
Acr	— „	optica (centralis retinae)	Ms	— Mesencephalon	
Aci	— „	carotis interna	Na	— Nares	
Acp	— „	cerebri posterior	Ns	— Nervus spinalis	
Adi	— „	diencephali	Nt	— „ terminalis	
Aen	— „	encephalica	Oc	— Eye	
Aep	— „	epibranchialis	Or	— Smell organ	
Ahe	— „	hyoidea efferens	Pld	— Plexus chorioideus ventr. III	
Al	— Aorta lateralis		Plr	— „ „ „ IV	
Ali	— Arteris lobi inferioris		Pq	— Palatoquadratum	
Am	— Aorta medialis		Pr	— Prosencephalon	
Amd	— Arteria medullaris		Ra	— Ramus anterior	
Ame	— „	mesencephali	Rb	— Rhombencephalon	
An	— „	nasalis	Rp	— Ramus posterior	
Ao	— „	olfactoria	Sd	— Spina dorsalis	
Aom	— „	ophtalmica magna	Sp	— Spiraculum	
Aor	— „	orbitatis	Sv	— Saccus vasculosus	
Apa	— „	pseudobranchialis affer.	Vau	— Vena auriculae	
Apb	— „	prosencephali basilaris	Vbo	— „ bulbi olfactorii	
Apd	— „	„ dorsalis	Vca	— „ cerebri anterior	
Ape	— „	pseudobranchialis effer.	Vcbl	— „ cerebelli lateralis	
Apl	— „	prosencephali lateralis	Vcbp	— „ „ posterior	
Arb	— „	rhombencephali „	Vch	— „ chorioidea	
Arbv	— „	„ ventralis	Vcp	— „ cerebri posterior	
Ar	— „	rostralis	Vdd	— „ chorioidea	
As	— „	subclavia	Vle	— „ lobi electrici	
Asl	— „	spinalis lateralis	Vmd	— „ medullaris	
Asv	— „	„ ventralis	Vpf	— „ praefrontalis	
Atc	— „	tecto-cerebellaris	Vpd	— „ prosencephali dorsalis	
Av	— „	vertebralis	Vpm	— „ „ media	
Au	— Auricula cerebelli		Vpv	— „ „ ventralis	
Bc	— Branchial clefts		Vrv	— „ rhombencephali „	
Br	— „ bars		Vsd	— „ spinalis dorsalis	
Cb	— Cerebellum		Vsl	— „ „ lateralis	
Cr	— Cranium		Vsv	— „ „ ventralis	
De	— Dentes		Vtc	— „ tecto-cerebellaris	
Di	— Diencephalon		Vtl	— „ tecti lateralis	
E	— Pineal organ		Vto	— „ „ optici (media)	

Wpływ wzrastającgo ciśnienia osmotycznego na kule żółtka białego z jaja kury. — *Influence of the increase in the osmotic pressure upon the white yolk spheres of the hen's egg.*

Mémoire

de M. *Z. GRODZIŃSKI* m. c.

présenté le 17 juin 1946

(Plate 1)

Problem and method

The yolk of the hen's egg is an emulsion of the O/W type. The dispersed phase consists of fine droplets of fat (about $1\,\mu$ in diameter) and much larger yolk spheres. Each yolk sphere is a vesicle with a wall built of a semi-permeable membrane. The vesicle contains the colloidal protein fluid and the drops of fat floating in it. When the drops of fat appear in great quantity, possess small dimensions and yellow colour (xantophil), we have to do with the spheres of yellow yolk (Fig. 2, pl. 1). The vesicles without colouring matter (Fig. 1, pl. 1), with one or a small number of the large drops of fat belong to the white yolk (Grodziński 1938).

During my researches on the digestion of the hen's yolk (Grodziński 1946) I paid attention to the turbidness of the yolk spheres and in connection with this to the optical disappearance of their drops of fat. This phenomenon has found an explanation in the osmotic processes. When a crystal of sodium chloride (NaCl) is added to the yolk suspended in a drop of some isotonic fluid upon the coverslip, we find after a short time that the white yolk spheres become turbid and opaque. At the same time their dia-

meter diminishes. Owing to the dissolving of the sodium chloride
the osmotic pressure has increased in the drop of fluid and caused
the dehydration of the yolk sphere.

These observations, made occasionally when dealing with the
problem of the digestion of the yolk, require further development
along several lines. The main task is to settle the successions of
the transformations of the sphere and their quantitative definition.
After the addition of a crystal of sodium chloride to a drop of
yolk, the osmotic pressure increases rapidly. The yolk sphere pas-
ses through all transformations in few minutes, driven in various
directions by the currents of diffusion. It renders observation and
measurement very difficult. When the drop of isotonic fluid
with the yolk evaporates, the osmotic pressure increases slowly
and everywhere uniformly. It is possible to condense by evapo-
ration the salts in the solution till they are converted into cry-
stals; this means that the yolk spheres can be completely depri-
ved of water. The whole process lasts a long time, on the average
one hour (62·6 minutes) and takes place relatively quietly, thus
rendering it possible to photograph the selected object at any
moment.

In our investigations we used as previously (1938, 1946) the
Tyrode's solution to dilute the yolk in the proportion 100 : 1.
For each experiment the suspension was newly made and the yolk
was obtained from a freshly laid egg. It turned out that the
yolk suspension reacted differently after 2—3 days than when
freshly made and that the yolk of a state egg (2—3 weeks)
also yielded other results. The drops of the yolk suspension were
placed on the coverslip. The latter was sealed, at the four cor-
ners, to the corresponding scaffold situated on the glasslide, with
the preparation facing down. In this way there were clefts bet-
ween the edge of the coverslip and the glas-slide. The air had
free access to the drop and changed constantly in its neighbour-
hood. Thus the yolk solution evaporated steadily.

In order to accelerate this process the air was sucked by
means of a water pump from beneath the coverslip or else the
preparations were kept at a temperature of $+ 35^0$. Transfor-
mations of the white yolk being in both cases similar, the heat-
ing only, by means of the electric table by Eisenberg (manufac-
tured by Leitz), was used during further investigations. The pic-

tures were taken with the aid of Miflex (Zeiss), using the standard microphotographic camera or Contax (Zeiss).

An effort was done to obtain a series of pictures to illustrate the whole transformation of one and the same yolk sphere. We got 22 series of pictures representing the fate of a white yolk sphere containing just one drop of the fat. But some of them are not complete, in others the yolk spheres underwent such malformation that they were not fit for measurement. In all 163 pictures were taken.

Observations

When the osmotic pressure of the fluid containing the yolk spheres increases, several changes in the latter are visible, which follow each other or increase in strength during the whole time of the experiment. The changes are concerned with the position of the drop of fat within the sphere, with the dimensions of the yolk spheres and with their optical properties.

The first change to be seen within the sphere of the white yolk, is the displacement of the internal drop of fat. This drop moves slowly from a central position toward the periphery and finally stops close to the surface of the sphere (Fig. 4 a, b, 9 a, b, 8, pl. 1). Sometimes it seems to touch the surface membrane directly. In any case it remains in this position till the end of the experiment, in spite of the eventual currents pushing the yolk sphere rapidly in the diluting fluid.

From the very beginning of the experiment till the final stage the circumference of the sphere diminishes visibly. It is very easy to trace these changes and to measure them on the pictures. In the accompanying table 10 cases, selected from 22 series of experiments, are enumerated. The volume of every sphere is calculated at various stages of the dessication of the drop placed upon the coverslip. The volume of the drop of fat contained within the yolk sphere, remains unchangeable, regardless of the osmotic pressure of the medium. The difference between the circumference of the whole sphere and the circumference of the drop of fat corresponds to the volume of the colloidal protein component of the yolk sphere. In these figures the volume of the semi-permeable membrane is also included, as we failed to measure it.

The volume of the colloidal protein component steadily diminishes. In the final anhydrous stage, when all salts contained in the fluid are converted into crystals, the volume drops on an average to 26% of the initial circumference. From our observations results, that the range of fluctuation extends from 12·7—39·3% of the initial volume. In any case the loss is significant and should be ascribed to the diffusion of water through the semi-permeable membrane from the yolk sphere in the medium, which becomes more and more hypertonic.

Parallel with the reduction in size, the optical properties of the yolk sphere also change. In the initial stage of the experiment the sphere is transparent; the drop of fat contained within it is clear visible because of the different refraction of light. The sphere becomes gradually turbid. The turbidness is uniform, without any traces of granules or flocculations. In such a yolk sphere the outlines of the drop of fat become less and less distinct, finally the whole becomes opaque and takes a uniform white colour. No traces of the drop of fat are visible in the sphere (Fig. 4 b, c, 9 c, pl. 1).

The uniformly white mass of the yolk sphere subsequently exhibits grey spots, which correspond optically to the coarse granules now being formed. They spread in a fine layer above the drop of fat, in other places they form thicker accumulations. Suitably focusing the objective, we can get a picture of the yolk sphere more or less smooth in the centre, with a coarsely granulated marginal ring. The outlines of the whole sphere however remain sharp and even (Fig. 4 d, 9 d, e, pl. 1).

One can distinguish several stages in the behaviour of the white yolk sphere. No distinct boundaries exist between them, but every stage exhibits distinct and peculiar features. The first stage (a), the initial stage is the transparent yolk sphere containing one drop of fat. In all subsequent stages the volume of the sphere diminishes. Simultaneously in the second stage (b) the drop of fat moves toward the periphery. The third stage (c) is characterised by the turbidness of the colloidal protein fluid within the yolk sphere and the optical disappearance of the drop of fat. In the fourth and fifth stage (d, e) the liquid protein component of the yolk sphere is converted into a granular mass.

Interpretation and discussion

The previously described transformations of the spheres of the white yolk find an easy explanation after having made the two assumptions. Firstly: in the evaporating yolk suspension and the Tyrode fluid the salts grow thicker until they become crystals; this means that the osmotic pressure increases more and more. Secondly: each yolk sphere is a vesicle built out of semipermeable membrane and filled with colloidal protein fluid containing one drop of the fat. By the nature of their structure, the spheres of the yolk play the part of an osmometer.

When the yolk sphere is placed in a fluid, which osmotic pressure increases steadily, the osmotic free water, i. e. water not connected with the protein molecules penetrates through the semipermeable membrane on the outside. If the water were diffused uniformly through the whole surface of the yolk sphere, the internal drop of fat would remain in a central position. The drop of fat however always moves towards the surface of the yolk sphere and remains definitely in this position. Probably the diffusion occurs more intensively here than elsewhere. The resulting currents in the protein fluid steer the drop of fat to the regions where the diffusion occurs more intensively than elsewhere. The wall of the sphere of the white yolk exhibits than different qualities as a semi-permeable membrane in different portions. When the displacement of the drop of fat within the sphere occurs, the volume of the protein components in the sphere drops on an average to $73\cdot4^0/_0$ (the limits of fluctuations are $59\cdot8 — 83\cdot3^0/_0$).

The displacement of the drop of fat may occur also from other causes. When in an isotonic fluid the currents shift the yolk sphere in any direction, the drop of fat will in consequence of inertness move to the side opposite to the direction of the current. When the sphere ceases to move, the drop of fat slowly regains its previous central position. In our experiments the sphere moved very slowly, the drop of fat was displaced toward the surface regardless of the direction of the movement and remained there definitely.

Further loss of osmotic free water induces such a condensation of the protein molecules within the yolk sphere that the light rays do not penetrate through it directly to the eye, but dispersed in the colloidal suspension give the illusion of the white colour. This phenomenon occurs when the volume of a protein

Number of experiment	Subject	stage	volume in microns					volume in percentages					Duration in minutes
			a.	b.	c.	d.	e.	a.	b.	c.	d.	e.	
7	whole sphere		8378	6313	6108	4288	3192	—	72·4	62·1	45·4	36·8	60
	protein comp.		7480	5416	5210	3390	2294	100	69·9	59·1	50·7	20	
9	whole sphere		7182	6108	5144	3192	?	—	83·3	68·6	37·5	?	23
	protein comp.		6418	5344	4380	2428	?	100					
10	whole sphere		9022	7182	6108	5144	?	—	76·7	62·1	49·2	?	55
	protein comp.		7628	5788	4714	3750	?	100					
11	whole sphere		7764	6630	5144	4814	2872	—	82·6	59·1	50·7	20	100
	protein comp.		6370	5236	3750	3420	1478	100					
12	whole sphere		1810	1212	1048	764	764	—	62·5	52	34	34	50
	protein comp.		1584	986	822	538	538	100					
16	whole sphere		1212	764	764	360	278	—	59·8	59·8	23·1	15·7	76
	protein comp.		1108	660	660	256	174	100					
17	whole sphere		3534	2516	1810	1048	764	—	68·9	47·1	23·3	14·9	102
	protein comp.		3256	2238	1532	770	486	100					
18	whole sphere		2306	?	1592	1394	536	—	?	64·9	50·2	12·7	55
	protein comp.		2026	?	1314	1016	2·8	100					
19	whole sphere		3534	2872	1810	1394	1394	—	80	48	34·9	34·9	50
	protein comp.		3308	2646	1584	1168	1168	100					
22	whole sphere		1810	1394	1048	898	816	—	74·6	53·4	44·4	39·3	55
	protein comp.		1636	1220	874	724	642	100					
	on an average							100	73·4	58·5	39·3	26	
	amount of water in the white yolk sphere							100	47·4	32·5	13·3	62·6	

component diminishes in the yolk sphere on an average to $58 \cdot 5\%$ (the limits of fluctuations are $48—69 \cdot 9\%$). In this stage the content of the yolk sphere is still liquid. One can see namely the white yolk spheres deformed under the pressure of other spheres, preserving however their continuous outlines and smooth surface. Released by chance from the compulsory position they acquire their previous spherical shape.

The liquid although condensed content of the yolk sphere loses a further amount of water. The protein molecules unite into granules, well distinguishable under the microscope. This proces is visible when the initial amount of the protein content of the yolk sphere drops on an average to $39 \cdot 3\%$ (limits of fluctuations $23 \cdot 1—50 \cdot 7\%$). The formation of granules continues till the complete dehydration of the sphere, which happens at the moment when the liquid medium evaporates and its salts become crystals. The volume of the protein component of the sphere then drops on an average to 26% of the initial state (limits of fluctuations $12 \cdot 7—39 \cdot 3\%$).

Simultaneoulsy with the precipitation of protein content in the form of granules, the colloidal liquid suspension turns into a more solid and rigid mass. Scars on the surface of the spheres confirm this event. The small drops of fat floating in the medium often stick to the yolk sphere. When by accident the current of the medium tears such a drop of fat from the sphere, a scar results in the form of a hollow with sharp rims (Fig. 6, pl. 1). Figure 3. represents a somewhat different case. One yolk sphere was pressed against two others and stuck to them. The strong current of the liquid medium tore the sphere from that position and put it aside. The traces of contact remain however in the form of two straight sharp edges. The shape of the sphere, so defaced is remained without change, neither did the sharp outlines soften; the content of the sphere had no more the character of a semi-liquid mass.

The figures presented above are far from being exact, for many reasons. Above all they are based on only 10 cases, photographically registered. Secondly there is the subjectivity of observations. The picture were taken at the moment, in which the state of a given sphere of yolk was estimated as the phase »b, c, d« by the observer. But every phase persisted for a few

or for several minutes; the picture taken at the beginning of the phase registers a larger yolk sphere than that taken in the middle or at the end. The observer cannot establish the moment representing the optimum of a given phase. Finally the figures also contain the volume of the membrane of the yolk sphere. It was impossible to determine the mass of the membrane in any phase of the experiment.

The figures concerning the volume, although accepted with some reserves to their exactness, may serve as general indicators in the problems interesting us, e. g. how much water the white yolk sphere contains and how the properties of the yolk sphere change in relation to the loss of smaller or larger amounts of water. The osmotic free water forms about 74% of the protein component of the white yolk sphere, because such are the losses of its volume in the final stage of the experiment. S p o h n and R i d d l e determine the amount of water in the white yolk at $86 \cdot 66\%$. The difference probably results from the method of measurement and may also be connected with the material used for the investigation. The authors used eggs of the white Wyandotte and Plymouth Rock, we of the Zielononóżki.

The quantity of water contained in the different tissues of man varies within large limits. The nervous tissue possesses 70% in gray matter, $83 \cdot 5\%$ in the white (P a r n a s), the muscles $72—79\%$ (O s t e r n), the erythrocytes 54% (S ł a w i ń s k i), the bones 13% (in atlas — P a r n a s). The white yolk takes in this regard an intermediate position between the blood corpuscles and the gray matter of the brain and equals the muscles.

The loss of half ($52 \cdot 6\%$) of the osmotic free water does not change the optical properties of the proteins in the yolk sphere (stage b). It induces of course the condensation of the colloidal protein suspension and that is probably one of the reasons, why the drops of fat do not return from under the surface into their former position towards the centre. The further dehydration to $32 \cdot 5\%$ (the loss of about $^2/_3$ of the water) is demonstrated by the complete turbidness of the protein component of the sphere (stage »c«). The precipitation of the colloidal proteins in the form of granules starts after the loss of $80—90\%$ of water (stage »d«). Simultaneously its consistency changes from a liquid and viscous into a more rigid and fragile one.

The changes in the osmotic pressure influence to some extent the semi-permeable membrane, which forms the wall of the yolk sphere. In the isotonic fluid two yolk spheres brought into contact with each other, even for the longer period of time, do not fuse together but move freely apart. The middle and large drops of fat floating within the dilutor of the yolk, the Tyrode's fluid, behave similarly. They do not stick to the spheres when brought into contact with them.

At the stage when the sphere loses more than half of the osmotic free water, i. e. when the turbidness is fairly well advanced, the yolk spheres very easily stick to each other or to a drop of fat. The qualities of the semi-permeable membrane have changed, the membrane has become sticky. The same holds true of the surface of the drop of fat, because one can see in the same samples (Fig. 7, pl. 1) two or more drops adhering to each other.

The myelin forms furnish some explanation of the changes occurring in the semi-permeable membrane, forming the surface of the yolk sphere. The membrane develops fine filaments of different lengths, which rotate, elongate or shorten. Finally they are torn off from the spheres or dissolve again in their membrane. When the osmotic pressure increases slowly, the myelin filaments appear comparatively seldom. During these investigations I have seen them just tree times (Fig. 5, pl. 1). But when the osmotic pressure increases rapidly in the yolk suspension, owing to the dissolving of a crystal of sodium chloride, the myelin filaments are present in every sample, in many of them abundantly.

Leathes believes that the myelin forms develop from phospha tides in water because of the hydrophilic qualities of the lecithin. The molecules become hydrated, which lowers the interfacial tension between them and the water and produces the filaments. This interpretation may be applied in our case, because the lecithin and cholesterin are probably present in the semi-permeable membrane (Hamburger, Marza & Marza). In the isotonic suspension the molecules of water either do not pass at all or pass only in a limited number through the membrane of the yolk sphere. When the osmotic pressure of the solvent increases, the water of yolk sphere penetrates its membrane and may hydrate the molecules of lecithin. In the case of a rapid increase of the osmotic pressure, the diffusion intensifies. The current of the

fluid causes an unequal attack upon the membrane. Local dehydration results, which may cause the change in the surface tension and in the viscosity of the membrane. In consequence convexities and concavities result, which are known as myelin forms. It is also possible, that an inversion of phases occurs within the membrane, as a result of the local dehydration. This may lead to the production of myelin forms.

The sphere of white yolk is a sensitive osmometer similar to the red blood corpuscle. The yolk spheres remain in the hypertonic fluid as spherical units, only their diameter diminishes. The surface of the erythrocytes in man shrinks and becomes covered with prickles. The structure of both bodies induces these differences of reaction. The erythrocytes possess an internal skeleton resting against the cell membrane. The skeleton and membrane consist of proteins and phosphatides insoluble in water. The colloidal suspension of proteins, mainly hemoglobin, constitutes the content of the red blood corpuscles. In the hypertonic solution the volume of the colloidal proteins diminishes notably, the skeleton on the contrary maintains the initial dimensions. When the erythrocytes shrink, the points of the skeleton protrude on their surfaces as prickles (Hamburger, Bürker). The yolk spheres are without an internal skeleton, so when contracting they maintain their regular, spherical shape.

Summary

The increase of osmotic pressure induces various changes in the sphere of the white yolk and reveals some of its properties.

The semi-permeable membrane forming the wall of the sphere allows the osmotically free water to pass, when placed in a hypertonic solution. Some regions of the membrane show better diffusing properties than others. During the diffusion of water the membrane becomes more viscous, which is demonstrated by the formation of myelin filaments and by the adhesion of two spheres to each other when brought into contact.

The drop of fat contained within the yolk sphere exhibits no changes in optical properties or size.

The colloidal suspension of proteins placed in the yolk sphere loses its osmotically free water parallel with the increase of the

osmotic pressure in the medium. It induces the condensation of protein molecules, which becomes optically demonstrated by turbidness. Further dehydration precipitates the proteins, and the colloidal fluid turns into a granulous and stiff mass.

The sphere of the white yolk contains about 74% of water. The sphere, losing it by diffusion, diminishes in size, preserving however a spherical shape.

Kraków. Institute of Comparative Anatomy in the Jagellonian University of Cracow. II. 1946.

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Description of plate 1

Eulargements of all pictures × 500. Figures in square brackets following the description of every picture indicate the numbers of the photographic records.

1. Spheres of the white yolk. a — containing one drop of fat, b — and c — containing more than one drop. (125, 193, 201).

2. A sphere of yellow yolk containing a great amount of droplets of fat (297).

3. The loss of about of 60% of water causes the precipitation of protein colloids and turns them stiff. a — a yolk sphere pressed against two bigger ones. b — the same sphere torn off and defaced (330, 331).

4. The changes of the yolk sphere induced by dehydration. a — initial stage, b — the drop of fat moves toward the surface, c — the sphere becomes entirely turbid, d — precipitation of the colloid component occurs (119, 120, 121, 123).

5. Myelin filament protrudes from the surface (164).

6. The surface of the turbid yolk sphere (because of dehydration), exhibits three scars in the shape of hollows. Drops of fat stuck in these places. They have subsequently been removed by the current of the medium (384).

7. The surface of the components of the yolk becomes sticky. a — initial stage. b — two fat drops stick to each other and to the yolk sphere. c — further dehydration reduces the size of the sphere and precipitates the protein content, the drops of fat remain unchanged (343, 346, 347).

8. Moving of the drop of fat from the centre of the sphere (a) towards its (b) surface (145, 146).

9. Similar experiment as in 4. e — picture was taken focusing the surface of the sphere in order to show granules present everywhere (387, 390, 391, 393, 394).
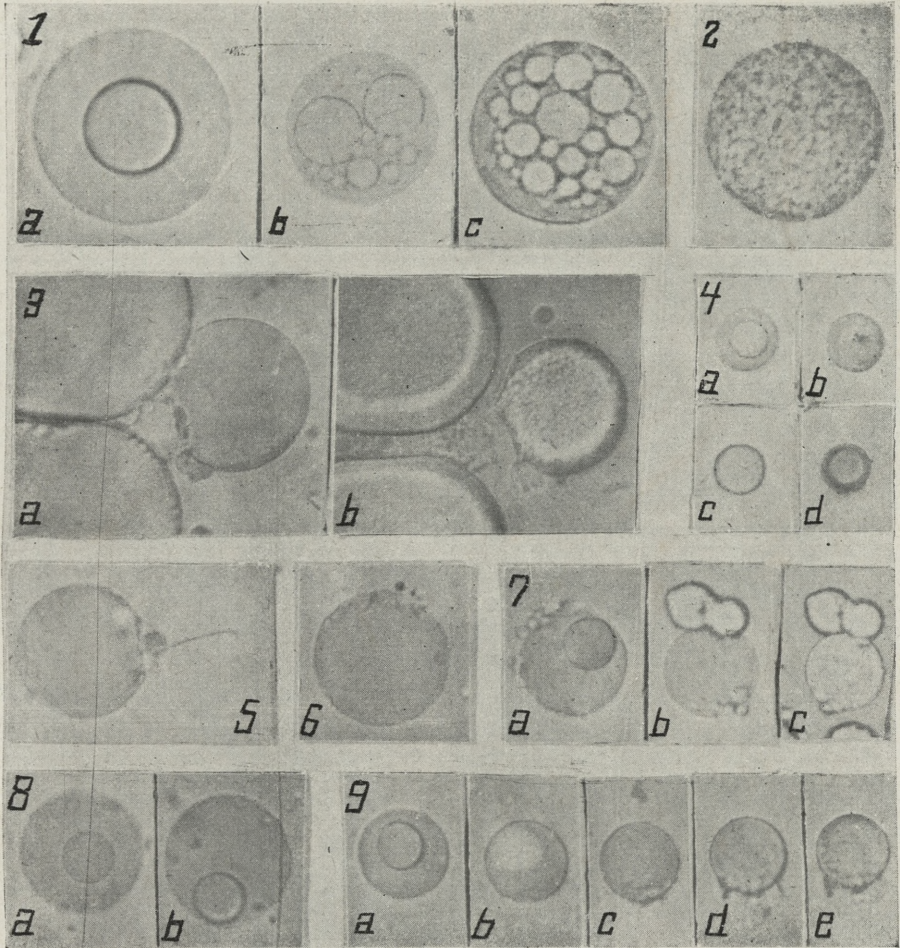
Z. Grodziński

*Badania nad zdolnością orientacji i szybkością lotu pta-
ków. VI. Doświadczenia nad wróblami (Passer arboreus
Bewick i Passer domesticus L.). — Homing experiments
on birds. VI. Investigations on the tree and house spar-
rows (Passer arboreus Bewick and P. domesticus L.)* [1]

Note

de M. **R. J. WOJTUSIAK,** Mᵐᵉ **H. WOJTUSIAK** et M. **B. FERENS,**

présentée le 15 Juin 1945 par M. H. Hoyer m. t.

All research-work on the homing and orientation in space of
birds referred so far to the migratory species such as: swallows,
storks, starlinks, wrynecks, noddy and sooty terns *(Anous stolidus
and Sterna fuliginosa)* etc. (Nearer literature see: W o j t u s i a k
R. J. & F e r e n s B. 1938). Among the »sedentary« birds resp.
those, which do not perform longer migrations, the only species
we had some information about was the gos-hawk *(Accipiter gen-
tilis)*, which according to R ü p p e l l (1937) shows suitable ho-
ming impulse. But whether its orientation in space is developed,
as strongly as with the migratory birds, the question remains so
far unsettled.

In summer 1939 we began experiments on the homing ability
of sparrows, which lead, as it is known, a settled way of life.
In our investigations we wanted to get evidence whether: 1. the
birds show homing impulse after being exported to definite
distances from their nests, 2. what is their ability of orien-
tation compared to that of the migratory birds. Besides this, two
problems were of special interest to us: the kind of their orien-

7*

tation in space and the degree of its development. Taking for basis the results obtained in our experiments on the swallows we assumed as a »field-work hypothesis« the existence of two kinds of orientation: 1. a visual-memory for distances to about 100—150 km, 2. orientation by means of a »direction-sense« or any other special »orientation-sense« unknown to us so far, for much greater distances. The question was whether the sparrows being »settled« birds, show both types of orientation or only the first.

From this largely conceived plan we could perform but the preliminary experiments in the beginning of summer 1939. The war stopped our further studies on the subject, so that we give here the obtained results only in form of a provisory note which may serve as a substratum for further investigations.

Two species have been used for the experiments: the t r e e s p a r r o w (*Paser arboreus* Bewick) and the h o u s e s p a r r o w (*P. domesticus L.*). The sparrows were caught at their nestings-botes in the Botanical Garden of the Jagellonian University, Cracow[1]). To catch them, we used with great success an instrument constructed by B. F e r e n s, which was composed of a long stick at the end of which was fastened a ring of wire. The ring had a sleeve of transparent stuff sawn to it, 6 m. in length and made out of marquisette used for ladies blouses. The two sides of the sleeve were open. We pressed the ring to the nestings-box. The bird on flying out was falling into the sleeve and gliding down the soft tube into our hands (fig. 1). Caught in this way, after being annealed with alluminium rings (of the Bird Migration Research Station, Polish Zool. Museum Warsaw), and marked on breast in different colours with ink (as in our experiments with swallows) the birds were exported by train or bicycle at a certain distance from Cracow and then released. During the experiments especially in watching the returning birds we were helped by some persons to whom we are very grateful.

The annexed table I. and figure 2 represent the obtained results. In all 7 sparrows were used for the experiments, one of which marked with a ring number 77775 was exported twice.

[1]) To the director of the mentioned Garden prof. dr. W ł. S z a f e r we wish to express in this place our greatest thanks for the permission to make our observations in so convenient a place.

We disposed together of 8 specimens, 7 belonging to the species
P. arboreus Bewick ane one to *P. domesticus* L. From these 8
specimens transported to different distances 4 returns have been
stated, it is exactly 50%.

The details of the experiments are as follows:

Tree sparrow nr 1, ♂ caught 17. V. 1939 about 6ʰ P. M. expor-
ted early next morning to the other side of the town western
direction and released at a distance 2,700 m near the dr Jordan
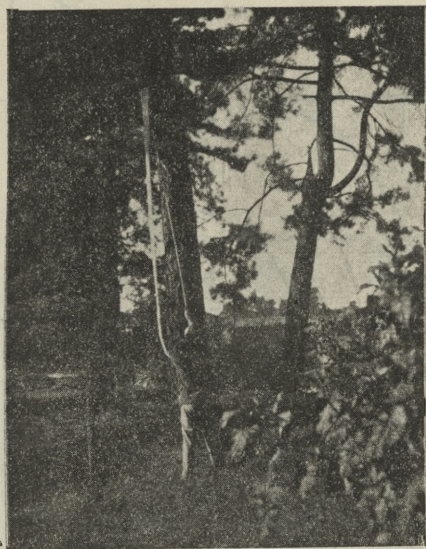
Fig. 1.

Park. After delivering it sat on the branch of a tree. Its return
was recorded only the 19. V.

Tree sparrow nr. 2 was a female of the former. It was caught
at the nest 19. V. P. M. at about 6ʰ in the morning exported by
bicycle west of Cracow and released next day on the highway
between Mydlniki and Balice. Its return was recorded the same
day. At 10ʰ30′ a great animation among the sparrows was no-
ticed, then 4—5 sparrows ran after one, that came from unknown
direction. About 11ʰ15′ we were able to state with all certainty
the return of the female.

The behavior of this female before and after the return of her male a day before was most interesting. Deprived of her companion she sat silently on a branch near the nest or on the box with her young, leaving only to bring them food. When the male returned, the welcome was rather sharp. The female pushed him off, stroke him with her wings and would not admit him at first to the nest. It was only after a time that peace was restored between them and they both fed the young. When in turn the female was caught and exported, no quarrell was recorded at
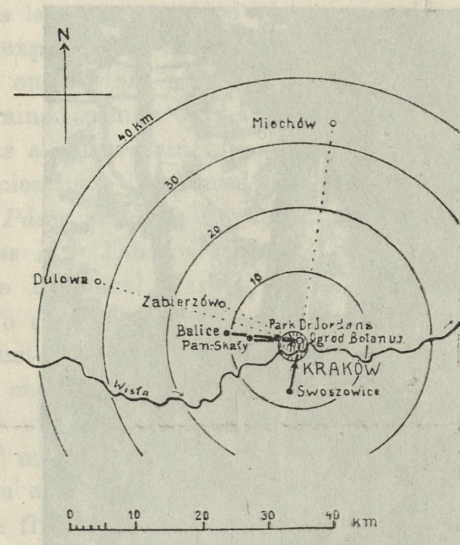
Fig. 2.

her return, except the above mentioned chase. At 11ʰ15 both birds were sitting quietly and feeding the young.

Meteorological conditions during the observations were rather good. For the individual days the are as follows [1]): 17. V. 1939 a sunny morning, then rain, sky a little clouded in the afternoon heavy clouds and fine drizzle several times, weak winds from WSW and S; then a lull, medium

TABLE I

Nr.	ring number and sex	nesting place	locality where released	distance from nest	date of releasing	date of return	time of return	velocity in km/h	species
1.	G. 77775 ♂	Cracow — Botanical Garden of the Jagellonian University	Cracow, Park of dr Jordan	2·700 km	18. V. 1939 3h00'	19. V. 1939 11h45'	32h45'	0·084 km/h	Passer arboreus
2.	G. 77776 ♀		Balice	11·000 km	20. V. 1939 6h30'	20. V. 1939 11h15'	4h45'	2·310 km/h	Passer arboreus
3.	G. 77777 ♂		Miechów	33·000 km	14. VI. 1939 0h48'	?	?	?	
4.	G. 77788 ♂		Zabierzów	13·000 km	16. VI. 1939 10h30'	?	?	?	
5.	G. 77789 ♀		„	„	„	?	?	?	Passer domesticus
6.	G. 77790 ♂		Dulowa	32·500 km	17. VI. 1939 20h45'	?	?	?	
7.	G. 77791 ♀		Cracow, »Panieńskie Skały«	6·800 km	19. VI. 1939 20h00'	20. VI. 1939 11h55'	15h55'	0·427 km/h	Passer arboreus
8.	G. 77775 ♂		Swoszowice	8·000 km	24. VI. 1939 1h20'	25. VI. 1939 11h05'	33h45'	0·237 km/h	

temperature 15·4⁶C. — 18. V. sunny morning, at noon the clouding of the sky increases, afternoon also sunny. Weak winds from WSW, medium temperature 18·5⁰C. — 19. V. Sunny morning, then cloudy increases, at 13ʰ a short rain, which reposts at 16ʰ and at 20ʰ20′. At 21ʰ lightnings on the WNW. At noon very weak winds from N, medium temperature 18·6⁰C. — 20. V. morning rather dull with periodical clearings, at noon sunshine, in the afternoon the sky cloudy again, weak winds turning from WSW to N, medium temperature 15·6⁰C.

Tree sparrow nr. 3, ♂ caught 13. VI. was exported by railway northward to Miechów, distant from Cracow 33 km in air line. Released, it flew down along the waggons of the train. Its return was not recorded.

Tree sparrow nr. 4 caught the 15. VI. PM was exported next day westward to Zabierzów, a spot distant from Cracow 13 km in air line. It was released at 10ʰ30′ from behind a church. Its return was not recorded.

House sparrow nr. 5 was caught the same day as the former, and together exported and released in Zabierzów at 10ʰ30′. Its return was also not recorded.

Tree sparrow nr. 5, ♀ caught in the afternoon 17. VI. was released the same day at 20ʰ45′ from Dulowa, 32,5 km westward from Cracow. The return was not recorded.

Tree sparrow nr. 7, ♀ was released in the day of the catch 19. V. at 20ʰ PM near the highway leading to Panieńskie Skały in the Wolski Las (Forest of Wola) 6·8 km westward from Cracow. Its return was recorded next day at 11ʰ55′.

Tree sparrow nr. 8, ♂ was the same specimen which a month ago 18. V. had been exported to the Park Jordan and had returned safely. This time it was caught the 23. VI. at 16ʰ30′ and exported by night southward to Swoszowice 8 km from Cracow in air line. It was released at 1ʰ20′. The return was recorded 25. VI. at 11ʰ05′ AM.

When observing this specimen we could state that the pair marked with rings nr. 77776 and 77775 after the young had left, kept staying in the same nest as before. The fact seems interesting, as J. S o k o ł o w s k i (1936) emphasised that what regards this circumstance at least with the house sparrow there are no available data. Owing to the rings with which the birds were provided we could state the fact in reference to the tree-sparrow.

Meteorological conditions in the days of the preceding observations from 13. VI. — 25. VI. were as follows: 13. VI. the day rather dull with clearings before noon, at 17^h and 18^h rain, weak winds from E and WSW, in the afternoon lull, medium temperature 19·3°C. — 14. VI. the day generally as the former, at 17^{h}30′ and 21^h it rains, AM weak winds from N and WNW, medium temperature 17·7°C. — 15. VI. at night a far away storm, the whole day cloudy and rainy, weak winds from NWN, NW and WSW, medium temperature 15·9°C. — 16. VI. morning rain, then clears up, sultry weather, in the afternoon storm from W, weak winds from SW, SSE and W, medium temperature 18·7°C. — 17. VI. morning fog and rain, then the weather becomes good. In the afternoon and in the evening it rains, storm noticed far away. At noon weak winds from ENE, medium temperature 21·1°C. — 18. VI. morning dull, at 9^h rain, then getting clear at 17^h lightnings in NO and O, at noon a very weak wind from ENE, medium temperature 20·2°C. — 19. VI. the morning rainy, then getting clear in the afternoon storm with heavy rain, weak winds from WSW, WNW and W, medium temperature 19·8°C. — 20. VI. the morning rainy then getting clear, at 19^{h}30′ storm and rain, weak winds from W, NW, medium temperature 19·3°C. — 21. VI. the morning dull, then the weather clears up, weak winds from EW and N, medium temperature 20·7°C. — 22. VI. clear till afternoon, at 19^h rain, weak winds E, medium temperature 21·6°C. — 23. VI. a rainy morning, then clear, weak winds from WSW and WNW, medium temperature 16·6°C. — 24. VI. clear, weak winds from ENE and ESE, medium temperature 21°C. — 25. VI. at night a distant storm, day-weather rather clear, at 13^{h}50′ storm and weak rain, then clear again, weak winds SE and SW, medium temperature 22·5°C.

Our observations, though still to be completed allow however to draw following conclusions:

1. Sparrows taken to definite distances from their nests, in which they had left their young or eggs show an impulse to return home. On 8 cases it was posible to state the return of 4 specimens, i. e. in 50%. In this respect no difference between sedentary and migratory birds can be seen.

2. The sparrows returned to their nests disregarding the direction in which they were exported. This fact agrees with the existing data obtained from other birds.

3. The maximum limit from which the birds returned was in our experiments about 11 km. From specimens, taken to greater distance (13—33 km) no one had returned. These facts did not show specially dependent upon the meteorological conditions. It would be highly interesting to find by further experiments whether indeed settled birds can return only from a small distance and whether in the case of a longer distance, their homing abi-

lity work or not. Unfortunately our observations are based on too small a quantity of material as to give a decisive answer.

4. The return of sparrows from small distances shows that an orientation memory based upon visual impressions plays with them the main role. The existence of another type of orientation by means of a direction sense or among other special orientation cannot be assumed on the basis of our experiments and will be so as long as further experiments mentioned under 3 do not bring the confirmation of the fact that sparrows do return also from much greater distances say 100—150 km. If the results of our experiments were confirmed that the sparrows return only from small distances, it would be possible to determine the limits of normal flights of these settled birds. To state this, it is necessary to know the exact distance from which they are able to return to their nests.

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Badania nad ewolucją dominacji u zwierząt ssących. —
Studies on the evolution of dominance in Mammals.

Note

de M. **T. MARCHLEWSKI,**

présentée le 17 juin 1946 par M. Z. Grodziński m. c.

The theory of F i s h e r (1923) on the role of subsequent changes of dominance in evolution, was for a long time lacking experimental confirmation.

Certain results of H a r l a n d (1933) obtained in his studies on cotton, seem to substantiate F i s h e r's (1923) deductions, though the evidence is not very critical.

V. S t e i g e r (1936) has submitted interesting results concerning the inheritance of yellow coat colour in certain breeds of dogs. It seems, according to the above findings, that yellow colour in Spaniels in consequence of selective processes, though originally a typical Mendelian recessive, becames a clear dominant.

These results have been confirmed by K u r z b a u e r and M a r c h l e w s k i (1938) who studied the genetics of the Australian Dingo.

It was found, that this form represents a black genotype, which owing to modifying genes turns into a yellowish red. This new colour behaves as a dominant in relation to the black colour commonly found in the domestic dog.

The writers were further able to correct certain deductions of L i t t l e (1936) and of M i t c h e l l (1937) concerning the behaviour of yellow coat colour in Toy Griffons and Collies respectively.

Later on, in a paper submitted to the VII. International Congress of Genetics at Edinburgh in 1939, I could prove, that by

appropriate mating i. e certain schuffling of genetic modifiers two yellow dogs may produce black offspring.

This of course was deemed impossible on the basis of hither to known genetical facts. In the present communication, I wish to point out certain facts connected with the inheritance of brindling in dogs.

According to the classical scheme of Little (1918) brindling is caused by an allelomorph of the extension factor and in consequence the coloration, as shown by Little and Little and Jones (1925) is recessive to black.

The alternative conception of Wriedt (1928) and Scot-Wattson (1934) acording to which brindling in cattle may be caused by the heterozygous condition of black and yellow, has not been confirmed by the results of Fraser-Darling (1936) in Irish Wolfhounds. On the other hand Queldrup and Dahl (1937) in their study of colour genetics in the Boxer, mention the occurence of a black bitch from brindle parents.

This would mean a certain »dominance« of brindle over black. My own observations may serve as an extension of the studies of Queldrup and Dahl (1937) and have been made during the war. Two brindle Great Danes, rather darker in shade than preferred by fanciers, produced a number of black offspring. The actual figures were as follows:

Litter Nr	Brindle	black	yellow
1	6	2	
2	7	1	
3	5	3	
4	7	2	
Totals:	25	8	

The results suggest a 3 : 1 ratio very closely, and the absence of a yellow class disproves the possibility, that brindling in Great Danes represents the heterozygous condition of black and yellow.

The black individuals from the above matings did show certain amount of yellow on their flanks and were very similar in appearence to the black individuals obtained in my former studies from a cross between the yellow Dingo and yellow Pointers.

A great analogy does exist between the colour of both types of dogs and certain Japanese rabbits, described some time ago by Marchlewski (1933).

My interpretation of the above results is, that originally brindle dogs are of very dark hue, just as certain primitive types of Japanese rabbits are.

The selective aims of breeders aiming of a bright »golden« hue of the yellow parts in a striped dog, result in the accumulation of modifiers of the same order. That tum a black Cocker Spaniel into a »golden« dog, cause the yellow coloration of the Dingo, or produce the »sable« colour in the Collie.

Our original »dark brindle« dogs were therefore heterozygous for the modifiers in question, thus producing »almost black« offspring which like the bitch of Queldrup and Dahl (1937) must correspond in appearence to the original brindle mutation of the extension factor.

The validity of my interpretation could be fortunately subjected to an experimental proof. All respective investigators agree that the »bicolor« factor, or tan coloring is recessive in relation to uniform coloring. Personally, when conducting experiments on breeding liason dogs, I conducted numerous crosses with black and tan dogs of various breeds, and always found the colour to be stricly recessive.

An exception from this behaviour was found as a result of mating the original Great Dane bitch to a black and tan Doberman. The results were as follows:

Litter	Brindle	black and tan brindled	black
1	5	4	
2	3	2	

These results must mean, that the action of the yellow enhancers discussed previously, causes dogs heterozygous for the bicolor factor to exhibit the pattern, in spite of its original recessivity. The modifiers in question act therefore not only upon black, but transmute the tan factor into a dominant phase.

Similar observations were made most likely by Iljin (1933) quoted by Hirschfeld (1936) though I was unable to study the original paper.

The main results of my observations proving an actual change of dominance of black colour and the bicolor factor in dogs, forms not only an addiditional proof of Fisher's theory, but do show clearly the role of modifying genes in this process.

The shifting of dominance in both ways, seams to be an especially active process both in natural evolution and in the moulding of our breeds of livestock.

From the Zootechnical Institute of Jagellonian University. Director: Prof. Dr T. Marchlewski Ph D.

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Spostrzeżenia nad pellikulą Euglena viridis Ehrbg. —
On the pellicle of Euglena viridis Ehrbg.

(Plate 2)

1. Problem

The Plant- and the Animal World are linked together by a number of forms. Such forms are to be found especially among the Flagellata (Pascher). The genus *Euglena* may be just regarded as one of such transition-forms.

Some species of this genus can in certain circumstances loose their chlorophyll (although in nature they appear always green); they are then very difficult to distinguish from animal protozoans.

A form deprived of chlorophyll was never reared from the species *Euglena viridis* — accordingly this form can be considered as more vegetal. It is well known that all species of the genus *Euglena* find their optimal conditions of development in a medium in which besides mineral salts, organic compounds are also present. These facts speak for a linking systematic position of the genus *Euglena*.

The animal and vegetal membranes are easily discerned. The vegetal membrane build of cellulose is a product of the living plasm, but a product already dead, stiff, and only loosely connected with the cell itself. Therefore the plasmolysis is possible. In the vegetal cell the permeability is regulated by a very deli-

cate protoplasmatical membrane lining the cellulose wall, and not by the cellulose membrane itself. In animal cells the cell membrane is simultaneously an osmotical filter and a mechanical shield. It is why the animal cell membrane can have very different structure — a delicate membrane surrounds the erythrocytes, a thick and strong one surounds the egg cell of the rabbit.

In the present paper I wished to examine the properties of the pellicle of *Euglena*, trying to show its vegetable and animal transition character. This form has been also interesting to me as an object of microdissection.

2. Material

The investigated flagellates were reared in a diluted pea extract prepared after Zumstein. The culture was standing in daylight, near a north looking window. The cultures multiplied feebly. Their growth was much better when the »artificial sun« of Pringsheim was used (an electric lamp of 150 W, 220 V). In cultures freely swimming forms and fixed cells are simultaneously found. For experiments I used only free forms, having a distinct sculpture of the pellicle.

3. Methods

The mechanical properties of the pellicle were studied with a Chambers' micromanipulator. Before a microdissection the flagellate must be fixed. This is reached by the use of cover slips coated with a thin layer of gelatine, that is subsequently dried. When a drop of fluid medium containing Euglenes is put on such cover slip, the water is soaked by the swelling gelatine, and the flagellates are fixed by its viscosity. One can also use the blotting paper boiled into rags in water. A drop of Euglenes containing medium is mixed with tissue paper rags, then the excess of water is soaked. The cellulose fibres form a netting, the meshes of which fix the flagellates.

The influence of chemicals on the pellicle was tested under the cover slip. On the margin of the cover slip a drop of the chemical was placed, from the opposite side the fluid was soaked by blotting paper. To ascertain the beginning of the action of chemical I mixed the fluid with some neutral dye. The alternation

of colour gives notice of the change of the medium. For prolonged observation of such preparations I enclosed them with a ring of vaseline, to prevent dessication.

The results are illustrated with photographs made with the Zeiss' microphotographing device »Miflex« adapted to the use of another camera (»Leica« of Leitz). The taking of pictures on a narrow film rendered possible a short exposure, even in the case when a preparation condenser was used. All pictures are taken with an apochromatic immersion obiective (60 × n. ap. 1,00 Zeiss) and compensating ocular (10 × Zeiss). The photographs allow easy and precise measurement, while using of micrometer eyepiece was highly inconvenient.

4. Observations

The body of *Euglena* is covered by a thick pellicle, clearly visible in the living cell. The pellicle is continuous over the whole cell and has no apertures. On the anterior extremity of the cell there is a blindly ending pouch (1 μ in diameter, 3—4 μ in length) making the appearance of a canal leading to the protoplasm. It is with this pouch that the flagellum is connected, but its precise course cannot be followed on living material. The surface of the pellicle is covered with delicate striations (ca 10—14 furrows on 10 μ) running spirally around the cell. The striations are sculptured on the outside of the pellicle and are not situated in the pellicle itself, as it is shown by the photograph (Fig. 2, pl. 2) representing the impression of the pellicle left in the layer of gelatine.

The pellicle has a great mechanical resistance. In spite of numerous trials (27 operations) I was not able to perforate it. When the *Euglena* is affixed to the surface of gelatine, it can be shifted with the aid of a needle introduced to the pouch of the pellicle, without causing damage to the pellicle (8 trials). When a protozoan fixed with gelatine is stuck even with a sharp needle, it bends on itself and can be pressed into the gelatine but does not burst (4 trials). After a prolonged stay on gelatine (over 1 hour), connected with dessication, the pellicle loses a part of its resistance and can be cut or pierced with a needle. It is however possible, that the lessening of the resistance is only appa-

rent. After the dessication the cell can stick so firmly to the gelatine, that the pellicle breaks when pressed down with a needle.

Distended with two needles, the pellicle shows little elasticity. It can be distended to $10-15\%$ in the long and short axes. Those figures were calculated for 7 cells only (4 in long and 3 in short axis), they cannot be very accurate, but give some idea of the small elasticity of the pellicle (Fig. 1, pl. 2). The cell can change normally in the shape, and it is therefore difficult to say whether the operation really distend the cell.

When the distention is too great, the pellicle bursts. The place of bursting cannot be foreseen (46 trials). Similarly, when the contents of cell are strongly pressed the point of bursting cannot be predicted. The margin of the burst pellicle is homogenous, it shows no special structures. The sculpture (striations) is visible also upon the torn pellicle. No changes of the sculpture are visible.

The posterior part of the body has peculiar properties. By distending, bursting or rounding of the cell the posterior part does not change its form. I believe that this can be sufficiently explained by its peculiar shape (pointed ending). It does not seem that the pellicle was here stronger than elsewhere. The operations of removing the pellicle or of pressing out the protoplasm from the burst cell show that the pellicle and the protoplasm adhere closely to each other but are not connected by special structures (14 trials).

Reactions for cellulose give negative results (sulphuric acid + iodine, zinc chloride + iodine, Schweitzer's solution). Reactions for proteins (Millon and xanthoprotein) gave no definite results. In both reactions essential is the change of colour of the investigated matter under the influence of chosen reagents (Millon's test, xanthoprotein test). The extreme delicacy of the pellicle does not allow to perceive the test.

The pellicle is completely digested by trypsin and partly by pepsin. The larger particles, as the particles of the dye »water blue« do not penetrate the living pellicle. Neutral red acts in vivo. The pellicle of dead flagellate is not destroyed by bacteria, even when the protoplasm decays.

In the acid reaction no apparent changes appear with the pellicle. Sulphuric and hydrochloric acids $2,5-5\%$ cause the dis-

appearance of superficial striations and a contraction of chroma-
tofores within the cell (Fig. 4). The acids destroy the semipermea-
bility, the water blue penetrates now freely the pellicle. Stronger
sulphuric acid (30%) acts violently on the content of the cell, but
does not destroy the pellicle even after 24 hours. Sulphuric acid
40% however dissolves the pellicle completely in 1—5 minutes.
The 35% acid dissolves the pellicle in the majority of indivi-
duals (about 70%) but not in all.

In an 0,5% solution of potassium hydroxide the cell swells
quickly augmeting its contents 200—400%, the pellicle is dissolved
and after 10 minutes it is hardly visible (Fig. 3, pl. 2). The swel-
ling of the pellicle itself was not observed. The swelling of the cell
is weaker and progresses more slowly in solution of 0,7% KOH
and 1,15% KCl, and does not occur at all in a solution of 0,7%
KOH and 5% glucose (the 5% solution of glucose is isotonic
with 1,15% of KCl). It therefore looks as if the swelling would
be a result of osmotic forces only. Further experiments prove
that it is not so. The swelling is present in hypertonic solution
of 5% KOH and 5% glucose, the pellicle is then quickly dissol-
ved. When the swollen cell is placed in an acid medium (5%
H_2SO_4) a sudden contraction occurs, after a replacement in the
alkali the swelling reappears.

All microchemical reactions were repeated three times.

The pellicle treated with a weak solution of the sulphuric
acid (2,5%) is easily distended and more difficult burst (4 trials).
The distention can attain 30% without bursting. Simultaneously
its viscosity increases, it does not slip out from the glass needles.
In a stronger acid (20%) its dilatability diminishes about to 10%.
The pellicle treated with solution of KOH and glucose (0,7%
KOH + 5% glucose) has no greater dilatability and is less resi-
tant to mechanical injuries (is easily torn, bursts etc.)

I fixed the flagellates with a saturated solution of picric acid
during 20 minutes and tried then to distend the pellicle. Its fra-
gility grows, the distension of 10% is not tolerated, although
some dilatability persists (4 trials).

70% ethyl alcohol after 2 hours dissolves the chlorophyll and
the pigment of the eye spot, simultaneously coagulating the pro-
teins. The pellicle has then a small dilatability and great slippe-
ness (3 trials). It slips easily out when caught with glass needles.

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5. Discussion of results

The sculpture of the pellicle was subject of some publications. Older authors believed the striations to be contractile elements (S t e i n), assertion, that has been however contradicted (K l e b s). G r a s s é found that in some flagellates of the order *Hyperma-stigina* (*Devescovina, Lophomonas, Polymastix* — commensales in the enteric canal of termites) the striations are caused by the symbiotic bacteria. The strations on the pellicle of *E. viridis* have another origin (J i r o v e c, L e f è v r e). According to J i r o v e c the striations on the pellicle can be impregnated with silver nitrate. I repeated the experiments of J i r o v e c and observed similar results (Fig. 2). With the same method the fibrillar structure in infusorians can be impregnated, to which the neuromotoric function is assignated. It is possible, that also in *Euglena* the striations are sensitive and conducting elements. It can be considered as stated, that their chemical composition differs somewhat from the composition of the pellicle because: 1. even in a weak solution of acid the striations disappear, 2. they are impregnated with silver nitrate. J i r o v e c consider them as supporting structures. Against this assumption speaks that: 1. by tearing the pellicle the striations burst simultaneously with the pellicle, 2. the dilatability of the pellicle is the very same in the direction parallel to the run of the striations as also across them or obliquely to them, 3. on the burst pellicle the striations run to the margin of the hole. The striations must be therefore tightly connected with the pellicle and do not differ from it in mechanical properties.

The place in which the pellicle bursts when the cell is pressed with the micromanipulator-needles cannot be predicted. It seems therefore that there is no aperture connecting the interior of the cell with outside world, and that there are no places of lesser resistance. According to some older authors (K e n t, T a n n-r e u t h e r) the *Euglena* has the ability of ingestion of solid particles. According to others (W a g e r) it is deprived of this ability. Lastly H a l l growing *Euglena* in an emulsion of india ink could not find the particles inside the cell, and is therefore of opinion that this protozoan cannot swallow solid particles. My experiments agree with the opinion of H a l l, as I could not find any aperture in the pellicle and was able to show its comparatively

great thickness. It exists however some discrepancy between the results of W a g e r and mine. According to W a g e r a vacuole situated at the anterior end of the body opens to the exterior by an aperture near the basis of the flagellum, to the recess of the pellicle. W a g e r's work being out of my reach, I cannot make out the cause of this discrepancy.

The experiments on the resistance of the pellicle show that it is a solid body, the protoplasm being liquid or semiliquid. With the aid of the microscope no structure in the pellicle is observable, the striations run on its outer surface. The micromanipulation gives also no evidence of structures having mechanical properties, the resistance and the dilatability of the cell being the same in all directions.

The pellicle of *Euglena* reminds by its physical properties the pellicle of certain amoebas (C h a m b e r s, H o w l a n d, M a s t, O k a d a). The pellicle of *Amoeba proteus* is invisible, the animal has the ability of producing many long pseudopodia. The pellicle of *Amoeba verrucosa* is much thicker, the animal is able to produce only one short pseudopodium. The pellicle of *Euglena* is the thickest of all, this protozoan has not the ability of producing real pseudopodia, but can greatly change its shape.

The similarity of the physic properties of the pellicle of *A. verrucosa* and *E. viridis* is striking, but their chemical composition and the origin can differ.

The chemical composition of the pellicle cannot be exactly determined. No traces of cellulose can be detected in the pellicle. The digestive reactions seem to prove that a great rôle is played by the proteins. The small dilatability of the pellicle fixed with picric acid points to the same conclusion. The resistance to acids and the dissolution even in weak alkali indicates that the proteins have an acid character.

The potassium hydroxide does not increase the dilatability of the pellicle. As the cell swells in alkali, I suppose the pellicle to be dissolved. The swelling, especially pronounced in the alkaline reaction is a well known phenomenon in the chemistry of colloids. The nature of swelling is not sufficiently explored.

It is generally assumed that the semipermeable cell membrane is built of lipoids and particles of proteins. The number of lipoidoproteic systems and its one- or many-layered arrangement deter-

mine the thickness and the mechanical property of the membrane. Very delicate semipermeable membranes are found on the red blood cells (S e i f r i z) and on yolk spheres (G r o d z i ń s k i). In both cases the membranes are so thin that they cannot exist longer after emptying their contents. The pellicle of *Euglena* is in principle similarly built, only the proteic substances are more numerous, giving to the pellicle greater resistance — it conserves its chape after taking off from the cell, it is not dissolved by alkali or ether. Between the pellicle of *Euglena* and the typical animal membrane a continual series of transitory structures can be established. The absence of cellulose is a striking difference between the pellicle of *Euglena* and the vegetable membranes.

6. Summary

1. The pellicle of *Euglena* surrounds the whole body as a thick, semipermeable membrane. As there are no aperture in it, the taking of solid food is impossible.

2. The outer surface of the pellicle is covered with striations. They do not differ from the pellicle in their mechanical properties. The pellicle is built from a physically uniform material.

3. The pellicle has a small elasticity and dilatability.

4. The chemical composition of the pellicle differ from that of the protoplasm. The pellicle is built of a small quantity of lipoids and a large quantity of acid proteins. There are no traces of cellulose in it.

Description of figures on Plate 2

Fig. 1. Dissecting of the pellicle.
    a. Protozoan caught with needles.
    b. Pellicle bursts.
    c. Pellicle taken off from the cell.
Fig. 2. Striations on the pellicle.

a. Living protozoan adhering to the surface of gelatine, shifted with a needle.

b. Striations impregnated with silver nitrate.

Fig. 3. Swelling in 0,5% potassium hydroxide.

a. Beginning of the experiment,　　b. 10 sec. later,
c. 20 sec. later,　　　　　　　　　　　d. 30 min. later.

Fig. 4. Action of 2,5% hydrochloric acid.

a. Beginning of the experiment,　　b. 15 min. later,
c. 23 h. 35 min. later.

The lines on the photographs indicate the length of 10 μ.

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1a   b

c

2a

b

3a   b   c   d

4a   b   c
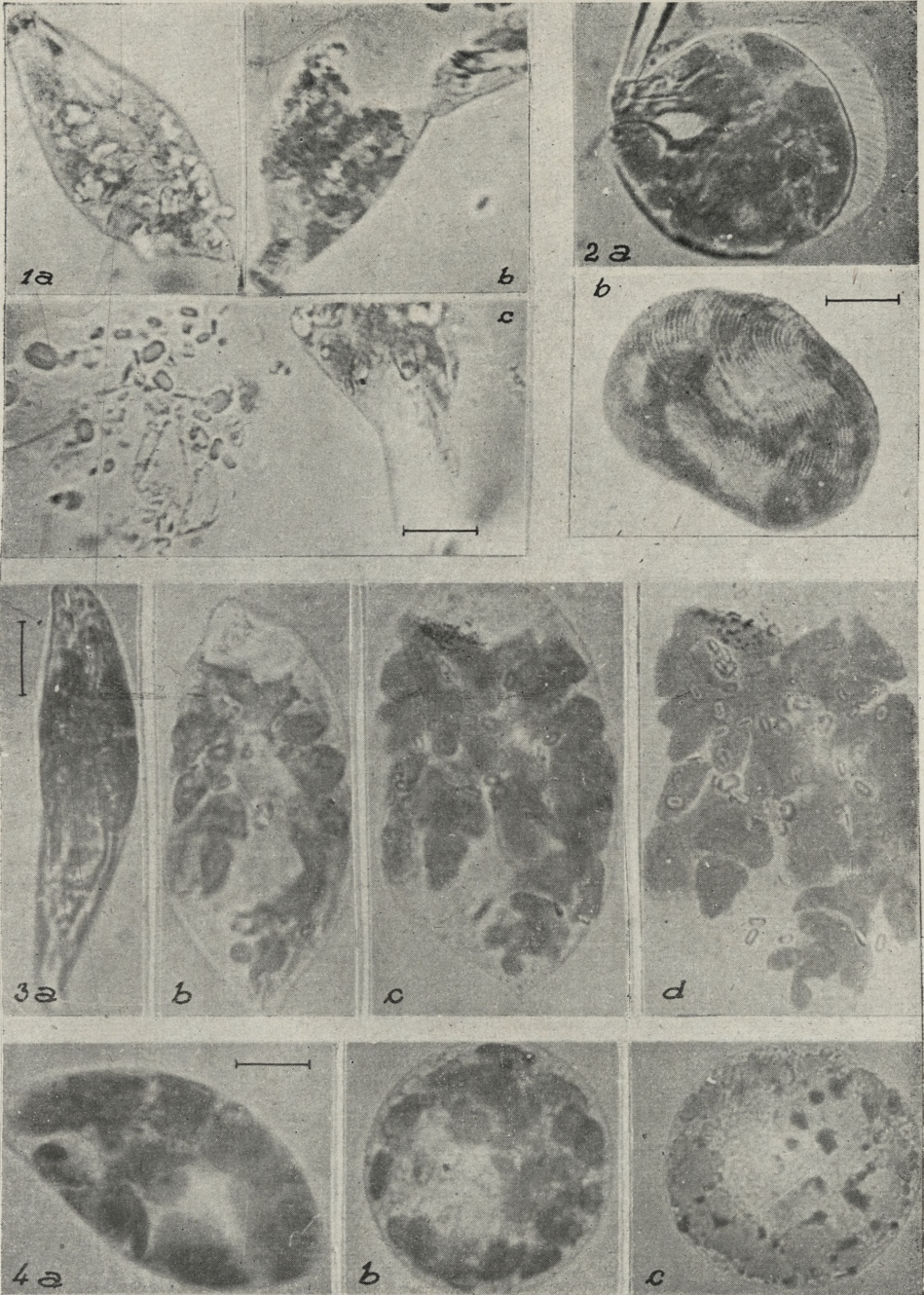
A. Pigoń

Badania struktury mineralnej spopielonych nerek myszy białej. — A study of the mineral structure of incinerated kidneys in the white mouse.

Mémoire

de M. *J. WILBURG,*

présenté le 11 juin 1945 par M. H. Hoyer m. t.

(Plates 3—4)

The spodographic investigations hitherto have dealt with the general topography of mineral salts in embryos (Horning and Scott 1932, Schultz-Brauns 1931) as well as in adult individuals (Okkels 1927, Schönholz 1929, Bagiński 1932). Other problems interesting the investigators were the localization of mineral substances introduced experimentally into the organism and the reaction of particular portions of the kidneys to the presence of these substances (Okkels 1927). The picture of the incinerated epithelium of the convoluted tubules in cases of artificial coagulation of blood in the kidneys was also studied (Schönholz 1929). The experiments were carried out chiefly on mammals such as rats, rabbits, mice.

Scott (1933) was the first to present detailed cytological investigations of incinerated kidneys. This author studied the structure of four parts of the nephron (expression coined by Möllendorff 1930) of the cat i. e. the gromerulus, the proximal convoluted tubule, the descending and the ascending limb of Henle's loop and the distal convoluted tubule. In his study the author did not consider the collecting tubule. According to Scott the mineral components appear within the body of the cell in the shape of evenly distributed granules whose degree of accumulation is characteristic of each particular part of the nephron.

S c o t t (1933) stated the presence of iron in the body of the cell, an the ground of the colour of the ashes. He considered the greater part of the ashes of the cell body as formed by iron. The purpose of the present paper was to find such a fixing solution and method of incineration as to cause the least deformation and dislocation possible in the mineral components of the cells. The results of the present investigations were considered as a basis for further research work upon the mechanism of excretion of colloid combinations of gold in the kidneys.

Method and material

In spodography there are two methods of making sections, that of ·S c h u l t z - B r a u n s developed by G e r s h, using fresh unfixed tissues and organs, and another introduced by P o l i c a r d, which consists of a previous fixing of tissues by means of adequate solutions. The second method is much simpler and, which is most important, enables one to produce very thin sections of the objects examined. It is only when their thickness does not exceed $1 — 2 \, \mu$, that the spodographic pictures are sufficiently clear.

In my investigations I have applied the second method and tried a series of various fixatives to find the best, one that would cause no shrinking and diluting of the tissues and would not dislocate the mineral salts. The Bouin liquid highly recommended by G o d l e w s k i (1937), whether containing acetic acid or not, proved to be wholly inadequate for our purpose. The picric acid combined with the mineral substances of the cell, while the acetic acid dissolved the salts within the cell, causing their dislocation. The fixative used by B a g i ń s k i (1932) — formol, concentrated sulphuric acid and absolute alcohol, mixed in the ratio $9 : 2 : 100$ — has the disadvantage of shrinking the kidney very strongly, as much as to $^1/_5$ of its former size, while the mineral salts in such kineys form a shapeless mass on the spodograms. Dioxan (G a g e 1938) shrinks the kidney strongly and changes its colour from red to pale yellow. In histological sections the presence of red blood corpuscles is difficult to detect, the blood pigment having been presumably wholly dissolved. The mineral structure of the protoplasm and of the cell nuclei of the convo-

luted tubule is blurred, and looks as if it were composed of a white cotton wool-like substance (Fig. 1, pl. 3). Absolute alcohol and 95% alcohol combined with formol in the ratio 9:1 (Scott 1933) gave better results, though the kidney shrank considerably and dislocations were clearly visible in the structure of the deposits. It was only when using 70% alcohol with neutral formol in the ratio 9:1 and $9^1/_2:{}^1/_2$ that I could obtain the pictures described in my present paper. The kidney was fixed »in toto« in fairly large quantity of this liquid for 12—24 hours then passed quickly through alcohols of higher concentration, xylol and 30° paraffin endeavouring to keep the kidney in 55° paraffin for as short a time as possible in order to avoid the destructive action of heat. The kidney embedded in paraffin was cut by aid of the Leitz-Wetzlar microtome into sections 2μ thick, this thickness having proved the most suitable for incineration (Fig. 2, pl. 3). In spodograms, mineral substances are melted down in thicker sections and thus give an erroneous picture of the structure and the localization of the ashes (Fig. 3 and 4). The paraffin sections were placed on a covering-glass (Kruszyński and Policard) and flattened with 70% alcohol. As the presence of paraffin is undesirable during incineration, it was removed from the sections by means of xylol, these sections having been previously well dried. The paraffin boiling at a higher temperature would carry away the mineral particles from one place to another. The slides thus prepared were placed on a metal slab made of an alloy difficult to fuse and burnt in a Policard incinerator, improved by Bagiński. The sections were burnt slowly and kept for one hour at a temperature of 18°—300° C and for 45 minutes at a temperature of 400° C. The range of mistake, according to the opinion of the constructor, did not exceed the temperature on the regulator by more than 10%. The spodograms were left in the furnace to cool completely, as when warm they absorbed moisture from the environment to high degree. The spodograms were inspected under dark ground illumination by the aid of an Ultropak Leitz-Wetzlar, the objective used being 75× immersion and ocular 8 comp.. The spodograms on cover-glasses were transferred for inspection to special glass frames, the deposits being turned upside down (Kruszyński). The pictures of the spodograms were made with a Macca Leitz-Wetzlar

apparatus and those of the control sections by means of a Miflex Zeiss. Microchemical reactions were carried out with Chamber's micromanipulator. White male mice, having a weight of
19—25 grammes, served as the material for experiments.

Appearance and interpretation of the incinerated sections

The Malphighian corpuscle

The photograph of the incinerated glomerulus (Fig. 5ıı., pl. 3)
shows a great number of cellular nuclei, differing greatly in
their intensity of colouring, size and topography. Similarly, on
the base of normal histological pictures (Fig. 5ı.), three types
of nuclei may be distinguished, belonging to cover cells, endothelial cells and fibrocytes.

The histological pictures and the spodograms were compared.
It is by comparison that an accurate localization of mineral salts
may be determined. The nuclei of the cover cells are distinguished by a strongly defined nuclear membrane, and an insignificant quantity of chromatin substance gathered close to the
membrane (Fig. 5ıı. E). The body of the cover cell does not
show any conspicuous features. In the spodogram the nuclear
membrane of cover cells form ring-like (Fig. 5ıı. E) deposits
having a dull white colour. Chromatin substances form glistening snow-white deposits. The plasm of the cover cells shows
very delicate deposits forming an irregular network (Fig. 5ıı. co).

The nuclei of the endothelial cells are distinguished by their
small size, their rounded shape and their uniformly dark colour
(Fig. 5ı. En). In the spodogram the deposits are bright, brilliant
snow-white (Fig. 5ıı. En). The deposits of the membrane so characteristic of the nuclei of the cover cells cannot be distinguished
among the ashes of the nucleus. The crosssections of the vessels
give dull white unhygroscopic deposits (Fig. 5ıı. ca). In the lumen
of the vessel the deposits have generally an irregular shape and
a glistening white light. These are mineral deposits from the
blood corpuscles (Fig. 5ıı. bl).

The nuclei of the fibrocytes, very small in number, are finally distinguished in histological sections from the two fore-

going by their larger size and paler colouring (Fig. 5ı. F). The mineral deposits of these cells are more loosely distributed than those of the endothelial nucleus. The outlines of the nuclei are jagged, the nuclear membrane showing no deposits in the spodogram (Fig. 5ıı. F). The mineral deposits of the fibrocytes plasm have not been detected.

The walls of Bowman's capsule adjoining the renal tubules appear as a fairly distinct dark line (Fig. 5ı B) on histological sections. On the wall of the capsule appear at long intervals nuclei uniformly dark in colour that protrude in the direction of the lumen of the capsule (Fig. 5ı. E'). The mineral deposits of the capsule form a white, indistinct line (Fig. 5ıı. B). Among the tiny grains that compose it, there are also some larger ones. In the incinerated nucleus it is easy to distinguish the dull deposits of the membrane of the nucleus from the brilliant white deposits of the chromatin (Fig. 5ıı. E'). Excretion may occasionally be seen in the lumen of the capsule in stained preparations, while in the spodogram the excretion does not leave any deposits.

The above observations deviate to a marked degree from the descriptions and figures which S c o t t gives of the incinerated kidneys of the cat. S c o t t did not notice the deposits and did not analyse them very closely. From his figures it would appear that the cells of Bowman's capsule protrude externally and not towards the lumen. The differences between our observations might be explained by the difference of the material used (cat, mouse) and in the technique. The tissues of the cat were fixed in a different way and cut $3—5\,\mu$ thick, which in my opinion gave less clear and certain pictures.

Proximal convoluted tubule

The proximal convoluted tubule is built from high cells provided with a brush border (Fig. 6ı. r, pl. 3) lighter at the base and darker at the edge. In the cell body the spots are light and dull (Fig. 6ı. f). The nuclear membrane and the adjoining layer of chromatin are both strongly coloured. The chromatin granulation though very tiny is also present in the centre of the nucleus (Fig. 6ı N). In the spodograms the nuclear membranes leave distinct deposits in the shape of dull white rings clearly detached

from the chromatin deposits by their shining snow-white light
(Fig. 6ıı. N). Within these ring-like deposits appear granulated
ones, generally two in number (Fig. 6ıı. Nu) resembling in colour
those of the nuclear membrane, though having a more pronoun-
ced greyish tinge. To determine their origin is not an easy task.
In all likelihood these should be regarded as the nucleole residue.
Inside the nuclei, in which there were fewer mineral salts of chro-
matin, were found tiny waxy-yellow granules. I have found them
not infrequently situated around the continuous line of ashes of
the nuclear membrane (Fig. 6ıı. g). The cell body as a whole left
ashes in the form of dull white network (Fig. 7ıı. re) with
small granules distributed within them (Fig. 6ıı. g'). In places
where on histological sections a brush border (Fig. 6ı. r) is found,
the ashes form a continuous white line (Fig. 6ıı. r), in which ap-
pear some lighter more conspicuous granules, forming often lar-
ger aggregations. By their waxy-yellow colour these granules
resemble those found in the nucleus. The ashes of the outer cell
limit do not differ in their appearance from the inner cell limits
of the tubule. It is only in places where the walls of two adjoin-
ing tubules touch each other that the ashes fuse into a conti-
nuous white line (Fig. 6ıı. l). In the phase of excretion (Fig. 7ı·
and 7ıı.) the spodographic cell the pictures of the proximal con-
voluted tubule undergoes a slight modification. The nucleus ashes
are similar to those described above (Fig. 7ıı. g). The chromatin
leaves far more snow-white substance. The cell body is filled
with ashes in form of irregular meshes (Fig. 7ıı. re), very distinct
and occupying spaces that are optically free from deposits
(Fig. 8ıı. sp and 8ııı. sp, p'. 3). Inside the meshes lie the waxy-yellow
granules (Fig. 7ıı. g), also encountered in the nucleus. The gra-
nules appear in larger quantities, in the processes of the cells in
their most peripheric portions, and often fuse into shapeless clods
(Fig. 7ıı. h). In longitudinal and transversal cell sections the de-
posits take the form of striations (Fig. 8ıı. and 8ııı. st.) perpen-
dicular to the base of the cell. Waxy-yellow granules adhere to
these striations, the larger granules being fused with them. Most
of these granulated deposits are accumulated in this brush bor-
der and the processes within the lumen of the tubule (Fig. 8ıı. pr).
The ashes of the excretion are found in the lumen of the tubule
(Fig. 8ıı. ex).

Scott (1933) found brown ashes in the brush border in the supranuclear zone and also in the basic parts of cells of the convoluted tubule and because of their colour look them to be iron. A similar brownish ash in spodograms, when treated with ferricyanate of potassium to detect iron, gave no microchemical reaction, and disappeared on longer incineration. Consequently I consider these ashes to be carbonized organic constituents, the optical spaces having no ashes described by Scott (1933) in the supranuclear zone of the convoluted tubule seem to be the artificial product of a different and less refined technique than mine. The rich deposits of the mineral salts upon the outer margin of the tubule are by no means constant as supposed by Scott (1933), but only appear where the walls of two tubules closely adhere (Fig. 6ɪɪ. 1, pl. 3).

Henle's loop

Histological sections of the descending limb of Henle's loop show high (Fig. 9ɪ. hi, pl. 4) and low (Fig. 9ɪ. lo) cells. The nuclei of the low cells take a more uniform colour (Fig. 9ɪ. N) than those of the high cells. The plasm of the former absorbs stain slightly, where as the periphery of the high cells takes a very strong colour. In the spodogram the nuclei of both high and low cells have deposits disposed similarly to those of the cells of the convoluted tubules, – that is to say, the ashes of the nuclear membrane form a ring, while the deposits of the chromatin are inside this ring (Fig. 9ɪɪ. N) although in the low cells the deposits are scattered equally. The body of the high cells does not contain any deposits in the nearest vicinity of the nucleus; this space is optically free from deposits (Fig. 9ɪɪ. ar). In low cells this free space occupies nearly the whole cell body. The cell body is filled with mineral granulations. The limits of the cell are determined by deposits forming continuous lines from which project large granules (Fig. 9ɪɪ. cl). In transverse sections the deposits from the thinnest part of the loop in the neighbourhood of the papilla demarcate the lumen of the tubule by a single line of deposits (Fig. 13ɪɪ. de, pl. 4), among which appear delicate mineral granules. The cell nuclei of the thinnest tubules are filled almost entirely with snow-white deposits (Fig. 13ɪɪ. N). The lumina of these tu-

bules show granulated or often lumpy deposits, the remains of excretion.

The cell body is striated (Fig. 10ı. st, pl. 4) and has no brush border in the descending limb of Henle's loop of the nuclei with a sharply marked nuclear membrane do not differ greatly from those of the proximal and distal convoluted tubules (Fig. 10ı. N and 11ı. N). In the spodogram the striated plasm is magnified as twig-like deposits of a dull white, set perpendicularly to the base of the cell (Fig. 10ıı. st). The deposits of the ascending limb of Henle's loop in the vicinity of papilla (Fig. 13ıı. as) have an exactly similar arrangement. The ashes of the nuclei distinctly recall the spodographic pictures of the proximal and distal convoluted tubules.

The Distal convoluted tubule

The cells of the distal convoluted tubule resemble in their general appearance the cells of the ascending limb of Henle's loop i. e. by the striations of the plasm and the distribution of the chromatin in the nucleus. The processes of low epithelial cells are the characteristic feature of this section of the kidneys (Fig. 12ı. pr, pl. 4). The nuclei leave ashes in the form of a ring with many granules inside (Fig. 12ıı. N). The plasm leaves deposits in the shape of striations perpendicular to the base of the cell (Fig. 12ıı. st). Besides this a great number of granulated deposits is found in the upper parts of the cells, in their processes and in the lumen of the tubule (Fig. 12ıı. g). The granulated deposits appear in larger quantity in the lumen of tubule. The deposits seem to be most probably incinerated excretion. In spodograms the processes give deposits in the form of continuous lines of the some colour as the deposits of the cell body (Fig. 12ıı. pr).

The Large collecting tubule

The cells of the large collecting tubule are marked by dissimilarity of size, very clear edges (Fig. 14ı. cl, pl. 4) contrasting with the pale body of the cell (Fig. 14ı.) and nuclei containing a small quantity of chromatin accumulated in the form of granules round the nuclear membrane (Fig. 14ı. N).

The spodogram is an exact reproduction of the structure visible in a histological section. The deposits of the nuclear membrane are almost invisible (Fig. 14II. N). Inside the nucleus there are tiny granules of chromatin glistening white. The cells demarcated by a continuous line of dull white deposits containing lumps of a mineral substance of a different colour. The body of the cell is optically almost entirely free from deposits (Fig. 14II. ar). The lumen of the tubules shows a large quantity of deposit left by excretion (Fig. 14II. ex).

The Microchemical analysis of ashes

The ashes of the kidney, treated with a solution of ferricyanate of potassium K_3FeCN_6 with concetrated hydrochloric acid HCl having a specific weight of 1,124 in twice distilled water in the ratio 2:1:100 gave a very small quantity of blue dye $[Fe(CN)_6]_2Fe_3$. This (Tirmann and Schmeltzer) would hint to a content of iron. Treated with concentrated sulphuric acid H_2SO_4 (conc. pro analisi) and twice distilled water (Aqua bidestillata) in the ratio 2:100, the ashes gave crystals of gypsum formed of the calcium contained in the ashes. In the first test as well as in the second the reaction was impossible to localize though the canules of the micromanipulator used for injections were the thinnest possible (Kruszyński 1934, 1938, 1939). The quantity of calcium Ca in the incinerated tissues of the kidney is markedly larger than that of iron Fe, of which latter very small quantities were found. Scott (1933) however, using the polarized analysis, a less sure method, saw iron filling the magnitude of the cytoplasm, which does not agree with the results of my investigations. Scott (1933) does not mention at all the appearance of calcium in the kidneys, no does he analyze the other mineral substances seen by him.

Summary

The results of my investigations of the kidneys of the white mouse are as follows:

a) The mineral substance left after incineration of a section of the kidney produces most exactly not only the characteristic

shape and size of its particular portions but also the mineral structure of the nucleus and the cell body.

b) The deposits of the nuclear membrane, chromatin substance and excretion are different in shape, size and colour. Those of the nuclear membrane are arranged in dull white in a ring, those of the chromatin substance present a shapeless snow-white mass, while those of the excretion form tiny granules of waxy-yellow colour.

c) The plasm of cells from the excreting parts of the nephron leaves deposits in the form of networks and striations. In the plasm of duct portions the structure ist quite different; there are spaces with no ashes round the deposits of the nucleus (the descending limb of Henle's loop) or no ashes at all within the whole cell body (the large collecting tubule).

d) The deposits forming the bulk of the cells in the excreting portions and the lumen of the tubules show waxy-yellow granules, which to judge from their localization and quantity may be regarded as the mineral remains of the excretion.

e) The ashes of the tissues of the kidney show a very small quantity of iron, but the quantity of calcium contained in them is considerably higher. The methods available do not permit the determination the place where these two components occur in the living cell.

f) The keeping intact of the mineral structure of the cells during incineration ist dependent upon 1) adequate fixative, 2) thin sections, 3) flattening of the section, 4) a special way of incineration.

To Prof. Hiller, I address my most heartful thanks for his kindness in receiving me in his Institute, where I found excellent conditions for work, and for his readiness to direct me throughout my investigations. I feel indebted also with no less gratitude to Dr J. Kruszyński for a truly friendly assistance in my work.

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Explanation of figures on the Plates 3 and 4

The kidneys were fixed in alcohol mixed with formol in the ratio 9:1, embedded in paraffin, and cut in sections 2 μ in thickness. The pictures from the spodogram were made with the Ultropack Leitz-Wetzlar and Macca apparatus, obj. 75× oel and ocul. 8 comp.. Natural scale (J. Kruszyński 1937). The pictures from histological sections were made with the

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microscope Zeiss and Miflex apparatus, obj. 40× and ocular 15 comp. These sections were coloured with haematoxylin after Carazzi and eosin. The figures are derived from two neighbouring sections, a histological and a spodographic.

Fig. 1. Spodogram of the convoluted tubule fixed in dioxan.

Fig. 2, 3, 4. Spodogram of the convoluted tubules. The thickness of sections $2\,\mu$, $3\,\mu$, $4\,\mu$.

Fig. 5$_I$. Malphighian corpuscle.

Fig. 5$_{II}$. Spodogram of Malphighian corpuscle.

Fig. 6$_I$. Oblique section of proximal convoluted tubule.

Fig. 6$_{II}$. Spodogram of an oblique section of proximal convoluted tubule.

Fig. 7$_I$. Transverse section of proximal convoluted tubule.

Fig. 7$_{II}$. Spodogram of transverse section of proximal convoluted tubule.

Fig. 8$_I$. Longitudinal section of proximal convoluted tubule.

Fig. 8$_{II}$. Spodogram of longitudinal section of proximal convoluted tubule.

Fig. 9$_I$. Longitudinal section of descending limb of Henle's loop (Istmus).

Fig. 9$_{II}$. Spodogram of longitudinal section of descending limb of Henle's loop (Istmus).

Fig. 10$_I$. Transverse section of ascending limb of Henle's loop.

Fig. 10$_{II}$. Spodogram of transverse section of ascending limb of Henle's loop.

Fig. 11$_I$. Transverse section of ascending limb of Henle's loop (thicker portion).

Fig. 11$_{II}$. Spodogram of transverse section of ascending limb of Henle's loop (thicker portion).

Fig. 12$_I$. Transverse section of distal convoluted tubule.

Fig. 12$_{II}$. Spodogram of transverse section of distal convoluted tubule.

Fig. 13$_I$. Transverse section of long portion of descending and ascending limb of Henle's loop from the vicinity of renal papilla.

Fig. 13$_{II}$. Spodogram of transverse section of long portion of descending and ascending limb of Henle's loop from vicinity of renal papilla.

Fig. 14$_I$. Transverse section of collecting tubule.

Fig. 14$_{II}$. Spodogram of transverse section of collecting tubule.

Fig. 15. Spodogram of piece of kidney.

Abbreviations for all figures

as — tubule of ascending limb of Henle's loop.

ar — areas free from ashes round nuclei.

B — wall of Bowman's capsula.

bl — blood corpuscles.

ca — capillary wall.

cl — cell limit.

co — cover cells deposits.

de — tubule of descending limb of Henle's loop.
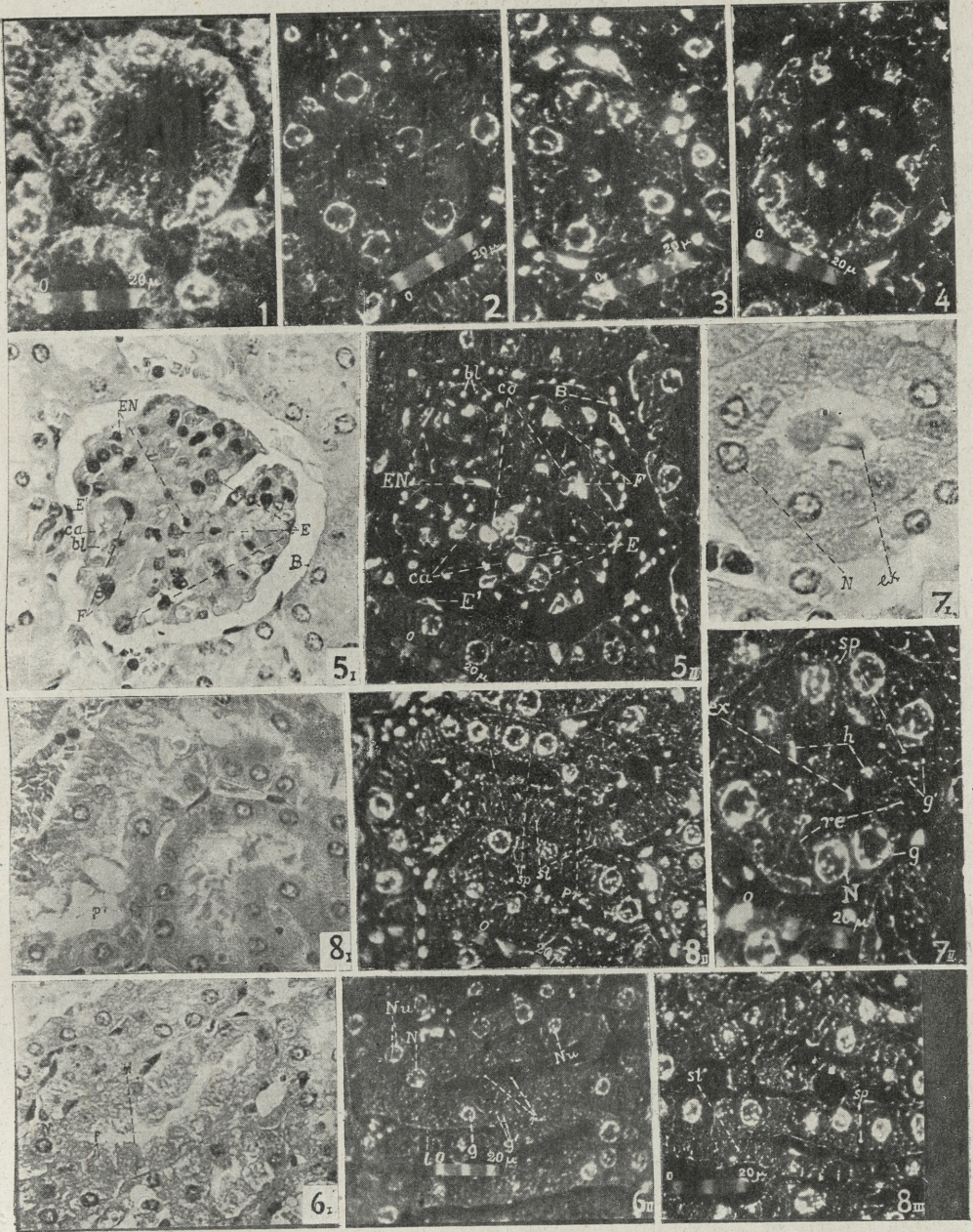
J. Wilburg
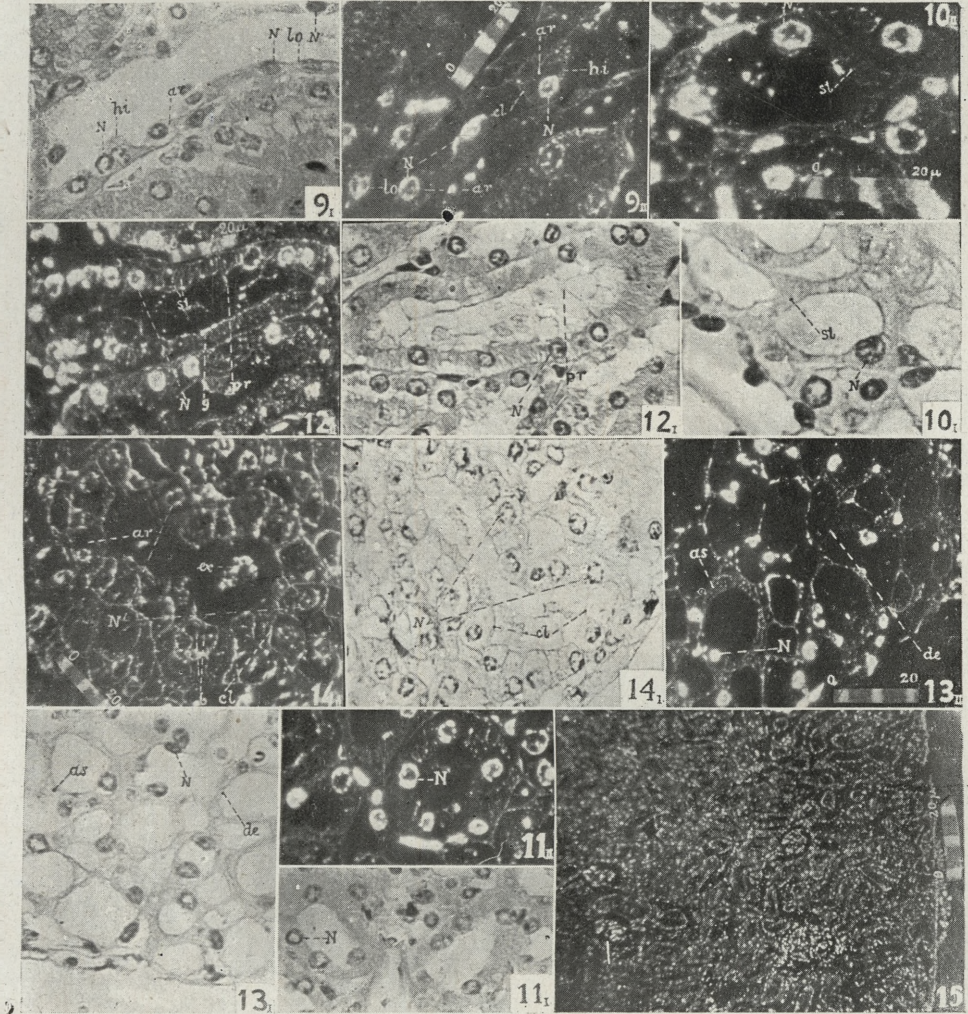
J. Wilburg

E   — nuclei of cover cells.
E′   — nuclei of epithelial cells of capsule.
En   — nuclei of endothelial cells of capillary.
F   — nuclei of fibrocytes.
f   — light and dull spots in cell.
g′   — waxy-yellow granulations.
g   — granulations in the cell body.
h   — clod deposits.
hi   — high cells.
N   — nuclei of tubular cells.
Nu   — nucleoli deposits.
lo   — low cells.
l   — deposits of wall the neighbouring tubules.
pr   — processes of cells.
r   — brush border.
re   — network in cell body.
sp   — free spaces between two neightbouring cells.
st   — striations.

Badania nad zdolnością orientacji i szybkością lotu ptaków. VII. Dalsze spostrzeżenia nad szybkością powrotu do gniazda i nad rolą pamięci w orientacji przestrzennej jaskółek dymówek (Hirundo rustica L.). — Homing experiments on Birds. VII. — Further Investigations on the Velocity of Swallows (Hirundo rustica L.) and on the Role of Memory in their Orientation in space.

Mémoire

de M. **R. J. WOJTUSIAK** et **B. FERENS**

présenté 11 juin 1945 par M. Hoyer m. t.

Problem

In a series of our foregoing papers on the faculty of orientation in space and homing ability of swallows we have shown that the homing velocity of birds is clearly dependent upon the distance (K. Wodzicki & R. J. Wojtusiak 1934, R. J. Wojtusiak 1934, R. J. Wojtusiak & K. Wodzicki & B. Ferens 1937, R. J. Wojtusiak & B. Ferens 1938). The return of swallows was slower when the distance was not great while the velocity increased with greater distances. The increase of homing velocity stood in direct ratio to the distance. The fact could be stated only for distances hardly exceeding 120 km. For distances over 120 km. the homing velocity shoved a slight decrease, kept more or less on the same level, and generally did not exceed 20 km/h. It must be added that in order to simplify the comparison of results the velocity was then calculated without deducing the time of rests, as if the swallows had flied for full 24 hours. To explain the fact, queer enough, that the swallows returned with a greater speed from further distances, we have

assumed (R. J. W o j t u s i a k 1934, R. J. W o j t u s i a k & B. F e-
r e n s 1938) a hypothetical existence in swallows of two sorts of
orientation in space: a) a sense of orientation resp. a sense of
direction, not sufficiently known, acting for all distances little
as well as great, and b) the same plus a memory orientation
consisting in some visual details of the surrounding fixed in the
memory of the birds during their daily flights and operating
for distances below 120 km. The increase of the homing velocity
of swallows in direct ratio to the distances from several up to
120 km. was explained by psychic phenomena occuring with
these birds, especially by the feeling of certainty and uncertainty.
Swallows taken to a little distance from their nests, feel familiar
in a region that is well known to them and thus do not grow
anxious and do not make speed when returning, hunting insects
a. s. o. The further they are taken from their nests the greater
is their restlessnes and their homing impulse influencing their
velocity of return. The distance of about 120 km. at which
expires the first hind of orientation and leaves place exclusively
to the „orientation sense“ has been determined by the experi-
mental results received from about 100 specimens of swallows
by L o o s (1907), W o j t u s i a k & F e r e n s (papers cited above)
and R ü p p e l l (1934, 1936; 1937). The cipher seemed fairly
sufficient to start with. We had however to complete it by the
greatest possible number of data, especially for distances approach-
ing the limit of visual orientation. It seemed also most desirable
to find out whether at distances exceeding 120 km. the homing
velocity of swallows did actually stop to increase.

As we have mentioned in our foregoing papers there is
a great difference between the orientation in space of swallows
and that of carrier pigeons, these latter showing a decrease of
homing velocity in direct ratio to the increase of distance they
are taken away from nests (R a b a u d 1927). The character of
orientation prevailing in pigeons is a visual memory one and
the further the birds are taken from nests the more time they
need to find their home. Carrier pigeons that are to be used
for sending messages should receive a training consisting in
flying many times a given track in order to fix in their memory
its optical details and make then their return so much more
easier. In swallows the question how they learn to remember

their homing track has never been so for investigated. This gave us the impulse to carry out some preliminary experiments in this respect.

The purpose of our investigations has been thus threefold:

1) to test the homing velocity of swallows for distances from 50 up to 260 km. using the greatest possible number of specimens;

2) to investigate whether and to what degree flights repeated on the same track influence the homing velocity of birds, that is to say whether learning and memory play an important role in the orientation of swallows;

3) to ascertain on the basis of our former and present experimental data whether the time of rests introduced into our calculations would have an influence upon the general aspects of the homing velocity in swallows.

For the possibility of carrying out our present experiments we feel indebted to our honourable colleague Prof. Dr. K. W o-d z i c k i who was kind enough to share with us the subsidy received by him from the P o l i s h N a t i o n a l C u l t u r e F u n d.

Material and Method

The experiments have been carried out in the month of June 1938 on C h i m n e y S w a l l o w s (*Hirundo rustica* L.) nesting in the farm buildings of the Agricultural Station of the Jagellonian University at Mydlniki in the vicinity of Cracow. Our former experiments being done in the same locality, some of the swallows were used for a second time a circumstance that was duly marked in our minutes as it could be a hint whether the specimens familiar with that sort of forced flight show some change in their homing velocity.

The birds were caught at the nests, in the evening, provided with rings of the Research Station for the Migration of Birds, State Zoological Museum at Warsaw and marked with coloured inks according to our previous method. During the night the birds were sent by train in cages shut with dark paper to the appointed locality and released. The localities were chosen so as

to represent the greatest possible variety of geographical direction (fig. 1). At the nests the time of return of each swallow was exactly recorded.

In our experiments we have been assisted by a number of persons, to whom we wish to express our most heartful thanks. In particular we feel indebted to Dr. W. Płoski manager of the Agricultural Station at Mydlniki, for the permission to use the swallows nesting at the farm and technical facilities, to Miss K. Sierosławska, Miss S. Skrochowska, Miss K. Wojtusiak, Mrss. M. Bielewicz, A. Miętus[1]) and W. Skorupa for their assistance during the experiments. The necessary meteorological data were furnished by the State Meteorological Institute in Warsaw, through the courtesy of Prof. Dr. T. Banachiewicz Direktor of the Cracow Astronomical Observatory of the Univ., to whom we express here our deepest gratitude.

Experiments

Experiment I. took place June 14. 1939 on the railway track Mydlniki—Cracow—Warsaw. The day before (13. VI.) eleven swallows nesting in the stables of the Agricultural University Station had been caught, ringed and marked with coloured inks. The numbers of the rings are given in table I. All birds were taken in night train (north line) to Warsaw and released in 3 groups. One of the swallows numbered G—66527 that took part in the experiments of 1937 returned to Mydlniki from Vilna.

The 1 group composed of 3 swallows was released at 2·45 in the morning at the railway station Kielce, 105 km. in air line from Mydlniki. Having recovered their freedom the birds flew very swiftly in west direction. The first swallow of this group returned as soon as 10·05 morning. Its homing velocity was 14·316 km/h. Other swallows returned all, the same day in the afternoon. The average homing velocity of this group was 9·396 km/h.

The 2 group composed of 4 swallows was released at 4·30 in the morning, at the railway station Radom, 172 km. in air line from Mydlniki. One swallow of this group was used already

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[1]) Murdered by Germans in 1940.

in 1937 in experiments on the track Vilna-Mydlniki. Having
recovered their freedom the birds flew very swiftly and made
a large circle in north-west direction. The first two swallows
returned only June 15. at 10·30 and 17·35, the two others June
16. The average homing velocity was 4·153 km/h. The »Vilna«
swallow reached 5·730 km/h.
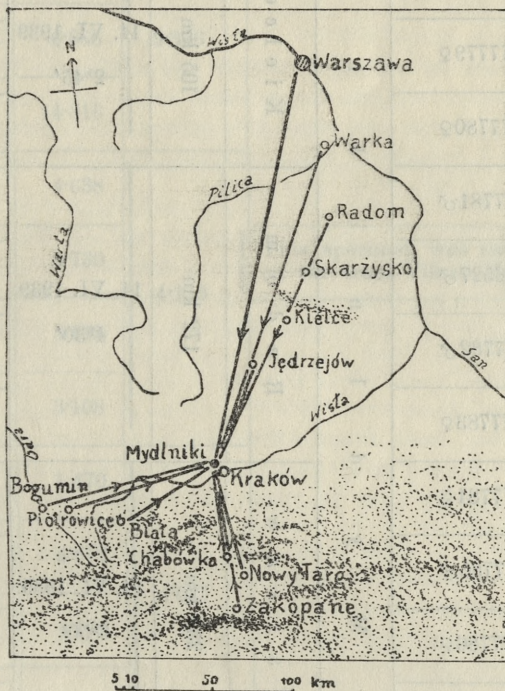
Fig. 1.

The 3 g r o u p composed of the remaining 4 swallows was
set free at 6·50 at Warsaw Napoleons Square (Plac Napoleona)
254 km in air line from Mydlniki. The first swallow returned
only June 16. at 10·35 a. m. covering the whole distance with
a velocity 4·908 km/h. The two others returned also the same
day before noon, the presence of the fourth one was noticed
only June 21 at 11 a. m. The average homing velocity was
4·027 km/h.

T A

Nr.	Ring number and sex	Nesting place	Locality where released	Distance from nest	Data of releasing	Data of return
1.	G—77778 ♀					14. VI. 17ʰ19′
2.	G—77779 ♀		Kielce	105 km	14. VI. 1939 2ʰ45′	14. VI. 18ʰ30′
3.	G—77780 ♀					14. VI. 10ʰ05′
4.	G—77781 ♂	M y d l n i k i				15. VI. 17ʰ35′
5.	G—66527 ♂		R a d o m	172 km	14. VI. 1939 4ʰ30′	15. VI. 10ʰ30′
6.	G—77782 ♂					16. VI. 11ʰ15′
7.	G—77783 ♀					16. VI. 11ʰ50′
8.	G—77784 ♀					21. VI. 11ʰ06′
9.	G—77785 ♂		W a r s a w	254 km	14. VI. 1939. 6ʰ50′	16. VI. 10ʰ55′
10.	G—77786 ♀					16. VI. 10ʰ35′
11.	G—77787 ♂					16. VI. 11ʰ02′

The atmospheric conditions of the day of flight on the track Mydlniki—
Kielce—Radom—Warsaw was for the two extreme localities, as follows:
M y d l n i k i June 13. all day long weather dull with clearings, afternoon
short drizzle, weak SE and SW winds, temperature in average 18·6⁰ C. —
June 14. weather very cloudy with temporal clearings afternoon and even-
ing short drizzle, weak NE, NW and SW winds. Mean temperature 15·6⁰ C. —
June 15. all day very cloudy, weak NE, NW and SW winds, mean tempe-
rature 15·5⁰ C. — June 16. morning and evening very cloudy, clearing at
noon, evening and night thunder, low SW winds, mean temper. 18·1⁰ C. —
June 17. dull with clearings, afternoon and night rain and storm, weak SW

B L E I.

Time of return	Velocity in km/h	Mean velocity in km/h	R e m a r k s
14ʰ34′	7·206		
15ʰ45′	6·666	9·396	
7ʰ20′	14·316		
37ʰ05′	4·638		
30ʰ00′	5·730		This specimen was used in the flight on the track Vilna—Mydlniki in 1937.
54ʰ45′	3·138	4·153	
55ʰ20′	3·108		
172ʰ10′	1·470		
52ʰ05′	4·872		
51ʰ45′	4·908	4·027	
52ʰ12′	4·860		

and NE winds. Mean temper. 20·0⁰ C, — June 18. dull day with occasional clearings, much lightning in the evening, weak W, SE and SW winds, mean temper. 19·0⁰ C. — June 19, morning foggy, many clouds, clearings at noon, thunder afternoon, weak SW winds, mean temper. 19·3⁰ C. — June 20. morning foggy, many clouds, clearings at noon, thunder and heavy rain afternoon and evening, weak W and N winds, mean temper. 18·7⁰ C. — June 21. morning foggy, moderately cloudy with clearings at noon, weak SW, SE and NE winds, mean temper. 19·6⁰ C. — June 22. fine day, afternoon more cloudy and fine drizzle, morning no wind, afternoon weak E and NW winds. Mean temper. 20·4⁰ C. — June 23. morning foggy, afternoon less

cloudy, clearings, weak SW, NW and N winds, mean temp. 14·9⁰ C. — June 24. sunny day, weak NE and SW winds, mean temper. 20·2⁰ C. — June 25. fine day, afternoon distant thunder. weak SE, SW and W winds, mean temper. 20·6⁰ C. — June 26. morning fine, afternoon more and more cloudy, thunder and strong rain, weak SW and NW winds, mean temper. 20·0⁰ C. — June 27. many clouds. shower at noon, weak W, SW and NW winds, mean temper. 16·1⁰ C. — June 28. morning fine, then more and more cloudy, weak N, NE and E winds, mean temper. 15·5⁰ C. — June 29. a few clouds, evening fine, weak E, SW and S winds, mean temper. 18·7⁰ C. — June 30. morning and evening cloudy at noon sunshine, no wind, then weak SE and E winds, mean temp. 23·3⁰ C. — July 1. fine morning, then more and more cloudy, afternoon and night thunder and rain, weak E and S winds turning W and very strong in the evening, mean temp. 24·4⁰ C. — July 2. very cloudy, afternoon rain, weak SW, W and SW winds, mean temp. 15·8⁰ C. — July 3. less cloudy with clearings, weak SW and NW winds, no wind in the evening, mean temp. 14·6⁰ C.

W a r s a w—B i e l a n y : June 14. many clouds and slight fog changing into haze, morning drizzle, weak or moderate NE and NW winds, mean temp. 14·8⁰ C. — June 15. many clouds and fine drizzle, morning foggy, before and afternoon far away thunder, moderate N and NE winds, mean temp. 18·7⁰ C. — June 16. less clouds clearings, moderate NE and E winds, mean temp. 24·4⁰ C. — June 17, more and more clouds, at evening rain and distant thunder, weak winds from E, evening strong wind from NNE and sandy blizzard, mean temp. 21·9⁰ C. — June 18. morning drizzle, clouds gradually dispersing, weak E and SE winds, mean temp. 22·1⁰ C. — June 19. sunny day with a few clouds then growing more and more cloudy, slight fog in the morning, moderate SE winds, mean temp. 24·8⁰ C. — June 20. strong clouds, slight fog in the evening, moderate winds turning from SE to E, temp. 22·8⁰ C. — June 21. morning sunshine, slight fog, very few clouds, weak and moderate winds turning from SE to E, temp. 21·5⁰ C. — June 22. morning foggy, then turning nice, growing more and more cloudy at noon, evening foggy again, weak E, SE and WS winds, temp. 22·4⁰ C. — June 23. very cloudy, moderate N winds, temp. 14·5⁰ C. — June 24. sunshine, moderate SE and SN winds, temp. 20·1⁰ C. — June 25. morning very cloudy with occasional clearings, afternoon rain and far away thunder, moderate SW and W winds, strong wind afternoon, mean temp. 21·0⁰ C. — June 26. very cloudy, afternoon rain, lightning in the evening, moderate S winds, strong wind in the afternoon, no wind evening, mean temp. 20·0⁰ C. — June 27. very cloudy, moderate NW winds, mean temp. 16·8⁰ C. — June 28. morning sunshine, then growing more and more cloudy, light fog in the evening, weak W and SW winds, mean temp. 17·0⁰ C.

The above given meteorological data refer not only to our first but to all experiments carried out in Mydlniki and Warsaw.

As seen from above, the atmospheric conditions of flight on this track were far from favourable. Despite of it all the swall-

ows returned to their nests. Their homing velocity shoved a pronounced decrease when compared with that of analogous experiments in 1937. The unfavourable flight conditions are to be accounted for this decrease.

Experiment II was carried out June 22. on the same railway track Mydlniki—Cracow—Warsaw. The evening of the previous day 15 swallows, caught in Mydlniki and marked were taken by the same night train in the direction of Warsaw. Two of the swallows had been used in the foregoing flight Kielce—Mydlniki. One of them had returned from Radom, and in 1937 from Vilna, the other had been used on the track Mydlniki—Warsaw. These two swallows were to be released at the same localities from which they had returned previously and we expected them to give an answer to the problem whether on a repeated flight of the same track their homing velocity would show some difference that could be interpreted as their learning abilities. Besides these two swallows there were two other specimens that took part in the experiments of the foregoing years, one released 1937 in Gdynia, whose return had not been recorded then (G—66521♀) and another (G—77702♂) whose return had been recorded 1936 from Liszki, and 1937 from Poznań. All these swallows were released in 6 groups.

The 1 group made of 3 swallows was released at 1·57 in the night at the railway station Jędrzejów, 72 km. in air line from Mydlniki (table II). The return of the first swallow was recorded the same day at 11·05 a. m. The homing velocity was in this case 7·878 km/h. The two other specimens returned soon after the first, the same day. The average velocity was 7·422 km/h.

The 2 group comprised but 2 swallows, that were released at 2·45 morning at the railway station Kielce, 105 km. from Mydlniki. Both swallows were flying that distance for a second time. The first returned at 8·00 a. m. the second at 8·10 a. m. of the same day. The average velocity was 12·989 km/h while in the first experiment it was 9·396 km/h.

The 3 group composed of 3 swallows was released at 3·41 a. m. at the railway station Skarżyska, 136 km. from Mydlniki. One of the birds returned the same day, evening at 18·20 p. m. showing a velocity 9,282 km/h. The two other followed the next day. Their average velocity was 5·734 km/h.

T A

Nr.	Ring number and sex	Nesting place	Locality where released	Distance from nest	Data of releasing	Data of return
1.	G—77793♂		Jędrzejów	72 km		22. VI. 11h45'
2.	G—77794♂				22. VI. 1939. 1h57'	22. VI. 11h05'
3.	G—77797♂					22. VI. 12h10'
4.	G—77780♀		Kielce	105 km		22. VI. 10h55'
5.	G—77778♀				22. VI. 1939. 2h45'	22. VI. 10h45'
6.	G—77799♀		Skarżysko	136 km		23. VI. 9h50'
7.	G—77798♀	Mydlniki			22. VI. 1939. 3h41'	22. VI. 18h20'
8.	G—63300♀					23. VI. 19h30'
9.	G—66527♂		Radom	172 km	22. VI. 1939. 4h31'	22. VI. 12h20'
10.	G—77795♀		Warka	210 km		22. VI. 17h50'
11.	G—66521♀					23. VI. 10h48'
12.	G—77702♂				22. VI. 1939. 5h24'	24. VI. 12h15'
13.	G—77796♀					?
14.	G—77792♀		Warsaw	254 km	22. VI. 1939. 7h00'	28. VI. 7h00' (?)
15.	G—77785♂					22. VI. 19h15'

B L E II.

Time of return	Velocity in km/h	Mean velocity in km/h	R e m a r k s
9ʰ48′	7·344		
9ʰ08′	7·878	7·422	
10ʰ13′	7·044		
8ʰ10′	12·852	12·987	Used in the flight on the track Kielce—Mydlniki 14. VI. 1939.
8ʰ00′	13·122		
30ʰ09′	4·506		
14ʰ39′	9·282	5·734	
29ʰ49′	3·414		
7ʰ49′	22·002	22·002	Used in the flights: in 1937 on the track Vilna—Mydlniki and in 1939 on the track Radom—Mydlniki.
12ʰ26′	16·890		
29ʰ24′	7 140	9·286	Used in the flight on the track Gdynia—Mydlniki in the 1937 its return at this time was not stated.
54ʰ51′	3·828		Used in the flights: in 1936 on the track Liszki—Mydlniki and in 1937 on the track Poznań—Mydlniki.
?	?		
(144ʰ00′ ?)	(1·758 ?)	(11·244)	
12ʰ15′	20·730		Used in the flight on the track Warsaw—Mydlniki 14. VI. 1939.

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The 4 g r o u p was made of 1 swallow, released at 4,31 a. m. at the railway station Radom, 172 km. from Mydlniki. The specimen was to make the filght for a second time, and had been used for experiments already three times. The return was recorded the same day at 12·20 a. m. The velocity shown by this bird was this time the greatest of the three experiments, it took part in 22·002 km/h.

The 5 g r o u p composed of 4 swallows was released at 5.24 a. m. at the railway station Warka, 210 km. in air line from Mydlniki. From these birds, only one (used 1937 in the Gdynia experiment) returned the same day at noon, showing a considerable velocity of 16·890 km/h. The two other specimens followed June 23 and 24, the return of the fourth one has not been recorded at all.

The 6 g r o u p composed of 2 specimens was released at 7·00 a. m. at Warsaw (Plac Napoleona — Napoleon Square) and had to cover a distance of 254 km. The swallow that was used on this track for a second time, returned the same day at 19·15 p. m. showing a velocity of as much as 20·730 km/h. The return of the other swallow was noticed only June 28.

In comparing the general results we see that out of 15 swallows there was but one whose return has not been recorded which constitutes a percentage of little importance. The mean homing velocity of birds used for the first time in the experiment did never exceed 20 km/h. In the case of the two specimens used for a second time on the same track, the velocity was higher bud did not exceed 23 km/h. The atmospheric conditions for Mydlniki and Warsaw during the days of flight have been given above. The first day of the experiment, these conditions were rather favourable, the second day much worse, yet better than during our first experiment. Consequently, the return velocity of birds from Kielce and Warsaw is a little higher than it was the case in our experiment of June 14.

E x p e r i m e n t III was done June 24 on the railway track Mydlniki—Cracow—Zakopane. June 23 in the evening 9 swallows were taken by night train in south direction and released in 3 groups in some submountaineous localities of West Carpathians (table III).

The 1 g r o u p comprised 3 swallows, released June 24 at 4·25 a. m. at the railway station Chabówka, 52 km. in air line from Mydlniki. Two of these swallows had been used for experiments, one on the track Jędrzejów—Mydlniki, the other on that of Radom—Mydlniki. Hawing recovered their freedom the birds flew rapidly NE. All specimens returned the same day between 11·55 and 12·40 a. m. The average velocity was 6·684 km/h.

The 2 g r o u p composed of 3 swallows was released at 5·25 a. m. at the railway station Nowy-Targ, 70 km in air line from Mydlniki. All the 3 specimens had been used formerly on the track Warsaw—Mydlniki, one also on the track Gdynia—Mydlniki 1937. Set free, the birds made several circles, one larger than the other and flew North, in proper direction. The return of the first swallow was noticed the same day at 13·05 p. m. The greatest velocity was 9·126 km/h. The two other specimens came back only June 26 and 30.

The 3 g r o u p of 3 swallows was released at the foot of the Tatra-Moutains in Zakopane, 85 km. in air line from Mydlniki. All specimens having made several circles flew North. The return of the first was recorded 12·40 p. m., the two others 18·00 and 18·50 p. m. of the same day. The greatest velocity was 13·596 km/h, the average 9·258 km/h.

Comparing the above data of homing velocity from these places we see that the highest is recorded from Zakopane, the greatest distance, the lowest from Chabówka which is the nearest to the nesting places. The fact agrees with the phenomenon, we spoke of in our former experiments that the homing velocity shows an increase in direct ratio to the distances up to 120 km.

Atmospheric conditions during the days of experiment were in Zakopane as follows: June 24. sunshine, more cloudy in the afternoon, weak NE winds, afternoon strong strokes of S wind, mean temp. 19·4⁰ C. — June 25. morning rather nice, then growing very cloudy, afternoon fairly strong rain and thunder, then SSW winds growing faint gradually, mean temp.17·5⁰ C. — June 26. morning nice then growing cloudy, rain and thunder, moderate SW winds, mean temp. 19·6⁰ C. — June 27 very cloudy, thunder at daybreak, before noon rain weak winds turning from W to N, mean temp. 13·1⁰ C. — June 28. growing less cloudy with clearings at noon, weak EN and E winds, mean temp. 11·1⁰ C.

The atmospheric conditions for Mydlniki have been given above.

T A

Nr.	Ring number and sex	Nesting place	Locality where released	Distance from nest	Data of releasing	Data of return
1.	G—77794 ♂					24. VI. 12ʰ40′
2.	G—66578 ♀		Chabówka	52 km	24. VI. 1939. 4ʰ25′	24. VI. 11ʰ55′
3.	G—77783					24. VI. 12ʰ02′
4.	G—77787 ♂					26. VI. 18ʰ50′
5.	G—77786 ♀	M y d l n i k i — 1	Nowy-Targ	70 km	24. VI. 1939. 5ʰ25′	24. VI. 13ʰ05′
6.	G—66521 ♀					30. VI. (5ʰ25′)
7.	G—66575 ♂					24. VI. 18ʰ50′
8.	G—66576 ♂		Zakopane	85 km	24. VI. 1939. 6ʰ25′	24. VI. 18ʰ00′
9.	G—66577 ♂					24. VI. 12ʰ40′

E x p e r i m e n t IV was carried out June 28. on the railway track Mydlniki—Bogumin. 8 swallows, caught June 27. in the evening were taken next day by an early train in W direction. The birds were released in 3 groups (table IV).

The 1 g r o u p of 2 specimens, one of whom took part in former experiments on the track Radom—Mydlniki, was released June 28. at 14·45 p. m. at Biała, 61 km. from Mydlniki (in air line). Having recovered their freedom the swalows flew South but were drifted to SW by the wind. Both returned only June 30. showing a very low homing velocity 1·281 km/h.

The 2 g r o u p of 3 swallows had been released earlier than the first group it is 8·50 a. m. at the railway station Piotrowice, 96 km. in air line from Mydlniki. All specimens flew South-east.

B L E III.

Time of return	Velocity in km/h	Mean velocity in km/h	Remarks
8ʰ15′	6·300		Used in the flight on the track Ję-drzejów—Mydlniki in 22. VI. 1939.
7ʰ30′	6·930	6·684	
7ʰ37′	6·822		Used in the flight on the track Ra-dom—Mydlniki in 14. VI. 1939.
61ʰ25′	1·134		Used in the flight on the track War-saw—Mydlniki in 14. VI. 1339.
7ʰ40′	9·126	4·130 (3·582)	„ „—„ „
(144ʰ—′)	0·486		Used in the flights: in 1937 on the track Gdynia—Mydlniki and Warka—Mydlniki in 12. VI. 1939.
12ʰ25′	6·840		
11ʰ35′	7·338	9·258	
6ʰ15′	13·596		

Two returns were recorded only July 3. The third swallows has never been recorded and is likely to have gone astray or to have perished. The average homing velocity was very low 0·774 km/h.

The 3 g r o u p composed of 3 swallows was released 9·10 a. m. in Bogumin, 115 km. West in air line from Mydlniki. All the three birds flew South-east. The return of the first was noticed next day 10·20 a. m. The two others only July 3. The highest homing velocity was 4·566 km/h.

Out of 8 swallows used in this experiment, 7 returns were recorded, that is a considerable percentage. Specially characteristic is the low homing velocity rate, much lower than in any other experiments of this and the former years on similar distances.

T A

Nr.	Ring number and sex	Nesting place	Locality where released	Distance from nest	Data of releasing	Data of return
1.	G 66527♂				28. VI. 1939.	30. VI. 18ʰ00′
2.	G—63304♀		B i a ł a	61 km	14ʰ45	30. VI. 10ʰ59′
3.	G—63301♀					3. VII. 12ʰ40′
4.	G—63302♀		Piotrowice	96 km	28. VI. 1939. 8ʰ50′	?
5.	G—63303♀	M y d l n i k i				3. VII. 12ʰ15′
6.	G—77702♂					29. VI. 10ʰ20′
7.	G—66477♀		B o h u m i n	115 km	28. VI. 1939. 9ʰ10′	3. VII. 12ʰ00′
8.	G—66526♂					3. VII. 12ʰ45′

Since the atmospheric conditions, as given below, were during the flight rather favourable, the decrease of velocity must result from the fact that the birds were tired. Because of the difficulties in railway connections, the swallows had namely to remain in cages from about 9 to 14·45 hours and were released late.

The atmospheric conditions in Mydlniki were given above.

From localities lying in the West, at which the birds were released we give only data refering to Bielsko near Biała Krakowska and for 3 first days alone. We could not succeed to obtain meteorological data from other localities. In Bielsko the weather conditions were as follows: June 28. moderately cloudy with occasional clearings, no wind, mean temp. 13·8⁰ C. — June 29. moderately cloudy, clouds disappearing, weak and moderate ES and SW winds, mean temp. 19·8⁰ C. — June 30. sunshine, slightly cloudy, growing more cloudy towards evening, very weak SE and SW winds, no wind in the evening, mean temp. 23·2⁰ C.

B L E IV.

Time of return	Velocity in km/h	Mean velocity in km/h	Remarks
51ʰ15′	1·188		Used in the 3 flights: on the track Vilna—Mydlniki in 1937, and Radom—Mydlniki in 14. VI. and 22. VI. 1939.
44ʰ14′	1·374	1·281	
123ʰ50′	0·774		
?	?	0·774	
123ʰ25′	0·774		
25ʰ10′	4·566		Used in the 3 flights: on the track Liszki—Mydlniki in 1936; Poznań—Mydlniki in 1937 and Warka—Mydlniki 22. VI. 1939.
122ʰ50′	0·936	2·144	Used in the flights: on the track Cracow—Mydlniki in 1936; and Warsaw—Mydlniki in 1937.
123ʰ35′	0·930		Used in the flight on the track Vilna—Mydlniki in 1937.

Discussion of results

The experiments presented above are based on 43 specimens of swallows. This number constitutes 40% of the general number of birds used in all former experiments, ours, as well as those of other authors. Out of these 43 swallows the return and velocity data of 41 specimens were obtained, that is 93%. The return of two specimens only could not be observed. The results are among the best of all experiments made thus far.

Comparing the data of our experiments for distances 52—254 km. with the corresponding data of former experiments we see clear, that the homing velocity was by no means higher than that of previous experiments. In general the data are even slightly lower than we could expect. As already emphasized the unfavourable atmospheric conditions are to be accounted for.

There are 3 cases only (fig. 2) in which the points expressing the highest velocity must be placed higher than the line drawn 1938 for maximal velocities on various distances. Two of these 3 cases refer to swallows Nr. G—66527 and Nr. G—77785 which were used twice on the same track. These two cases will be discussed below. The velocity of the third swallow Nr. G—77795 though higher is not much different from those examined in our former papers.

Our last experiments confirm thus our former results. The homing velocity of swallows shows an increase in direct ratio only to the distances up to 120 km. Above that limit the homing velocity ceases to increase and when calculated according the method of our former experiments does not exceed 20 km/h.

As for the ability of learning the track and for the memory of swallows, our experiments supplied us also with a material that can provide a ground for further investigations on this matter. We mean the data referring to 4 swallow that have flied the same distance twice. The number seems rather low but can be explained by technical difficulties. Despite of our persistent efforts to catch for our second experiment on the track Mydlniki— Cracow the greatest possible number of birds that took already part in the flight on that track we were unsuccessful to get more than 4 specimens. The majority of the birds were new and not yet used in experiments. Out of the 4 »old« specimens two swallows: G—77780 and G—77778, both ♀ ♀ were released for a second time at Kielce 105 km. from Mydlniki, one Nr. G—66557 a male, at Radom 172 km. from Mydlniki, the last G—77785 at Warsaw 254 km. from Mydlniki. Table V and fig. 3 giving the obtained results, show clearly that the swallows learn the homing track and returning for the second time cover the same distance in a shorter space of time than the first. The only exception was the swallow G—77780 from Kielce, with which the homing velocity was on the second return slightly inferior to that on the first. The difference in this case is quite insignificant and may be regarded as accidental. The second swallow of the experiment Kielce—Mydlniki returned for the second time nearly twice as
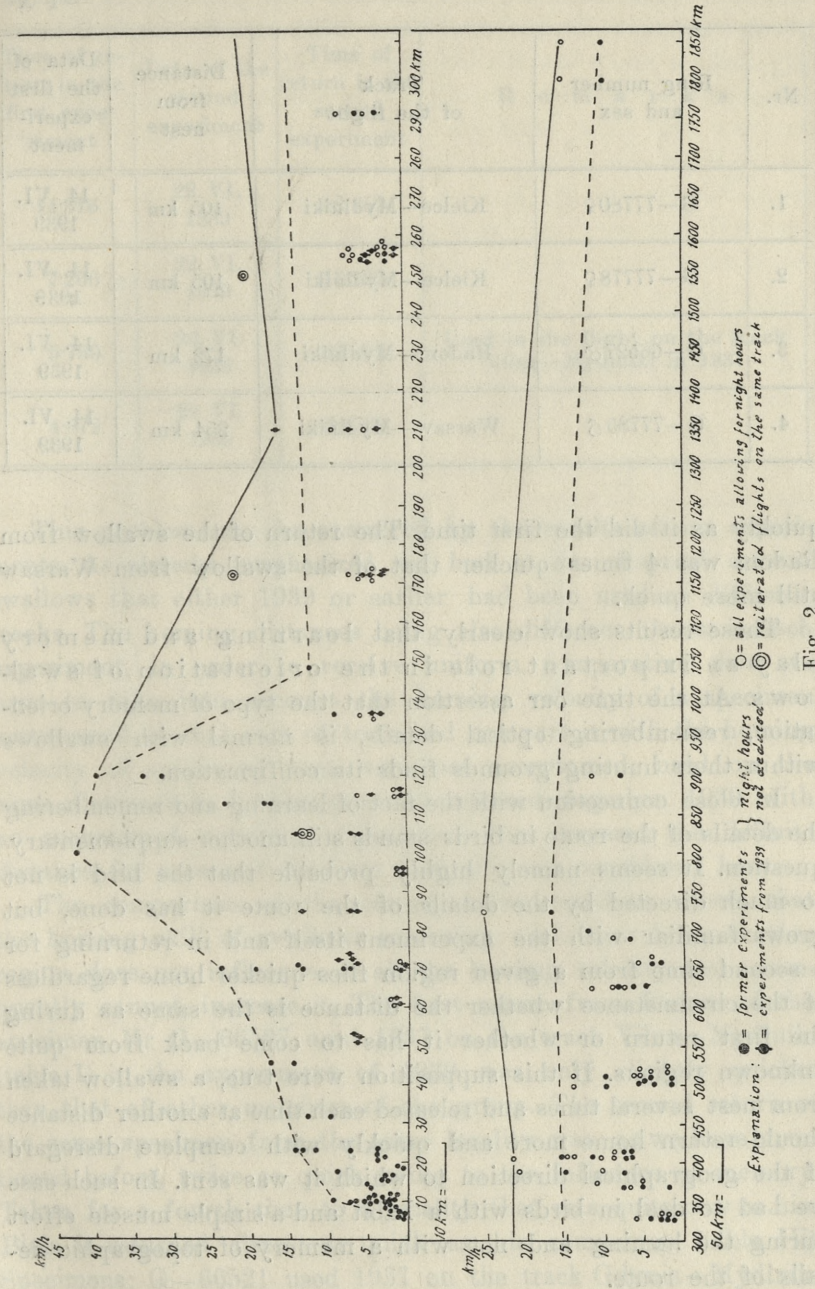
Fig. 2.

Explanation:
○ = all experiments allowing for night hours
◎ = reiterated flights on the same track
night hours { dedicated }
{ not dedicated }
● = former experiments
◆ = experiments from 1939

Nr.	Ring number and sex	Track of the flight	Distance from nest	Data of the first experiment
1.	G—77780♀	Kielce—Mydlniki	105 km	14. VI. 1939
2.	G—77778♀	Kielce—Mydlniki	105 km	14. VI. 1939
3.	G—66527♂	Radom—Mydlniki	172 km	14. VI. 1939
4.	G—77785♂	Warsaw—Mydlniki	254 km	14. VI. 1939

quickly as it did the first time. The return of the swallow from Radom was 4 times quicker that of the swallow from Warsaw still more quick.

These results show clearly that l e a r n i n g a n d m e m o r y p l a y a n i m p o r t a n t r o l e i n t h e o r i e n t a t i o n o f s w a l - l o w s. At the time our assertion that the type of memory orientation, remembering optical details, is normal with swallows within their hunting grounds finds its confirmation.

In close connection with the fact of learning and remembering the details of the route in birds stands still another supplementary question. It seems namely highly probable that the bird is not so much directed by the details of the route it has done, but grows familiar with the experiment itself and in returning for a second time from a given region flies quicker home regardless of the circumstance whether the distance is the same as during the first return or whether it has to come back from quite unknown regions. If this supposition were true, a swallow taken from nest several times and released each time at another distance should return home more and quickly with complete disregard of the geographical direction to which it was sent. In such case we had to deal in birds with a habit and a simple muscle effort during the homing and not with a memory of topographic details of the route.

T A B L E V.

Time of return in the first experiment	Data of the second experiment	Time of return in the second experiment	R e m a r k s
14·316	22. VI. 1939	12·852	
7·206	22. VI. 1939	13 122	
5·730	22. VI. 1939	22·002	Used in the flight on the track Vilna—Mydlniki in 1937
4·872	22. VI. 1939	20·730	

This problem can be answered by the results of our experiments. As already emphasized we had at our disposal several swallows that either 1939 or earlier had been used on different tracks. The homing distances being also different almost in each experiment, in order to receive uniform comparative data we have to take into account the homing velocity of a specimen accustomed to that sort of tests and compare it with the homing velocity of specimens that were not accustomed, used on the same distances in former years or released together 1939 with an accustomed specimen. Specimens that covered the definite distance for a second time are naturally not considered here.

The comparison of the corresponding numbers shows that the homing skill of swallows returning each time from another region does not influence at all the homing velocity and consequently cannot increase it. The first return from Radom of the specimen Nr G—66527 used 1937 on the track Vilna—Mydlniki (table I) in the experiment of 1939 was only slightly quicker than that of other members of the group. The second return of the same specimen from the same locality was, as we have mentioned before, twice as quick owing to the influence of memory. Taken for a fourth time to a locality that was quite new to him Biała it returned almost as quick as his companion (table IV). Specimens: G—66521 used 1937 on the track Gdynia—Mydlniki

and G—77702 (table II) that returned 1936 from Liszki and 1937 from Poznań to Mydlniki showed 1939 on the track Warka—Mydlniki a much smaller homing velocity 7·140 and 3·828 km/h whereas the velocity of a third specimen G—77795 used for the
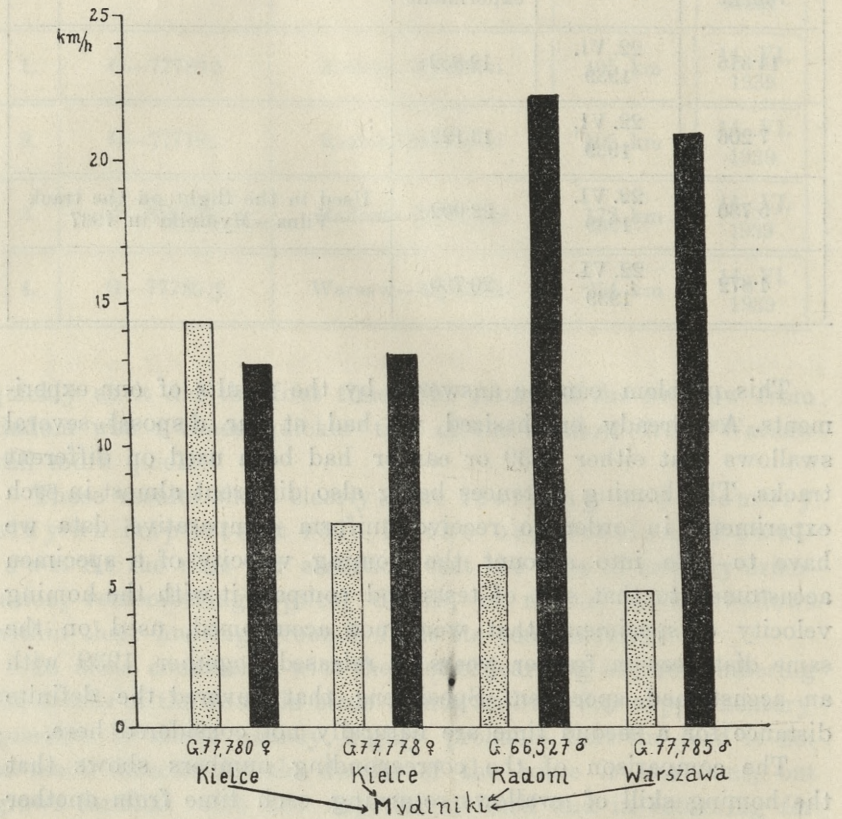

Fig. 3.

The influence of memory upon the orientation in space.
The velocity of the first return — dotted diagrams.
The velocity of the second return dark diagrams.

first time together with them in this experiment, was 16·890 km/h. The same can be noticed in the above mentioned specimen G—66521 used for the third time on the track Nowy-Targ—Mydlniki (table IV). Finally the specimen G—77794 used in the flight Jędrzejów—Mydlniki, in another experiment returned from

Chabówka (table III) with a velocity almost equal or even slightly inferior to that of two other swallows released with him at the same time and at the same locality.

From the above statement it appears that neither specimens used in former years and in 1939 nor those that were used several times in 1939 alone show any increase of homing velocity. This is so much more striking as the majority of »old« swallows used in 1939 had to cover smaller distances than was the case in former experiments and their task seemed thus to be an easier one. We can infer from it only that:

The increase of homing velocity can be observed only with swallows that repeat their flight on the same track and not with specimens that fly every time a new region.

Consequently, we have to deal here exclusively with the phenomenon of learning and the ability of remembering the details of the covered route that have nothing to do with mere taking skill in homing.

The fact that some birds used several times on different distances return later than those used for the first time in our experiments, can be explained easily by a fatigue of a long flight without rests. The role of memory in the homing orientation of swallows used repeatedly on the same track is so much more put in evidence by the fact. Their homing velocity on a second flight shows, despite of their fatigue, a notable increase.

Our experiments should of course be continued. It would be particularly interesting to observe swallows flying the same track more than twice. Such experiments would most probably give an answer for the problem of maximal velocity in these birds.

As we have already mentioned in the introduction of our paper, the homing velocity of swallows was calculated for the whole period between the moment of their release and that of their return to the nest. The time of rest was not taken into account and the velocity calculated as if the swallows had flied for full 24 hours. We have done this because of two reasons. Firstly, the results obtained in this way were much more simple and could be applied both to the birds returning from the nearest and further distances, what enabled us to make the necessary comparisons between them. Secondly, this kind of calcula-

tion was dictated by the difficulty to determine exactly the time
of rest. Further computations that necessarily would approach
the reality only very inadequately, had to be postponed till the
time when we shall be better provided with observation data
from various distances.

The problem seems to be not without importance for the ge-
neral results of investigations concerning the orientation in space
and the velocity of swallows. According to our former night
experiments (1937) it is highly probable that these birds never
return to their nests during the night. On the other hand, the
obtaind homing data for swallows returning from various distances
show that the birds return in one day only from distances up
to 120 – 150 km. Only one specimen covered the distance of
210 km. (Warka—Mydlniki) in one day. (This refers of course
to swallows used for the first time on a given track. On repeating
the flight of a same distance, memory and learning can play
a role). To return from a distance above 120—150 km. the
swallows need more than one day. In this case the night rests
deduced from the time of flight would give data approaching
more the reality. The further the distance the greater is the
number of night rests.

A mere glance on the diagram of the homing velocity of
swallows returning from various distances (fig. 2) suggests the
necessity of a correction. As already emphasized the increase of
homing velocity in direct ratio to the distance can be observed
on distances below 120 km. Above that limit the velocity not
only ceases to increase but on the contrary shows a decrease
keeping for all distances nearly on the same level never exceed-
ing 20 km/h. Since the decrease of homing velocity begins on
distances over 120 km. just those which the swallows cover in
more than one day it seems natural to suppose that the decrease
results from the fact that the night rests of the birds had not
been deduced from their time of flight. Allowing for night rests
would make the time of flight shorter and the homing velocity
higher. The question was whether, in correcting in this way the
calculations the homing velocity for distances above 120 km.
would be equal with the highest velocity data obtained by us
for distances 100—120 km.

In order to find an answer to that question we have computed the homing velocity data of all specimens of swallows used by us since 1933 till 1939, as well as of those used by L o o s and R ü p p e l l in their experiments, whose comparison was made in our former papers. The greatest difficulty consisted in determining properly the night rest. To simplify the task we assumed the duration of day and night as given in the annuals of » *Przyroda i Technika*« for the particular decades of each month. The average length of night for the month of June appeared to be 7 h. 20 m. The results obtained from this computation are presented in the diagram 2 in form of rounds. For comparison, former mean volocities and those obtained in the present experiments (without deducing the night hours) were repeated in this diagram in form of dots. Table VI gives comparison of results obtained from various distances above 120 km. using both kinds of computation. In this table were considered only specimens having shown the greatest homing velocity. As seen from the above given comparisons the mean homing velocity allowance made for the night rests, shows a pronounced increase and a curve delineated trough the points representing the highest volocities lies higher than the analogous checked line taken from our former papers. An equalisation of the decrease of homing velocity (observed in distances above 120—150 km.) is here noticeable but far from being complete. The numbers are altogether lower than those of the homing velocity from distances 100— 120 km. This difference may be easily explained if we assume that the swallows returning from distances over 120 — 150 km. rest not only by night but also during a portion of the day. Birds flying for several days must every day use a certain amount of time for searching food.

Allowance made for all these corrections we receive a clear diagram of the homing velocity of swallows returning from different distances. Up to 120 km. approximately, the velocity increases in direct ratio to the distance, on distances lying between 100 and 120 km. the velocity reaches its maximum point, for further distances up to the greatest ones used in experiments the velocity keeps on the same level. Specimens covering distances over 120—150 km. are directed exclusively by a sense of orien-

T A

Ring number and sex	Track of the flight	Distance in km	The date of flight	The number of night
G—77799♀	Skarżysko—Mydlniki	136	22.—23. VI. 1939.	1
G—77787♂	Radom—Mydlniki	172	14.—15. VI. 1939.	1
G—66521♀	Warka—Mydlniki	210	22.—23. VI. 1939.	1
G—66512	Warsaw—Mydlniki	254	23.—25. VI. 1937.	2
G—77740♂	Lwów—Konary	292	25.—27. VI. 1937.	2
G—66519	Poznań—Mydlniki	332	16.—19. VI. 1937.	3
G—77735♂	Jaremcze—Konary	374	25.—27. VI. 1937.	2
(G—218739♂)	Rheise a. d. Ems—Pausin	390	28.—29. VI. 1934.	1
(G—281378♀)	Bentheim—Pausin	410	28.—29. VI. 1934.	1
G—77713	Gdynia—Mydlniki	504	16.—20. VI. 1937.	4
G—77709♂	Vilna—Mydlniki	628	23.—26. VI. 1937.	3
1.	Bukuresti—Butyny	660	23.—30. VI. 1937.	7
—	Gliwice—Deinste	723	22.—24. VI. 1935.	2
—	Madrit—Berlin	1850	16.—24. VI. 1936.	8

B L E VI.

Time of flight allowing for night hours	Time of flight, night hours not deducted	Time of return allowed for night hours in km/h	Time of return, night hours not deducted	R e m a r k s
30ʰ09′	22ʰ49′	4·506	5·960	
30ʰ00′	22ʰ40′	5·730	7·371	
29ʰ24′	22ʰ04′	7·140	9·516	
46ʰ42′	32ʰ02′	5·436	7·929	
47ʰ45′	33ʰ05′	6·114	8·826	
76ʰ01′	54ʰ01′	4·362	6·145	
46ʰ47′	32ʰ07′	7·992	11·644	
25ʰ35′	18ʰ15′	15·240	21·369	after Rüppell
25ʰ10′	18ʰ30′	15·870	22·161	„ „
102ʰ25′	73ʰ05′	4·920	6·895	
73ʰ25′	51ʰ25′	8·550	12·213	
161ʰ15′	100ʰ55′	4·092	6·004	
42ʰ30′	27ʰ50′	17·010	25·975	after Rüppell
178ʰ00′	119ʰ20′	10·392	15·502	

tation whose nature is wholly unknown to us, and which brings the bird back to the nest with almost the same mean velocity per day. Differences in velocity appearing here should be attributed to external influences chiefly to atmospheric conditions. With swallows covering distances above 120—150 km. for a second time the situation is different. Their sense of orientation is combined with another factor, that of memory leading them by optical details remembered during their first flight of the same track. Accordingly their homing velocity becomes greater than that of specimens returning from the same distance for the first time who are guided exclusively by sense of direction.

The last thing to consider woult be how much the homing velocity from various distances may be influenced by the memory. Swallows released at distances inferior to 120 km. show so much lesser homing velocity the nearer from their nests they had been released. On the contrary the greater the distance the quicker the velocity. To explain this, we keep to our auxiliary hypothesis, that specimens released not far from nests, in a region familiar to them are dominated by a feeling of certainty. The increase of distance causes in swallows a growing anxiety to return to the nest and they return so much quicker the greater the distance. Slow returns are observed up to 120 km. a distance to which the swallows may extend their daily flights and which consequently is well known to them. The area corresponding to this distance can be called their ordinary flight region. Beyond that area, that is over 120 km. the birds try to get back home as quickly as possible. The phenomenon was known to us from our former experiments but only in reference to birds that found themselves for the first time in a »new« region. The results of our last experiments compel us to assume that the same phenomenon repeats with birds released for the second time from the same locality which does not belong to their own area of daily flights and whose distance is above 120 km. In this latter case, instead of a slow return due to the feeling of certainty in an ordinary flight region, the swallow would accelerate his return on his recognizing the way to the nest. We regard this explanation as a supplement to our former subsidiary hypothesis. A better criterion can be furnished only by further investigations in this respect.

Summary of Results

1. Swallows taken from nest to distances from 52 to 254 km. show a homing velocity inferior to 20 km/h. which confirms the fact observed in former years, that the increase of homing velocity in direct ratio to the distance takes place only on distances up to 120 km., the increase on higher distances keeping more or less on the same level.

2. Swallows covering the same distance twice, show a pronounced increase of homing velocity, whereas swallows used for experiments several times but on different distances do not return quicker. Learning and memory seem thus to play an important role in their orientation.

3. Swallows released for the first time in a region that is not known to them at distances above 120—150 km. need more than 1 day to return home. Therefore, besides their time of flight longer night rests and the time necessary to obtain food should be taken into account. It is only when these periods are allowed for that a true aspect of homing volocity can be gained, which at distances above 120—150 km. is equal to the highest rate of velocity observed in swallows returning from distances between 100—120 km.

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specimens through a number of years it was possible to com-
plete what was known so far about the faculty of orientation
in these birds.

To illustrate things better we give here a table containing
all data referring to swallows used repeatedly during several
years. The biological available material was discussed already in
our former papers and what we give here are the most typical
examples. The number of years through which it was possible

[...]

the nesting place for more summer seasons. On comparing the
respective data we see that maximum period is 4 years.
Especially in [...] periods above
4 years, though as mentioned before, our experiments raged in
time between [...] be deduced
from the fact that swallows live in freedom for about 4 years,

When discussing in our former papers the question

Badania nad zdolnością orientacji i szybkością lotu u pta- ków. VIII. — Spostrzeżenia nad przywiązaniem do gniaz- da, wiekiem i zdolnością orientacji przestrzennej dymó- wek (Hirundo rustica L.). — Homing experiments on birds. VIII. — Observations on the nest, the age and the faculty of orientation in space of chimney swallows (Hirundo rustica L.)

Note

de MM. **R. J. WOJTUSIAK** et **B. FERENS**,

présentée le 11 Juin 1945 par M. H. Hoyer m. t.

Several years of experiments upon the homing velocity and
the orientation in space of chimney swallows (*Hirundo rustica* L.) [1]
have furnished us besides the answer of the problem investi-
gated, a number of facts referring to the general biology of these
birds.

Our observations of 1933—1939 concerned mostly swallows
nesting at the Agricultural Station of the Cracow University at
Mydlniki in the vicinity of Cracow. We were able thus to follow
the daily routine of some swallows, ringed by us, for a space of
time longer than one summer season, and could learn something
about their attachment to the nesting grounds as well as make
some inferences regarding the age of the birds. Moreover on
putting together and comparing the observations of the same

one. Despite of the fact that the individuals specified in our

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[1] See our last paper: W o j t u s i a k R. J. and F e r e n s B.: Homing
experiments in birds VII. — Further observations on the homing velocity
and preliminary experiments on the role of memory in the orientation in
space of chimney swallows (*Hirundo rustica* L.). Bull. Acad. Polon. Sc.
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specimens through a number of years it was possible to complete what was known so far about the faculty of orientation in these birds.

To illustrate things better we give here a table containing all data reffering to swallows used repeatedly during several years. The abundant available material was discussed already in our former papers and what we give here are the most typical exemples. The number of years through which it was possible to observe a particular specimen is given in a separate column. Another column gives the direction in which each specimen was taken and from which it had to return.

A glance at the table would tell that the swallows show a great attachment to their nesting places. 10 specimens were stated to return to Mydlniki from their hibernating quarters for at least two subsequent years. Some swallows even appeared at the nesting place for more summer seasons. On comparing the respective data we see that the maximum period is 4 years. Especially interesting it is that we never met with periods above 4 years, though as mentioned before, our experiments ranged in time between 1933—1939, that is full 8 years. It may be deduced from the fact that swallows live in freedom for about 4 years, or at least exceed that period rather exceptionally. The fact is confirmed by Stresemann (1933)[1] who stated that out of 77 chimney swallows ringed as nestlings only one reached the age of 5 years.

When discussing in our former papers the question of the orientation in space in swallows we emphasized that the different groups of birds were taken by us into different geographical directions. The results proved to be identical for the individuals returned to nest with complete disregard of the direction in which they were taken as a group. Yet the question arose whether each particular swallow taken several times from nest and each time in another direction, would return without difficulty. As shown in our table the answer to this question is also a positive one. Despite of the fact that the individuals specified in our table were each time released in another locality all did return to the nest independently of the circumstance whether the lo-

[1] Stresemann E.: Aves. im: Handb. d. Zool. v. Kückenthal, Bd. 7 1933.

T A B L E I.

Nr	Ring number and sex	Nesting place	Date of experiment	Locality where released	Distance from nest	Direction from which the bird is returned		Time of observations
1.	G - 58073		27. VI. 1933	Frydek Č. S. R.	120		WSW	4 years
			26. VI. 1936	Morskie Oko	100		S	
2.	G—66477 ♂		10. VI. 1936	Cracow	6·5		E	2 years
			23. VI. 1937	Warsaw	254		NEN	
			28. VI. 1937	Bogumin	115		W	
3	G—66521 ♀	i	16. VI. 1937	Gdynia	504		N	3 years
			22. VI. 1939	Warka	210		NEN	
			24. VI. 1939	Nowy Targ	70		S	
4.	G—66526	k	23. VI. 1937	Vilna	628		NE	3 years
			28. VI. 1939	Bogumin	115		W	
5.	G—66527 ♂	i n	23. VI. 1937	Vilna	628		NE	3 years
			14. VI. 1939	Radom	172		NEN	
			22. VI. 1939	Radom	172		NEN	
			28. VI. 1939	Biała	61		WSW	
6.	G—77702 ♂	l d	23. VI. 1936	Liszki	10		WSW	4 years
			16. VI. 1937	Poznań	332		NW	
			22. VI. 1939	Warka	210		NEN	
			28. VI. 1939	Bogumin	115		W	
7.	G—77707 ♂	y M	23. VI. 1936	Rybna	16		WSW	2 years
			26. VI. 1936	Nowy Targ	70		S	
			16. VI. 1937	Gdynia	504		N	
8.	G—77713		26. VI. 1936	Morskie Oko	100		S	2 years
			16. VI. 1937	Gdynia	504		N	
9.	G—77701		23. VI. 1936	Liszki	10		WSW	2 years
			16. VI. 1937	Poznań	332		NW	
10.	G—77709 ♂		23. VI. 1936	Alwernia	22		W	2 years
			23. VI. 1937	Vilna	628		NE	

TABEL.

cality was situated on the line north-south, being approximately that of their seasonal migrations, or on the line east-west. The homing faculty and the orientation in space are not confined thus exlusively to territories the birds got familiar with during their seasonal migrations but seem to be grounded in a special unknown homing impulse.

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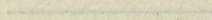
Przyczynki do trawienia żółtka kurzego jaja. — The digestion of the yolk of the hen's egg.

Mémoire

de M. **Z. GRODZIŃSKI**,

présenté le 11 Juin 1945 par M. H. Hoyer m. t.

(Plates 5—8)

Contents

The problem

Virchow (1891), Dubuisson (1906), Remotti (1927—1930) and Thomas (1933—1938) have contributed to our knowlegde of the digestion of the hen's yolk. Owing to their work we are now quite well instructed on the significance of the yolk sac for the digestion of the yolk. They settled that its entodermal cells contain and slowly digest the yolk spheres. During further incubation the same cells secrete into the yolk sac various ferments, which are able to break down the yolk chemically. The derivatives of this complex process are again taken up by the cells of the yolk sac and delivered to the embryo by the intermediation of the blood vessels.

Konopacka investigated histo-chemically the fate of a single yolk sphere during intracellular digestion. Also Thomas paid a good deal of attention to this problem. His observations of the living cells cultivated *in vitro* can be summarized as follows. Roundish, oval or irregular yolk spheres are present within the entodermal cells. He assumed those which were granular and brown to be the yellow yolk spheres and the others, more homogeneous and colourless to be the white ones. Both sorts of yolk spheres are characterized by similar histo-chemical reactions. The intensity of these processes is different, depending on the higher content of proteins and water and the lower content of fat in the white yolk spheres.

The yolk sphere consists, according to Thomas, of the lipoprotein complex, which splits during the digestion into relatively simple substances. Finally there remain numerous drops of fat, which originated in the above mentioned complexes and which consist of glycerides. The boundaries of the yolk spheres are sharply defined against the protoplasm until the moment when the drops of fat are formed. Lipase and protease probably regulate the digestion. The process is of varied duration even in the cells of the same culture. The unbroken yolk spheres take no neutral red; this occurs only after the lipoprotein complex becomes hydrolysed during the digestion. Thomas was able by means of exact histo-chemical methods to discover the following chemical compounds in different stages of digestion: lecithin, cholesterol, fatty acids, glycerids, various proteins, glycogen, iron, peroxidase and phenolase.

Cultivating *in vitro* the tissues from the yolk sac of a chicken (1930). I was able to distinguish the entodermal cells, containing yolk spheres, among other types of cells. I was impressed by the fact, that the yolk spheres possessed fat droplets of different size. I thought (1933), that one of them (with coarse droplets) was a white the others (with the fine droplets) yellow yolk spheres.

Watching one and the same yolk sphere during several days I came (1935) to another explanation of this phenomenon. At the beginning of the observation the yolk sphere contained very fine fat droplets only and its boundaries were sharply defined against the other components of the cell. After several hours the fat droplets fuse into larger bodies and finally into coarse

fat globules. Simultaneously the clearly defined boundaries of the yolk sphere are obliterated. Its granular content spreads slowly within the protoplasm and becomes optically identical with the other fat globules of the cell. The yolk spheres with both fine and coarse granules belong then to the yellow yolk, and exhibit different stages of digestion.

The differences between the views of T h o m a s and myself require some elucidation. They are based on the kind of the material observed, or on the methods of investigation, or on the individual explanation of the observed facts. I repeated than my experiments. On this occasion some details in the morphology of the fresh yolk were worked out. Three sorts of yolk spheres were distinguished (1938): the white, the yellow, and the transitory. The first two are vesicles with the wall of a semipermeable membrane. They contain colloidal protein fluid and fat droplets, differing in size and number. The question remains open, if the protein fluid is purely protein, or if it is formed by lipoprotein complexes, as is accepted for the protoplasm (L e p e s c h k i n) and Golgi appartus (P f u h l). The yolk spheres with numerous but fine droplets of fat and with yellow colouring matter belong to the yellow yolk. The white spheres contain one or a few larger fat drops and lack the yellow colouring matter. Both sorts of yolk, when uninjured take up neutral red and other vital dyes very easily. The elements of transitory yolk are much smaller and consist of a lump of fat, in which pink spots shine. The presence of all components in the yolk and some of their physico-chemical properties were proved unequivocally in different ways.

These observations proved very useful for the research on the digestion of the yolk. The problem was approached along three lines. First of all the intracellular digestion was investigated. Further studies dealt with the appearance of the yolk elements within the yolk sac during the whole time of incubation. Finally the experiments were undertaken in order to elucidate the significance of some ferments for the digestion of the yolk spheres.

The intracellular digestion of the yolk

The tissue culture method is very well suited to the study of the intracellular digestion. The material for cultures was derived from the extravascular area of the yolk sac of the 36—144 hours incubated chickens. Tyrode's fluid was the medium, in which the explantat grows very well at $+37^0$ C, when left in a sitting drop on the coverslip. The method of the double coverslip, that of M a x i m o w (1925), proved favourable. It is namely possible to take the tissue culture with its coverslip after two days growth and to put it into a bath of the Tyrode's fluid for 30 minutes. Afterwards the culture gets a drop of plasma with heparin and can be again put upon the slide, together with the external coverslip. Thanks to this method, one and the same cell is accessible for constant observation, uninterrupted by the usual transferring of the culture. Plasma as a medium stops the proliferation of the cells (F i s h e r & P a r k e r, 1929) and favours the quick digestion of the yolk spheres. One entodermal cell, selected with a plainly visible yolk sphere, was every 3—5 hours drawn with the aid of the apparatus of Abbé or photographed with a »Miflex« camera.

When the cultures were derived from a very young chicken (36—48 hours of incubation) and allowed to proliferate no longer than 40 hours, many cells contained the uninjured yellow spheres (Fig. 1 A, 2 A, 7, pl. 5). They show up against the body of the cell because of their sharp boundaries, fine granulated content and yellow or dusky colour. They are but seldom spherical, mostly elliptical or oviform, depending on the state of the cell stretched out on the coverslip. In cells stretched flat they are elliptical, in others more spherical. When the isotonic medium is replaced by a hypotonic, some entodermal cells round off and their yolk spheres acquire a spherical shape.

All three components of the yolk sphere can be very easily distinguished optically *in vitro*. The sharp, smooth outlines of the sphere indicate the presence of the superficial membrane. In cultures treated with Perenyj's fixative and later with dioxan and Sudan III, the membranes of the yolk spheres become reddish (G r o d z i ń s k i 1938). The homogeneous colloidal protein fluid filling up the yolk sphere takes up some vital basic dyes

e. g. neutral red, nil blue sulphate, brilliant cresyl blue. The spheres observed within the cells are then identical with the yolk spheres contained in the fresh hen's egg. The white yolk spheres appear extremely seldom in the cells proliferating from the explanted yolk sac; their digestion was therefore not investigated.

The progress of digestion is remarkable in the tissue culture especially by the behaviour of the membrane and of the fat droplets. The membrane dissolves on one spot of the yolk sphere, which changes there its smooth outlines into undulate ones, because the fat droplets protrude from the yolk sphere into the protoplasm (Fig. 1 B, 2 B, C, pl. 5). The dissolution of the membrane advances quickly, which uncovers the fat droplets and causes their dispersion in the protoplasm.

Simultaneously with the dissolution of the membrane the fat droplets of the yolk sphere fuse together into bigger units. In different cells they acquire a different size. When the cell is stretched out flat on the coverslip and exhibits just a very little migration, the droplets remain small (Fig. 2 A—D). In other cases the cells being in full movement, the fat droplets fuse into large globules (Fig. 1 A—C). At the same time they intermingle with other fatty inclusions of the protoplasm to such a degree that their distinction from one another is impossible (Fig. 1 D, pl. 5).

I observed the digestion of 20 yolk spheres from the very beginning till the dispersion of the fat droplets in the protoplasm. In the accompanying table ten cases, selected freely from the protocols, are assembled, and the time of duration of some stages

Number of observation		1	2	3	4	5	6	7	8	9	10
Dissolution of the membrane	Hours of observations	14	14	12	25	6	25	20	25	5	28
Coarse fat globules		23	23	16	40	12	32	25	35	7	48
Dispersion of fat		39	63	26	48	32	40	48	121	26	—

of digestion is put together. From this it is seen, that the dissolution of the membrane, the formation of the coarse fat globules and their dispersion in protoplasm lasts for different periods of

time. When the cell exhibits no movement on the coverslip, the
fat drops may fail to disperse in the cell (Nr. 10). The time of
the observed processes depends probably on the state of the
yolk sphere at the beginning of the observation, on the capacity
for digestion of the cell itself and on its mobility. The last factor
influences the above mentioned process mechanically, the first
two physico-chemically. The medium, which consisted in all cases
of Tyrode's solution and plasma derived from the same hen,
exercised an equal influence upon the processes which occurred
within the cells.

The third component of the yolk sphere, the colloidal protein
fluid, becomes red in the tissue culture after addition of neutral
red. The colour varies significantly even in the same culture;
pink, brick-red and dark red are visible (Fig. 3, 7, pl. 5). It depends
on the concentration of colouring matter (M ö l l e n d o r f) and
probably on the physiological state of the yolk sphere (pH — G r o-
d z i ń s k i 1930) In the stained sphere the fat drops appear as
darker spots. During the initial stages of the digestion the appear-
ance of the protein component changes very little optically.
Even after the dissolution of the membrane the dye sticks to the
protein fluid for several hours and covers the fat droplets as
previously. The latter fuse into bigger globules and disperse
within the body of the cell. The fluid stained with neutral red
fills up the space between the colourless fat globules (Fig. 4, 5, pl. 5).
It finally looses its colour, which is ascribed to the incorporation
of the protein component of the yolk sphere into the protoplasm.
The speed of this process is connected with the mobility of the
cell. The currents of the protoplasm accompanying the migration
of the cell carry away the protein content of the dissolved yolk
sphere and facilitate in this way the process of dispersion.

The introduction of neutral red helps to follow the further
stages of digestion, especially of the fat droplets belonging prima-
rily to the protoplasm or derived from the yolk spheres. In the
beginning they do not take up neutral red (Fig. 3). But when
the culture, explanted from a chicken incubated for 36 hours,
survives for 5—7 days, some fat droplets appear, which can be
stained with this colouring matter (Fig. 7, 8, 13, pl. 5). Their
number increases steadily later.

Similar pictures can be seen in the cultures derived from older embryos. Within the cells, cultivated from the yolk sac of the 6 days-old chicken, the uninjured yolk spheres are only exceptionally present, though their remains are very often found. In these cultures neutral red stains the partialy digested yolk spheres in the manner described above and also many fat drops (Fig. 6). Observing some cultures freshly submitted to the influence of the neutral red, it is impossible to foretell which of the fat drops will react to the colouring matter.

It is however possible to make some general remarks valid for this problem: 1. When the protein content of the digested yolk sphere keeps the neutral red, the fat droplet of this sphere do not take up the colouring matter. 2. The biggest fat drops are the last of those in the same cell to take up the colouring matter. 3. The cells of the same culture exhibit one, two, many or all fat drops, which take up the dye. 4. The fat drops of the same culture are stained in lemon-yellow, pink, brick-red and purple. 5. The shape of the fat drops remains mostly spherical, but deformed ones exist also. 6. Besides the normal fat drops other fatty substances appear within the cells. They refract the light in a different way from the normal fat (polarising microscope) and take the shape of elongated bands or of stretched lacunes. They become light pink when treated with neutral red. Their supposed significance will be discussed below in the chapter on lipase.

In the same cells as contain the stained fat drops, exist very often vacuoles reacting also to neutral red (Fig. 12, pl. 5). Some experience is needed in order to distinguish both inclusions of the protoplasm, when stained. The vacuoles are dull, the fat shiny. The first are always separated from each other by a protoplasmic rampart, the latter may move towards and away from each other. The outlines of the fat drops are sharper than these of the vacuoles. Any doubts are cleared up by adding the neutral formaldehyde (10 %) to the culture. In dead cells the vacuoles fade completely the fat drops preserve their colour for several days (observed up to 15 days). The fixing solution of Perenyj, which reacts acidly, discolours instantly the fat drops stained with basic neutral red.

Neutral red reveals then, that during the intracellular digestion a fundamental chemical transformation of the neutral fat of the yolk sphere occurs. Further inferences are based upon the observations carried out with the aid of a polarising microscope. The undigested fat drops prouve in the crossed Nicols to be an isotropic sub-tance, the digested however to be anisotropic. The interpretation of this observation must be made with caution (L i s o n). The fresh neutral fats (glycerids) and fatty acids are always isotrop in the living cells. When digested, the phosphatides or their derivatives are probably responsible for the aniso-tropal light reaction. In any case two sorts of fat are present in one and the same cell, the isotropic which furnishes the material for the production of the other, which is anisotropic.

The yolk within the yolk sac during incubation

The yolk of the freshly laid egg is an emulsion: it consists of a fluid (continuous phase) in which microscopically visible drops of some other fluid are suspended (dispersed phase). The dispersed phase comprises small fat droplets, transitory yolk and the white or yellow yolk spheres (G r o d z i ń s k i 1938). Both the latter bodies also bear the character of an emulsion, consisting of the continuous and dispersed phases, and being separated by the membrane from the rest of the yolk. The yolk is thus a multiple emulsion of the O/W (oil in water) type.

The fresh yolk derived from an unincubated egg consists of a great number of yolk spheres, a few fat droplets and a small amount of suspension fluid. The yolk exhibits a ductile consistence. When observed under the microscope in the undiluted state, the specimen exhibits such an accumulation of the morphological units that their exact outlines can not be established. The flattened and often defaced yolk spheres can be distinguished only on the edge of the yolk drop, which is spread on the coverslip. When some isotonic fluid is added, all yolk spheres become at once visible.

The volume of the yolk increases rapidly during the incubation of the egg. It doubles its former bulk between the 4 th—9 th days, but later on it diminishes rapidly (G r o d z i ń s k i 1934). The remnant of the yolk, which is not utilised by the embryo

during the incubation, remains for a few days in the belly of the hatched chicken. The increase in size of the yolk is performed at the expense of the albumen (Białaszewicz, Byerly), which dilutes the continuous phase of the yolk. The yolk is at this time very fluid and exhibits under the microscope all its morphological units. In the last week of incubation the yolk becomes very thick and turns into a yellow-green grease. It appears in that state under the microscope as a homogeneous non granular mass. After the Tyrode's solution is added, the morphological components of the yolk appear again.

The drops of fat and the white and yellow yolk spheres are present during the whole time of incubation. Till to the fourth day of incubation the white yolk spheres occupy the centre of the yolk (latebra). Later on they spread peripherically and from the 7th day of incubation they are mixed completely with the yellow yolk. The turning every day of the egg in the incubator is probably responsible for this, because it displaces the yolk spheres suspended in the yolk, very fluid at this time.

The number of the yolk spheres decreases continually during incubation, until at the time of hatching they are present only in small numbers. This is due to the membrane of the yolk sphere, which becomes less resistant. Yolk spheres incubated for more than 12 days burst very easily, when placed into isotonic fluid on the coverslip. This happens very seldom with the fresh yolk. It is probably that they also burst in the yolk sac and free their granular content. The result of this process is, that the number of small fat droplets in the yolk increases continually and finally almost no yolk spheres are present in the obliterating yolk sac.

The granular content, especially of the yellow yolk spheres, fades optically towards the end of the second week of incubation and the sphere acquires a turbid appearance. The spheres often become defaced and take an elongated or irregularly curved shape (Fig. 17, pl. 6). When a small amount of water is added to such a specimen some of the spheres then assume rounder outlines and show their granular content distinctly. The usual »vitelloptyse« (Grodziński 1938) may also occur. Turbid yolk spheres, with optically disappeared granules, were also observed, when the fresh yolk samples were prepared in the usual manner on the

coverslip and kept over a long period of time (14—20 days) at
room temperature. The fine drops of water adhered to the glass
slide at the bottom of the chamber containing these samples.
The drops of water probably resulted from the evaporation of
the yolk. It seemed highly probable, that the evaporation changed
the isotony of the sample of yo k into hypertony. This state
of the suspension fluid induced the dehydration of the yolk
spheres and consequently the optical fading of their fat droplets.

In order to check this supposition a crystal of sodium chloride
(NaCl) was added to a sample of yolk spread on the coverslip
and the result was watched under the microscope. The selected
yellow yolk sphere (Fig. 21, pl. 6) reacted against the increase of
osmotic pressure of the medium at first by changing its shape. It
was stretched in one spot, after a few seconds the place became again
rounded off. At the same time its diameter was diminished. The
fat drops faded optically more and finally the whole sphere
became white with dark spots. The yellow colouring left the yolk
sphere and formed for a short time a yellow halo round the
sphere, subsequently dispersed in the fluid medium. The white yolk
spheres (Fig. 20, pl. 6) changed in a similar way. Their diameter
diminished significantly, their fat also faded optically and the
whole sphere became dull white. Both kinds of yolk in the dehy-
drated state can be distinguished by an experienced observer.
The degree of the osmotic pressure attained by the medium in
these experiments was not directly determined. It probably equals
1—2 molar NaCl solution in water, because the yolk spheres pla-
ced in this kind of fluid behave similarly (Fig. 19, pl. 6). For better
understanding of this problem it should be remarked that the
1/6 molar water solution of NaCl is isotonic with yolk spheres.
The appearance of the yolk spheres derived from the yolk sac
towards the end of the second week of incubation also depends,
in the light of these experiments, on their dehydration by the
medium.

The hypertonic fluid also influences the membrane of the
yolk spheres, which is stretched in one or more places into fine
transparent threads (Fig. 18 A, pl. 6), ending often mace-like. They
swing or tremble, they may tear off from the sphere and move
freely in the medium (Fig. 18 B) Their length and strength vary
considerably. Some of them are minute, others are longer than

the diameter of the sphere. After some time they all vanish from the samples under observation.

These threadlike formations appear again in the yolk towards the end (19 th day) of the incubation, especially in the material derived by washing the walls of the yolk sac with the aid of the Tyrode's solution. There are suspended many transparent and strongly refractive threads. Some of them are connected with the yolk spheres.

These formations, experimentally induced and existing in the incubated yolk, are comparable with the myelin filaments. The threads are transitory formations, which disappear from the specimen after some hours. They probably fuse into minute drops, as was observed in a few cases. While sticking to the yolk sphere, they become incorporated into its membrane.

During incubation some fat components absent from the fresh egg in the yolk appear. First of all it must be emphasized, that among the fine fat droplets, situated outside the yolk spheres, great numbers of coarse fat globules are present after the 13th day of incubation. They are doubtless partially derived from the bursted white yolk spheres. They may however originate by the fusion of small drops of fat. Observations of the yolk incubated for more than 15 days support this supposition. Under the microscope the fat globules are visible with small fat droplets adhering to their surface. At the beginning of the incubation no fat drops stick together, although they may come into touch with one anothers. Some of the fat globules have pink spots, rendering them similar to the transitory yolk.

Very characteristic fat bodies appear in the last (17 th—20 th) days of incubation. They are large fat globules (25—50 μ), which are oval, biscuit-like or sharply tied (Fig. 14, 16, pl. 6). They consist of a yellow homogeneous mass of fat, in which pink lines in the shape of circles and elipses are present. Sometimes digitated outgrowths protrude from their surface. In the yolk of the hatched chicken there appear besides that, special bodies of fat (Fig. 15), marked by concentric light and dark rings and by similar but scarce rays. They resemble to a certain degree the Liesegang's rings. Similar formations were found by J. E. S w e e t in the biliary stones of the human liver.

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Some insight into the processes occurring in the yolk during incubation can be gained by adding neutral red to the samples of the yolk of various ages. The uninjured yolk spheres always take up colouring matter, provided the reaction takes place in the isotonic solution. The spheres with the fat droplets more or less faded optically do stain not so well. The dehydrated do not take up the neutral red. Some free fat drops react also to this dye. They are usually middlesized bodies and appear in the yolk from the 17 th day of incubation. The number of these fat drops is small, never exceeding $3^0/_0$ of the total amount of fat. Similar reactions were found in the cultures of the wall of the yolk sac. The reactions of some fat drops to neutral red were ascribed to the transformation of their fatty character by digestion. Neutral red has no influence upon the large homogeneous bodies of fat, whose shape is irregular and which are marked by pink lines. These always keep their yellow colour.

Significance of some ferments for the digestion of the yolk

We used in our experiments two ferments lipase and protease. The first one was Steapsin pulv., manufactured by Dr T. Schuchardt-Görlitz, the other Pepsin, manufactured by E. Merk-Darmstadt. The saturated solution of the steapsin in the Tyrode's fluid was added to the normal samples in the ratio of $4:100$. Freiwald's mixture ($H_2O - 100\ cm^3$, $HCl - 0·05\ cm^3$, Pepsin — 5 gr) was used for proteolitic experiments. Both agents were kept some weeks in the refrigerator at temperature of $+8^0$.

Freiwald's mixture digest the coagulated medium of the culture and also attacks the cells at room temperature, but the process is quicker in the incubator at $+37^0$. After 6 hours some cells are so severely damaged that the yolk spheres protrude out of their bodies (Fig. 23 B, pl. 6). After prolonged digestion all the fat drops of the protoplasm and the yolk spheres fell into the medium (Fig. 23 A). Very often irregular remmants of the body of the cell adhere to the latter (Fig. 23 C, pl. 6). These denudated spheres remain unhurt even after five days stay in the digesting mixture. They preserve their former outlines, but their colloidal protein content becomes coagulated. It does not swell in the hypotonic fluid and does not tear the membrane of the sphere.

Similar results were obtained with the free and fresh yolk spheres. The yolk was diluted with Freiwald's mixture, or the yolk was added to this mixture dried out on the coverslip. After stay of 4—6 hours at $+37^0$, all organic particles of the medium coagulate and stoped producing the Brown's movements. The colloidal protein content of the yolk sphere passes into more solid phase, the fat droplets fade optically at different degrees (Fig. 22 B, pl. 6). Later spherical granulations appear in the white yolk, which surround all the fat drops (Fig. 22 A). This state remains during the whole time of observation; even after 70 hours the spheres are undigested. The hypotonic fluid induces no »vitelloptyse«, because the protein component of the sphere is coagulated. The membrane of the yolk spheres whether free or enclosed in the cell is resistant to the influence of the pepsine. It does not consist therefore of protein molecules only. Provided they are present, they form with the lipoids such complexes, as cannot be destroyed by the ferment used.

The other ferment employed here, steapsin, yielded no satisfactory results when added to the medium of a culture exhibiting a good proliferation. After several minutes the whole zone of proliferation contracts powerfully, that neither cells nor their yolk spheres can be distinguished.

On the contrary, the addition of steapsin to the free yolk proved to be very advantageous. The samples kept in the incubator at $+37^0$ exhibited after 4 hours only a few uninjured yolk spheres. The majority were destroyed, and their fatty content floated as fine or coarse drops in the medium. At the room temperature ($+18^0$) after two days many yolk spheres remained (Fig. 29, pl. 7), after 5 days the sample equaled the drop of yolk which had been kept during 4 hours at a temperature of $+37^0$.

The transformations of the yolk under the influence of the steapsin were more accurately observed while using Eisenberg's heating table for the microscope (E. Leitz). This instrument renders possible the continuous observations at a constant temperature of $+37^0$. After 10—40 minutes some yolk spheres undergo the previously described »vitelloptyse« (Grodziński 1938), later on the rest of them. Their membranes burst in one spot and contract into shadows, after shedding the granular content into the medium. The fat droplets display a tendency to fuse together

when brought into touch with each other. The number of the small ones diminishes, that of the bigger ones increases. The outlines of the latters are often irregular, because the fine droplets adhere to their surface.

The fat drops undergo further changes, which can be followed optically day by day. Their dimensions increase because of their continuous fusions (Fig. 26 A, pl. 7). In the samples extended out upon the coverslip as a shallow drop they acquire the shape of a ribbon. In the same conditions they may also unite into chains or formations resembling the leaves of the clover (Fig. 27). When the thick deep preparations are regularly looked over and shaken on these occasions, the fusion of the fat drops proceeds faster. Their uneven outlines become regular, which may be ascribed to the better liquefaction of the fat substance and to the increase of the surface tension. The action of iodine vapours accelerats these processes. When a tiny piece of iodine is placed in the air chamber, between the slide and coverslip (G r o d z i ń s k i 1938) the whole transformation, lasting normally several days, is accomplished in 20—30 minutes.

Pink spots appear in the majority of the irregular fat globules. Their shape varies from round or oval to that of a narrow ribbon (Fig. 26 B C, pl. 7), extending to the border of the globule. They are abundantly distributed between third and fifth days of digestion at $+37^{\circ}$. Later their outlines fade and about the 12th day just the traces of a few are to be distinguished. This process of disappearance of the pink spots coincides with the acquisition of a spherical shape by globules. The pink spots of the *in vitro* digested fat can be compared with similar formations of fat derived from the yolk sac incubated over 14 days (Fig. 28 C — 14 A, 28 D — 16 B).

In the yolk digested by steapsin during 7—10 days at $+37^{\circ}$ new changes are visible. Round every fat globule, regardless of their shape and diameter a light outer zone forms which refracts light strongly (Fig. 28, pl. 7). When connected with a free swimming globule, it exhibits a sharp, round outline. When however the drop sticks to the coverslip, it takes an irregular shape. In both cases the outer zone increases steadily, always surrounding the included fat bodies on all sides. The material of this outer zone exhibits, as the experiments below demonstrated prove, a fatty

character. It originates from the fat of the yolk, which becomes digested by the steapsin at temperature of $+37^\circ$. This material resembles, optically and stained with neutral red, the previously mentioned fatty lacunes appearing during intracellular digestion. Their identity was chemically not determined, but in any case they are products of the highly advanced digestion of the fat.

The formation of myelin filaments often occurs. In this case the material of the outer zone streches into wide bands, but the fat drop contained within remains spherical (Fig. 30 A, pl. 7). The liberating impulse of these morphological changes is the addition of physiological salt solution or even of distilled water. But the myelin filaments were observed also without this interference.

All applied basic vital dyes (Nil blue sulphate, Brilliant cresyl blue, Neutral red, Brown of Bismark, Methylen blue, Janus green) give a lasting stain to the new fat bodies. The colouring matter penetrates the whole material; even the finest myelin filaments maintain the acquired colour. The addition of neutral formadehyde has no influence upon this. Acid colouring matters (Trypan blue, Trypan red) show no affinity to the digested fat.

Fine, elongated highly refracting needles appear in the liquid medium of the sample from the 7 th days of digestion. Their number increases steadily, they fuse into clusters (Fig. 24) and may adhere tangentially to the fat drops (Fig. 25 B, C). The solution of steapsin alone produces no formations of this kind on the coverslip. They are always present in the yolk digested by steapsin. Their appearance cannot be changed, by cooling to $+18^\circ$ or by heating to $+70^\circ$. The increase or decrease of osmotic pressure is also without significance. They do not take any vital basic or acid colouring matters. In the polarising microscope they prove to be anisotrope

The formation of myelin filaments and crystals and their behaviour in polarised light furnishes some rather uncertain hints on the nature of this fatty matter. Previously it was accepted that cholesterin only produces the filaments under the action of alkalies. Today it is asserted that without fatty acids no myelin filaments exist. Lecithin protrudes them especially easily even in the presence of water (Tunman & Rosenthaler). The needle-shaped cristals of fat originate from saturated fatty acids (Tunman & Rosenthaler) or from phosphatids (Lison).

Because the incubated hen's egg possesses lecithin and its derivatives in the considerable amount (H a n e s) we may assume that in the yolk digested *in vitro* lecithin (phosphatids) is also present. It forms the outer zone (staining, myelin filaments). The denser fat drops contained within take the colouring matter too, which argues agains their being glycerids. The crystals consist either of lecithin and its derivatives or of fatty acids.

The new fatty bodies, which consist of a fat drop surrounded by a lighter outer zone, fuse very easily together. Such a process has been observed several times under the microscope and has once been microphotographically registered (Fig. 32, pl. 7). Two fatty bodies of unequal size approach each other slowly. They come into touch and remain in this position 2—3 minutes (Fig. 32 B). Suddenly the surface of contact of both bodies dissolves and a common outer zone originates (Fig. 32 C), with two fat drops floating within. In a few seconds the latter unite into one sphere (Fig. 32 D) and after a certain period of time the whole body acquires its former appearance (Fig. 32 E). This process lasted 10 minutes at a temperature of $+37^0$. The clearing up of the outer zone continued over one hour. In the description of this process we hawe disregarded the changes in outline of the fatty bodies, the pink spots of the inner fat drop and the transparency of the outer zone. In some samples these processes advanced to such an extent that after 5—10 days in the medium large fatty bodies prevailed, visible with the naked eye as white spots.

Behaviour of fat at various temperatures

The fat of the yolk digested *in vitro* proves to be very sensitive to a lowering of the temperature. The temperature was regulated with the aid of an Eisenberg's electrically heated table. The fatty body interesting us is characterized at a temperature of $+37^0$ by the smooth surface of the inner fat drop and of the outer zone. Clusters of cristals usually adhere to the surface of the fatty body. The temperature is slowly reduced to $+18^0$, which takes 60 minutes. The pink spots appear as the transitory formations (Fig. 37 B, C, pl. 8) in the inner fat drop. Their number

decidedly diminishes at $+ 27^0$. Focusing such a spot, we remark at first that the pink colour changes into white. At the same time the spot steadily diminishes and finally disappears.

At a temperature of $+ 24^0$ the first threadlike cracks project on the surface of the outer zone. At $+ 37^0$ the superficial layer of the outer zone does not differ from the rest of the fat body. It acquires at the reduced temperature the character of a crust, is becoming more rigid than the rest of liquid fatty material. The crust wrinkles at $+ 24^0$ forming cracks (Fig. 37 C, D, E). Similar phenomena can be followed with the naked eye on the surface of the melted paraffin or butter, when cooling. Simultaneously the inner fat drop wrinkles slightly. When the temperature is further reduced new cracks are formed in the crust and small drops of liquid fat protrude on its surface ($+ 21^0$). The latter increase in size parallelly with the wrinkling of the crust ($+ 20^0$), which presses out the liquid fat (Fig. 37 C, D, E). When the sample remains 3 hours longer at a temperature of $+ 18^0$, the changes described gain but insignificantly in degree.

The reactions of fatty bodies observed are much stronger when the temperature drops rapidly. When a specimen of yolk comes directly from the incubator ($+ 37^0$ on to the table of the microscope, it reaches the temperature ($+ 18^0$) of the latter in few minutes. At this moment the fatty bodies have (Fig. 39 A, pl. 8) a crust covered with strong cracks and small fat droplets on their surface. The inner fat drop becomes strongly wrinkled and after a considerable period of time (2—24 hours) divides into fine regular spheres (Fig. 38, 39, C—E). The liquid fat of the outer zone protrudes through the crust in the shape of several big drops (Fig. 39 B, C). It sometimes occurs under high pressure that the pieces of crust with the crystals adhering to them are torn off and removed far from the original position (Fig. 39 D, c). Some of the liquid drops of fat may lose their contact with the crust.

They remain in the medium as independent formations, of which the surface is covered by the usual crust, but they do not contain any more solid fat drop (Fig. 36). The reaction of these fatty bodies to the rapid cooling resembles the changes induced by the slow reduction of the temperature, but however exceeds them in the degree of change. The speed of the transformations renders

it imposible to establish the sequence of the different stages of this process.

The transformations represented above can be reversed. When the table of the microscope is slowly heated $+(30-33)^0$, the crust bocomes smooth and the inner fat drop becomes spherical (Fig 37 F, G). The fine fat droplets of liquid fat which adhere to the outside of the crust vanish completely at $+37^0$; the same holds true of the large fat drops pressed through the crust. The initiation of this process can be ascribed to the increase in fluidity of the fat under the influence of the raised temperature. The currents in the outer zone appear. The transitory drops are also visible developing, becoming displaced and vanishing. They give the pricipally homogeneous and transparent outer zone a spotted appearance, because they refract the light differently (Fig. 37 F, 32 B, C). Gradually the whole fatty body becomes smooth optically. Even the wrinkles of the inner fat drop disappear, a phenomenon connected with the slow currents exhibited by the fat. The whole, inner sphere may move floating and oscillating within the outer zone (Fig. 37 B—E).

In the case when the inner fat drop has been divided into several small spheres, their fusion occurs at a temperature of $+37^0$ after a longish period of time. This process occurs at various speeds even in the same sample of the yolk. The delay in the transformations described may last more than an hour.

The heating of the samples, observable under the microscope, may be accomplished with the aid of the heating table or of an arch lamp. In the second case a Zeiss point microscopic lamp was used, from which Wolfram electrodes emanate intense light and heat rays. A thermometer placed on the table where the preparation was, exhibited in ten minutes a rise of temperature from $+18^0$ to $+30^0$. The lamp was kept at a distance of 15 cm from the mirror of the microscope. With the aid of the lamp the temperature of the sample of the yolk can be easily changed. Putting a flat glass dish filled with water (which works as a heat filter), between the microscope and the source of light or taking it away, induces in 1—2 minutes the changes in the appearance of the fat corresponding to heating or cooling. On the base of these observations the temperature of the curdling of the digested yolk fat was established it lies between $+(21-24)^0$.

The corresponding figures for other sorts of fat are inacessible, therefore I am not able to infer from this temperature the nature of the yolk fat investigated.

Discussion

The digestion of the yellow yolk spheres was studied in the yolk sac, in cultures of the entodermal cells and *in vitro*. The three components of the yolk sphere visible under the microscope, namely the semipermeable membrane, the colloidal protein fluid and the fat drops, behave differently during this process, as could be expected from their chemico-physical properties.

Thanks to the membrane, when uninjured, the yolk sphere exhibits smooth sharp outlines. The membrane dissolves during intracellular digestion and the superficially situated fat droplets form an uneven line with their boundaries. The intracelllar lipase are responsible for the dissolution of the membrane. The protease has no influence upon this process. From this fact we can infer that the membrane consists for the most part of lipoids. The dissolution of the membrane is the first sign, that the yolk sphere has been digested. This process proceeds rapidly.

The behaviour of the fat droplets can be optically followed without difficulty. The fine fat droplets fuse gradually into bigger ones and in many cases 3—5 exccedingly large fat globules take the place of the former yolk sphere. This process starts before the dissolution of the membrane is completed and continues after its disappearance. In consequence, the fat drops spread all over the protoplasm and intermingle with the others fat components of the cell. The fats derived from both sources cannot be distinguished from each other.

The fat drops are so crowded in the free yellow yolk spheres that they are pressed against each other. They can remain in such a state for weeks and do not normally fuse together. The intracellular digestion changes their disposition I have previously (1938) reported on the character of these alterations as follows. The micrurgical experiments of Chambers and Ludford and also my own on the affinity of the fat drops to some vital basic dyes indicate, that a fine film covers their surface. This film is the cause of the fact that the fat drops

do not fuse into larger bodies although they are pressed against each other. When this film is destroyed by iodine-vapour and ether, or by dioxan and mercuric chlorid, the surface tension of the fat drops increases and favourable conditions for the fusion result. During the intracellular digestion the ferments are probably engaged in destroying the above mentioned film and induce the fusion of the fat drops.

This conception remains in full agreement with the present theory of the structure of emulsion. The yolk spheres represent a typical emulsion of the O/W character. The emulgator forms a fine film on the surface of the yolk sphere and of every single fat drop. The fusion of the dispersed phase is induced by its destruction (C l a y t o n), which is not synonymous with the reversal of the emulsion into W/O type. In our case the type O/W is preserved.

The accumulation of fat in the cells of tissue cultures has often been investigated from different points of view. It was been determined among other things, that the freshly proliferated cells contain in the beginning tiny fat drops, which later increase in size (L e w i s, L a u c h e, S z a n t r o c h, Z w e i b a u m, H a s z l e r, W y l e s c h a n i n). H o r n i n g and R i c h a r d s o n often observed *in vitro* the fusion of small fat drops into bigger bodies. These observations can be satisfactory explained only in the light of the theory of emulsion.

In the first stages of digestion the membrane of the yolk spheres dissolves and the fat drops fuse into bigger globules. During the further stages of digestion the chemical properties of the fat are changed, as it is proved by its affinity to neutral red. Before discussing this problem, the fate of the third component of the yolk sphere — of the colloidal protein fluid — will be considered.

This substance is entirely homogeneous and transparent. As also its index of light refraction also equals that of the protoplasm, this fluid cannot be optically distinguished from the body of the cell, when shed out from the yolk sphere. It reacts however positively to several basic vital dyes, which do not stain the protoplasm. The uninjured yolk sphere colours uniformly and intensively. During and after the digestion of the membrane of the yolk sphere the colouring matter fades slowly. This process

is connected with the currents in the protoplasm, which aid the diffusion of these components of the yolk into the protoplasm.

My observations confirm those of Konopacka. She established that the yolk spheres contained by the entodermal cells consist of two main components: fat drops and protein substance, which are connected with phosphatids. The latter take part in the production of protoplasm and nucleins. After a prolonged incubation no traces of these substances can be detected within the cell, even with the best histological methods.

When the membrane of the yolk sphere is dissolved and no reaction occurs between its protein remnants and neutral red, the entodermal cells of the yolk sac exhibit a peculiar appearance. Numerous large fat globules fill up the protoplasm with the exception of its peripheric parts and the place occupied by the nucleus. They react very easily to Sudan III and OsO_4, they do not show affinity to neutral red. In the polarising microscope they exhibit isotropic qualities. From all these observations it may be infered, that the fat drops consist of neutral fat (glycerids).

The morphological properties of the fat remain unchanged for a fairly long period. Its physico-chemical qualities show a remarkable transformation, which is manifested by the affinity to basic vital dyes e. g. to neutral red. Some small and middle sized fat bodies react in this way in young cultures, in older cultures, and especially those derived from the chicken of 6—10 days of age, the reactions become more frequent.

When a series of cultures of the same origin are treated with neutral red at intervals of a few hours, different numbers of fat drops become stained. They appear in the young cultures in some cells only and as single inclusions. In the suitably old ones they are numerous in all cells. The experiments carried out *in vitro* on the influence of lipase upon the yolk spheres, gave similar results; after the destruction of the membrane of the yolk sphere the fat drops take neutral red after some period of time. The affinity of the fat drops to neutral red is a sign of advanced digestive processes within the cell, which are dependent on the action of lipase. One is obliged to assume that the entodermal cells produce this lipase. A part of it is secreted into the yolk sac, where the usual digestion is performed. The problem remains undetermined whether the entodermal cells produce these ferments

during the whole time of incubation, or this production varies with the age of the cell.

The yolk digested by the lipase *in vitro* acquires under certain conditions a spherical shape and consists of a drop of fat, which is surrounded by an outer zone of more liquid fatty substance. It resembles in this state, when superficially observed under the microscope, the white yolk spheres. Both formations are however fundamentally different, as is visible from the accompanying table. The yolk spheres are emulsions of the O/W type, the continuous phase being the protein fluid, and the dispersed, the neutral fat. The others result from the yolk spheres digested by lipase and consist of fat substances only. This kind of fat differs profoundly from the fat in the yolk sphere, which is manifested by its affinity to the basic vital dye, by its behaviour in polarising light and its behaviour at various temperatures.

The enumerated characters serve as a basis in determining the chemical nature of the new fat, which is derived from gly- cerids probably with the addition of small amounts of cholesterols and phosphatids. The new fat is not uniform, it appears in three forms. The needle-like crystals adhere to the surface of the fat bodies in bundles or singly ones. The very liquid outer zone shares, by the formation of myelin filaments and the reaction to polarised light, the properties of liquid crystals. The central drop of fat is more ductile, forms no myelin filaments, and at a temperature of $+21^0$ to $+24^0$ acquires no crust. It reacts however to neutral red similarly to the outer zone and behaves in the same way in the polarising microscope.

These three forms of the new fat can be differently estimated. Either they form three phases of the same chemical matter, or their chemical nature is different. In the first case we should like to ascribe to them the character of phosphatids, probably of lecithin. In the second the crystals can be considered as fatty acids or lecithin and the remainder as similar or different phosphatids.

Similar bodies to the final products of yolk digestion *in vitro* appear within the cells of the yolk sac and in the sac itself. The entodermal cells digest the included yolk spheres and convert the drops of neutral fat into two kinds of bodies. Some are spherical drops, which react very easily to basic vital dyes. The

Inducing factor	Free yolk spheres	Fat of yolk digested *in vitro*
Temperature + (21°—24)°	— — —	Curdling
Hypertony	Optical fading of the fat drops	— — —
Iodine-vapour	The membrane of the sphere expands, the protein component coalugates, the fat drops stain brown	The whole body fuses into one sphere and stains brown (Fig. 35, pl. 8)
Ether Dioxan	Vitelloptyse as a result of the dissolution of the membrane	The whole body fuses into one sphere (Fig. 33, pl. 8)
OsO₄	The membrane and fat drops stain, the protein component coagulates	The whole body acquires a brown colour; no changes in outline
Nile Blue Sulphate Brilliant Cresyl Blue Neutral Red Brown of Bismark	The yolk spheres are stained. After the addition of formol, they become colourles	All colouring matters give stains. Addition of formaldehyde without effect (Fig. 31, 34, pl. 7, 8)
Methylen Blue Janus Green	They yolk spheres are stained after a longer period of time. After the addition of formaldehyde, they become colourless	As above
Trypan Blue Trypan Red	The fat drops of the burst sphere take the stain	— — —
Sudan III	Only the fat components are stained	The whole body is stained (Fig. 33, pl. 8)
Polarising microscope	Isotropic substance (18—60)°	Anisotropic substance
Fat crystals	— — —	Appear singly or in bundles

others display similar affinities to the colouring matter, but are
more liquid and spread as lacunes between other components
of the cell (Fig. 5, 11 b, pl. 5). Similar bodies were found in the
yolk sac itself. In the cells no myelin filaments and crystals were

observed. According to T u n e m a n n and R o s e n t h a l e r this cannot be expected before the fatty matter lies outside the cell. The fatty matter developed experimentally *in vitro* and *in vivo* derive from the same initial substances (yolk) and exhibit similar reactions. Therefore they are recognized as chemically similar or even identical substances. The views on the chemical nature of the yolk digested *in vitro* as represented above are fully valid for similar formations *in vivo*.

T h o m a s relates that the digestion of the white yolk spheres occurs in a similar fashion to that of the yellow. In my cultures the white yolk spheres appeared very seldom, therefore their digestion was not investigated. Generally speaking, it seems that the views of T h o m a s and myself on the structure of the yolk spheres are not identical. T h o m a s asserts that the yolk spheres consist of the liquid lipoprotein complexes, which in the yellow are granular and in the white more homogeneous. He does not mention the presence of the membrane, he just remarks that the yolk sphere (enclaves de vitellus) possesses sharp outlines, at the beginning but later no exact boundaries are visible.

In his pictures various formations are present. One of them (Pl. III 1, 2. Pl. IV 3) corresponds exactly with our conception of the yellow yolk sphere. But not all of them are in a perfectly uninjured condition. The others (Pl. VI. VII) are merely the accumulation of the fat drops, which resulted in our experiments from the digested yolk spheres and from the fat originally present in the protoplasm.

According to T h o m a s the fat drops do not appear in the yolk spheres till the lipoprotein component becomes hydrolised and forms the final product of these complexes split up by digestion. Contrary to this, we have seen the fat drops in the fresh yolk spheres from the very beginning. T h o m a s seems to ascribe a great value to the reaction of the yolk spheres to neutral red. He repeats several times (Pl. 264, 289, 383) that those yolk spheres, which undergo the digestion within the cell take neutral red. He failed (Pl. 264) to stain the spheres from the fresh yolk with the same colouring matter. But he succeeded in doing so with the yolk from an egg incubated for 8 days.

In reality all yolk spheres from the freshly laid egg and from the entodermal cells stain with neutral red. They must

be however uninjured, i. i. surrounded with the membrane (G r o d z i ń s k i 1938). The fresh and undiluted yolk is not suitable for the investigation of its morphological components, because the yolk spheres are so crowded together that it is almost impossible to distinguish them optically. By the addition of some isotonic dilutor to the yolk, the yolk spheres reacting to neutral red become revealed. In the case of T h o m a s observations, on the yolk spheres from the yolk sac, a similar explanation is valid. During incubation between the 4 th—9 th days the yolk becomes diluted at the expense of the albumen and the yolk spheres appear distinctly. T h o m a s was able to stain them at this time, but he thought that it was because the properties of the yolk were changed by digestion.

The observations of T h o m a s, that the digested yolk spheres when present within the entodermal cells cultivated *in vitro* take up colouring matter, were confirmed in some respects by me. 1. The red drops in his pictures (especially Pl. XXV, 1) probably correspond to the fat drops, which react to neutral red having just been digested by the lipase. 2. The protein content of the sphere deprived of the membrane also takes this dye for a short time. 3. Among the pictures of T h o m a s (Pl. XXIV, 4, 5) they are some bodies, which were also observed by me. He draws large vacuoles, oval or irregular in shape, not containing fat drops, which are for me enigmatical. They react to neutral red and exhibit a few fine red granules. They seem to possess a fine membrane. Their derivation and further fate is unknown to me.

The observations of T h o m a s are very precise and deserve no critisism, but his interpretation is not satisfactory. In his cultures he found exceptionally the unbroken yolk spheres, and frequently their derivatives resulting from the digestion. In his studies he missed then the early stages of digestion, though he analysed the later very well.

Summary

The yolk of the hen's egg is an emulsion of the O/W type. The continuous phase consists of a protein colloidal fluid. The dispersed phase is formed by the fat drops (glycerids). The yolk sphere consists of both phases and of the superficial semi-permeable membrane.

The digestion of the yolk spheres was studied *in vitro*, in the incubated yolk sac and within the entodermal cells explanted from the yolk sac for cultures. The results of these threefold investigations agree with each other.

The lipase dissolves the membrane of the yolk sphere and uncovers its content.

The protease acts upon the continuous phase of the emulsion. Its incorporation in the protoplasm was followed. The chemical transformations connected with this process were not studied.

The lipase strongly influences the fat drops. At first it destroys the emulgator, which covers their surface with a fine film. As a result of this the small fat drops fuse into bigger bodies. Simultaneously it transforms them chemically from glycerids probably into the phosphatids.

Various properties of both kinds of fat were established: 1. their affinity toward some colouring matters, 2. their behavior in polarised light, 3. influence of some fat solvents, 4. the reactions to hypo- and hypertonic solutions, 5. the resistance to the exposure to a temperature of $+(18 - 60)^0$.

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Explanation of figures on the Plates 5—8

All pictures were taken with the aid of the »Miflex« apparatus, manu-
factured by Zeiss. The pictures representing similar observations are formed
into groups under one number and are marked with Latin capital letters.
The small letters are reserved for special details in the morphology of the
cell and the yolk sphere. The figures 1, 2, 32, 37, 39 contain series of
snapshots, which represent some continuous process.

The black lines drawn on the tables correspond to a length of 30 μ in
the photographed objects. The numbers in square brackets concern the
protocol of the experiment.

1. The digestion of a yellow yolk sphere with fine fat drops, contained
by the entodermal cell migrating on the coverslip. A — smooth outlines
of the sphere, B — the membrane is dissolved after 7 hours, C and D —
the fat drops fuse into bigger bodies after 19—25 hours (Nr 238, 239,
245, 251).

2. The digestion of a similar yolk sphere, present in the cell, which
migrates very slowly in the culture (A). The dissolution of the membrane
clearly visible (arrow — B, C). The fat drops fuse only to a limited degree
(Nr 186, 193, 195, 196).

3. The undigested yolk sphere, stained with neutral red (a), a 3-day old
culture obtained from a chicken incubated for 64 hours. No fat drops in the
body of the cell react to the colouring matter (Nr 377).

4. The same culture. The cell contains the yolk sphere deprived of the
membrane. The digested yolk sphere takes up the dye and deposits it
between the colourless fat drops (Nr 376).

5. The digested yolk sphere within the cell. Neutral red is present between the fat drops (a) and in one fat body stretched as a lacune (b). A four-day old culture from a 56 hours incubated chicken (Nr 358).

6. Many fat drops of the entodermal cell are stained with neutral red and appear black in the picture. A four-day old culture from a 144-hour incubated chicken (Nr 366).

7. The culture described under number 3. Within the cell the yolk sphere (a) and one fat drop (b) of the cell body react to neutral red (Nr 378).

8. In a ten-day old culture, derived from a 56 hour incubated chicken, cells are present with large bright (a) and small fat drops (b) reacting to neutral red (Nr 381).

9. One entodermal cell in a six-day old culture, from a 104 hour incubated chicken, contains numerous neutral red vacuols (a) and bright (b) fat drops (Nr 386).

10. In a cell belonging to the four-day old culture derived from a 144 hour incubated chicken, a fat body (a) stretched as a lacune and coloured with neutral red is present, beside the usual bright fat drops (Nr 367).

11. Culture from the same series. Some fat drops of the digested yolk sphere (a) and a fatty body stretched as a lacune (b) react to neutral red (Nr 379).

12. The same series of cultures. Neutral red vacuols (a) and one fat drop stained with neutral red (b) within one cell (Nr 380).

13. The culture, five days old, derived from a 56 hour incubated chicken. The small fat drops only (black in the figure) react to neutral red (Nr 363).

14. Different morphological types of fatty bodies from the incubated yolk sac. Some (D, E) exhibit red spots. They do not react to neutral red and preserve their natural yellow colour. A — 17, B, C, D, E — 19 days of incubation (Nr. 142, 121, 391, 389, 390).

15. Fat body from the yolk sac of a freshly-hatched chicken (Nr 211).

16. Other fatty bodies from the yolk sac of 19-(A) and 17-(B) day incubated chickens (Nr 394, 146).

17. The defaced yellow yolk spheres from the 17-(A) and 21-(B) day incubated chickens. The fat droplets optically faded (Nr 407, 217).

18. Myelin filaments formed by the membrane of the yolk sphere in the hypertonic fluid. A — the filament adheres to the yolk sphere. Molar solution of the glucose. B — free filament executing movements. 3 molar solution of NaCl (Nr 93, 106).

19. Yellow yolk spheres defaced by the molar NaCl solution, with optically faded fat drops (Nr 99, 98, 94).

20. White yolk spheres, which were exposed to a slow increase of the osmotic pressure. A — at the end, B — at the beginning of observation (Nr 410, 411).

21. The yellow yolk sphere in the same condition. A — beginning, B — end of the experiment (Nr 414, 412).

22. The influence of pepsin on the white (A) and yellow (B) yolk spheres (Nr 257, 348).

23. The same conditions for the yolk spheres in tissue culture. The cells are completely (A) or partially (B, C) digested, their yolk spheres remain uninjured a, — yolk sphere, b — rest of the body of the cell (Nr 133, 134, 218).

24. The bundles of the needle-like crystals which originated from the yolk digested by steapsin (Nr 415, 347).

25. The crystals developed in similar conditions (a), which adhere to the fat drops radially (A) or (B, C D) tangentially (Nr 293, 290, 292).

26. The first phase of the influence of steapsin upon the fat of the yolk spheres at a temperature of $+ 37^{0}$. A. — the fine fat drops fuse into bigger ones, — B, C they increase steadily and acquire the appearance of fat bodies with red spots (Nr 170, 267, 266, 116).

27. Similar processes, which occur in a shallow drop on a coverslip. The fatty bodies adhere to the coverslip. A — resembles the clover-leaf, B, C — fat bodies form chains (Nr 278, 269, 262).

28. Later phase of transformations of the fat under the influence of steapsin at $+ 37^{0}$. A bright outer zone (a) is formed round every fat drop (C, D, E) or round several drops together (Nr 370, 362, 346, 396, 369).

29. The yolk after 4 days exposure to the influence of steapsin at $+ 18^{0}$. Small fat drops scattered round a yellow yolk sphere under digestion (Nr 256).

30. The new fatty bodies resulting from the influence of steapsin, stained with Janus green. B — through the crust (b) protrude small green droplets. A — they form myelin filaments (Nr 400, 402).

31. Methylen blue stains similar bodies, a — the fat of the outer zone protrudes through the (b) crust, c — the inner fat drop (Nr 406).

32. The fusion of two fatty bodies. A, B — two bodies come into contact, C — their outer zones and the crusts fuse, D — the inner fat drops unite, E — the outer zone spreads and becomes optically smooth, a — inner fat drop, b — outer zone (Nr 319, 320, 321, 322, 323).

33. Cold dioxan causes the fusion of both components of a fatty body (similar to 32 A) Sudan III stains it simultaneously. The crust remains unchanged (Nr 351).

34. Similar fatty bodies are stained with Nile blue sulphate.. The addition of 40% formaldehyde has no influence upon their colour, they merely become round (Nr 405).

35. A similar body fuses under the influence of iodine-vapour to a brown sphere (Nr 399).

36. The isolated portion of the outer zone of similar fatty body submitted to room temperature (Nr 334).

37. The slow lowering of the temperature of a similar fatty body. A — the inner fat drop (b) and the outer zone (a) at $+ 37^{0}$ are smooth, B — the first red spots appear at $+ 21^{0}$ in the fat drop, also the first cracks in the crust. C — the fat drop wrinkles at $+ 19^{0}$, D, E — upon the crust appear at $+ (18—10)^{0}$ cracks and fine droplets (c), a portion of the outer zone (d) is pressed through the crust, F, G — the outer zone acquires at

+ (30—37)⁰ its original shape after having exhibited strong internal currents (Nr 326, 327, 328, 329, 331, 332, 333).

38. A similar fatty body preserved at + 18⁰ during two weeks. The inner fat drop was divided into several small ones (Nr 345).

39. The rapid lowering of the temperature from + 37⁰ to + 18⁰. A — crust with cracks and fat droplets (a), bundles of needle-like crystals (c) adhere to it, the internal fat drop (b) wrinkled. B, C — portions (d) of the outer zone are pressed through the crust, D — the inner fat drop divides into several drops, the crystals (c) are carried away together with the crust (d). E — a secondary crust covers the whole body again (Nr 335, 336, 338, 340, 343).

Z. Grodziński

Pl. 6.
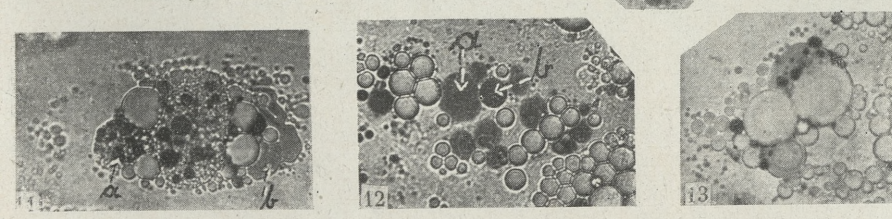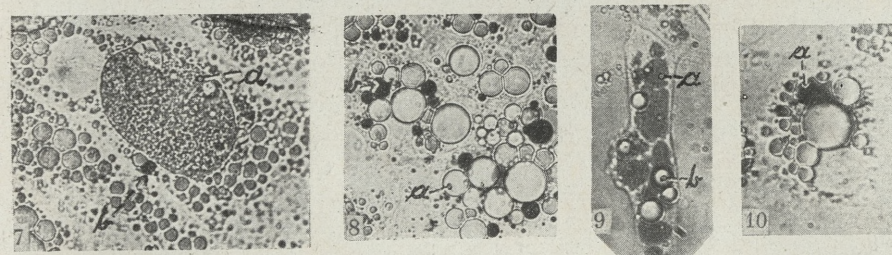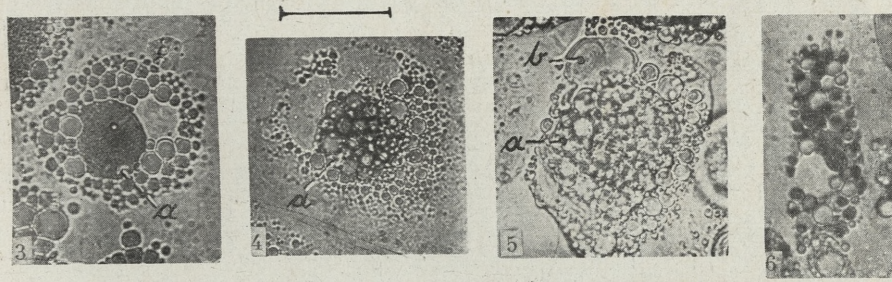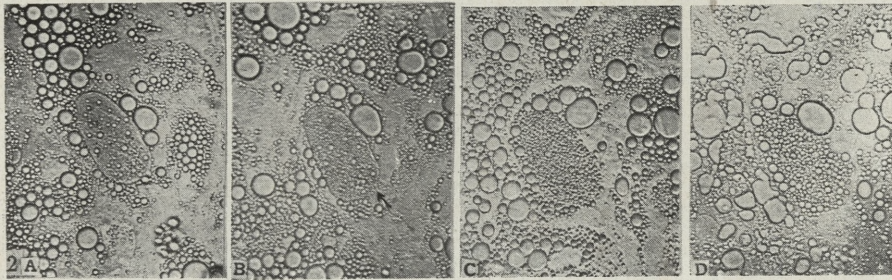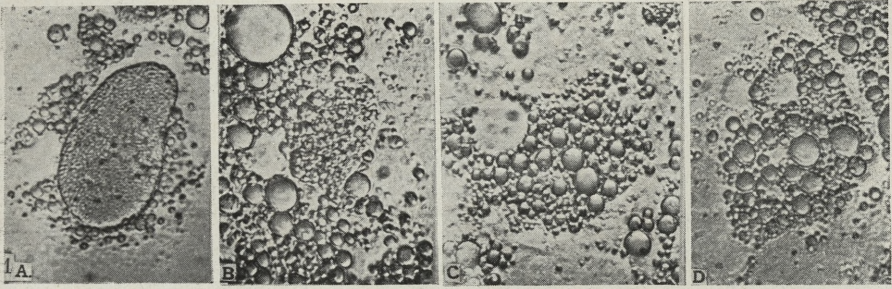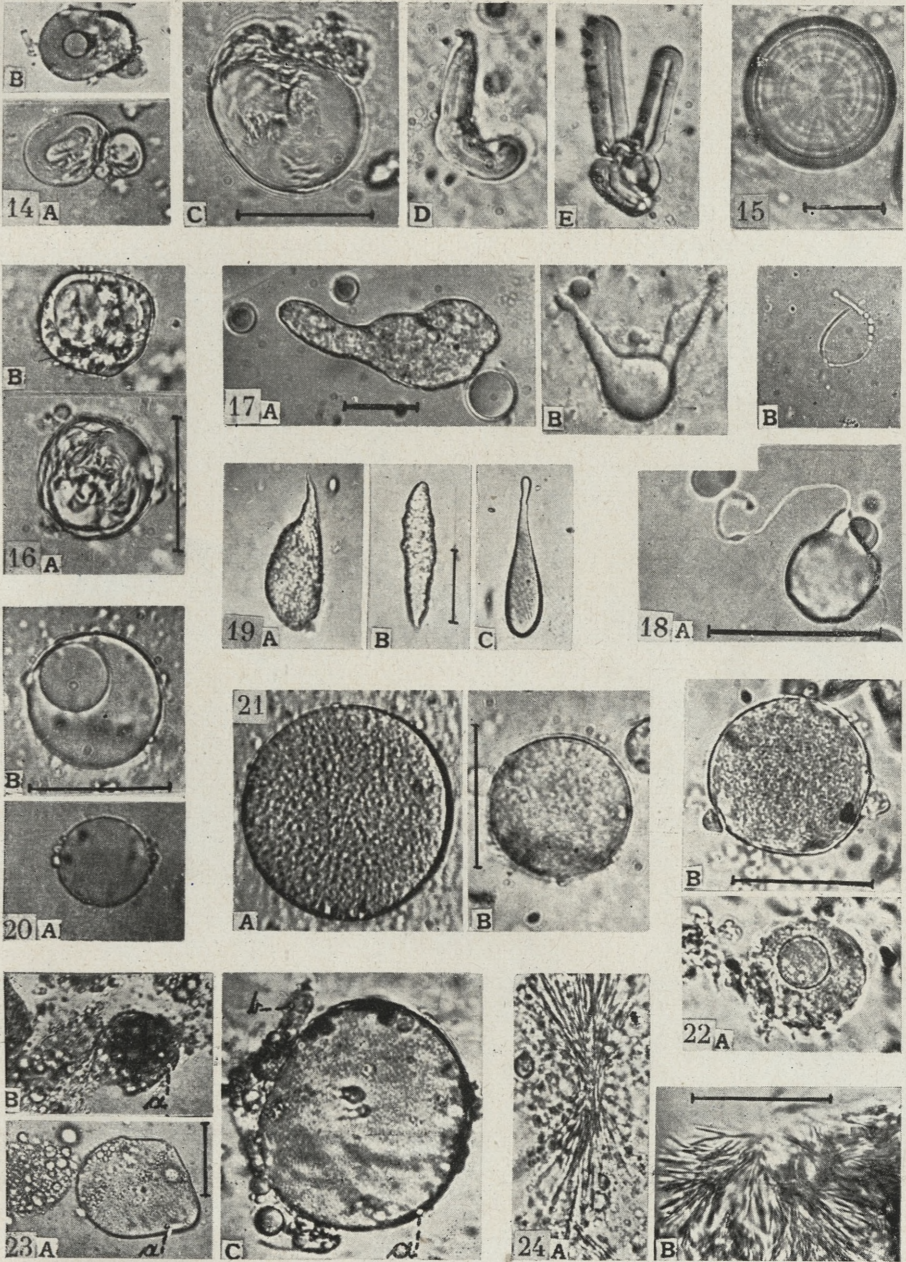
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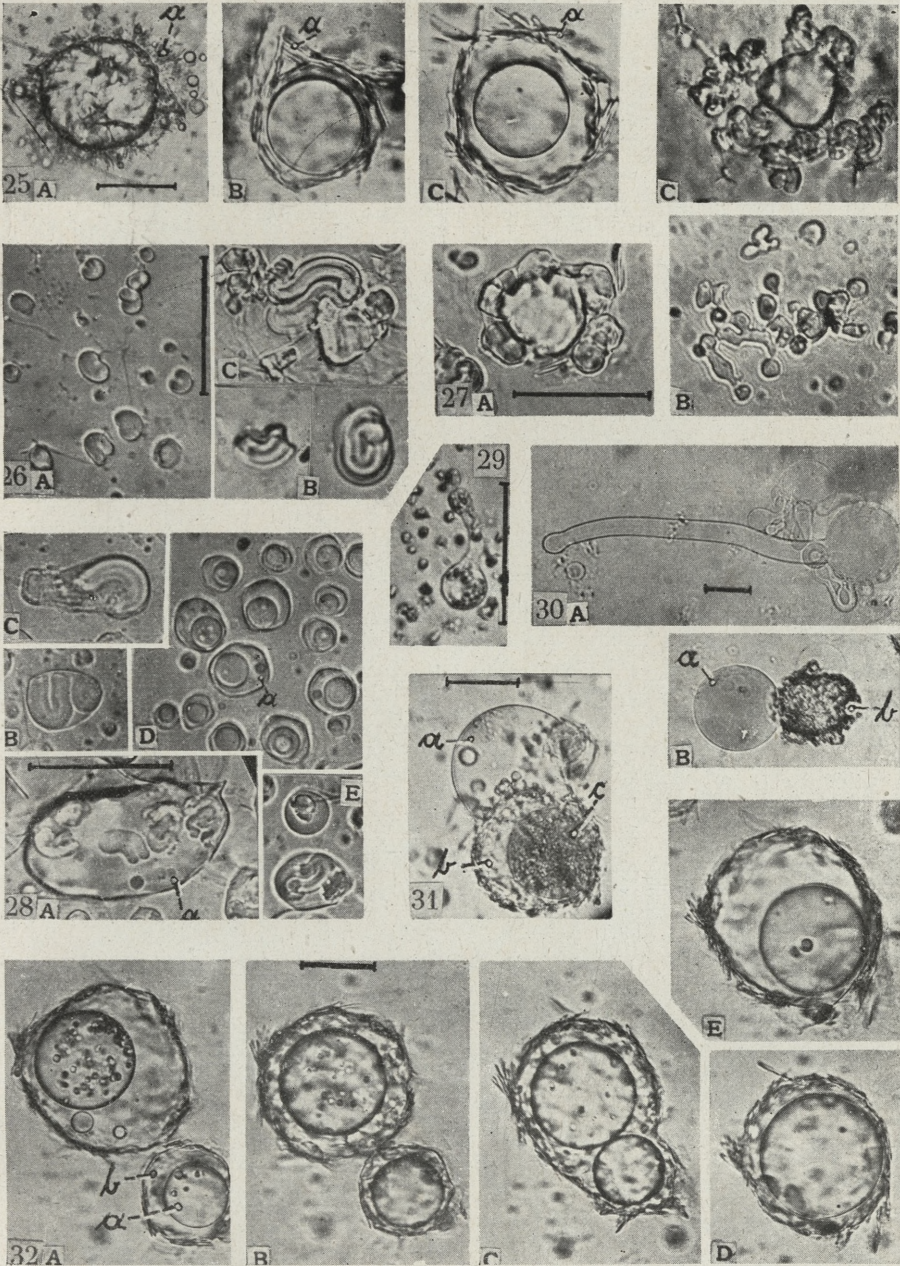
Z. Grodziński

*Doświadczenia nad zmianą ubarwienia skrzydeł u Ly-
mantria dispar L. metodą injekcji Zaćwilichowskiego. —
Experiments about the changes in colouring of the wings
of Lymantria dispar L. by the method of Zaćwilichowski.*

Note

de M. *J. RYMAR,*

(Plate 9)

Until present only Hirschler and Zaćwilichowski obtained
changes in wing colour of the moth (*Lymantria dispar*), as a result
of the action of chemical agents upon larvae or pupae.

Hirschler (2) subjected larvae to the action of ammonia and
acetic acid from the second moult up to pupation and obtained the
following changes: Females showed darkening of colour and an
elongation of the lateral anterior rami, males on the other hand,
exhibited light spots on wings of a feminina type and female
type of scales. The writer supposes, that these sexual anomalies
are a consequence of changes in oxidisation and not a result of
specific action of acid or alkalia.

Zaćwilichowski (6) obtained melanic females of *Lymantria
dispar* after injecting young pupae with phospho-wolframic acid,
oxidated bioxyphenylalanine, with coccinin and phospho wol-
framic acid with bioxyphenylalanine. Formely, this writer descri-
bed extremely active influence of phospho-wolframic acid and
phospho-molibdene acid upon colour and wing pattern in *Va-
nessa urticae* L. In my own experiments I was able to prove
a similar action of brown oxidation products of hydroaromatic
bodies, and enzymatically oxidated plant tannine substances.

In the present paper, I tried to compare the biological react-
ions after injections of active substances with the methods of

Zaćwilichowski into pupae of *Vanessa urticae* L. and *Ly-mantria dispar* L.

It was found, that some chemicals are very active in one species and not in the other.

Materials and methods

My experiments were performed by injecting pupae in the first hours after pupation. The larvae were partly collected from free living populationts and partly from eggs laid in captivity by L. *dispar*. The injected pupae had a soft chitinous cover, that did jet not show the usual brownish black colour.

The amount of the injected matter varied considerably but in all cases the extreme extension of the injected pupae was avoided.

In the majority of cases pupae *Lymantria dispar* L. were used, and for comparative experiments pupae of *Euproctis chry-sorrhoea* L. and *Vanessa urticae* L. For injections the following reagents were used: 1) extract from unburned coffe grains, enzymatically oxidated, 2) a brown product of oxidation of tannine ammonia solution after previously mentioned recipe [(4) new group of organic substances with change the wing patterns of moths injected according to the method applied by Zaćwilichowski], 3) a mixture of peroxydase from horse radish with tannine and hydroaromatic bodies made after following recipe: iuice from horse-radish was treated with tannine in small doses, so as to obtain green reaction with 1% solution of ferri chloride. To this tannine peroxydase in some experiments small amounts (2—5 mg in 30—50 cc solution) tirosine, adrenaline and pyrokatechine was added (preparation I), in other cases small quantities of hydrochinon pyrokatechine resorcine, pyrogallol, gallidacid and floroglucine were added (preparation II), immediately before injection the fluid was titrated and mixed in the injection caniule with aproximately the same amount of 3% oxidated water. After the addition of oxidated water the fluid assumed a pale yellowish or pale violett colour. These solutions were injected before the fenoles became totally oxydated. Both preparations contain free fenolic bodies detectable with 1% ferri chlorid and peroxydase showing the benzidine test. The reaction in aqueous solution of

benzidine with 3°/₀ perhydrol showed slowly a greengrey than greyish blue an than brown colour. Horseradish juice without tannine and polyphenoles gave with benzidine at once a dark blue colour. My basic hypothesis in preparing solutions was the assumption, that wing pattern is connected with the presence of polyphenoles acting as propigments an of oxidating enzymes.

Comparative experiments on pupae of *Lymantria dispar* L. and *Vanessa urticae* L.

In the first experimental series a number of *L. dispar* L. pupae in varying stages after pupation, were injected with an enzymatically oxidated extract of raw coffe grains. In no case was an effect observed, although the preparation is very active in *V. urticae* L. causing such modifications as ab. *ichnusa* Bon., ab. *atrebatensis* Boisd, ab. *ichnusoides* Sel., ab. *coniuncta* Neub., and a number of intermediate forms. The enzymatically oxidated coffe grain solution is active in *Vanessa urticae*, but totally without effect in *Lymantria dispar* L.

In further experiments the biological activity of peroxydase preparation with the addition of tannine and phenoles was tested. Pupae of *V. u.* injected with both preparations showed only small enlargement of the blue spots on the ocelar bands and a darkening of the light spot on the anterior margin of the posterior wing. The great majority of the treated images dit not show any changes whatever.

The same preparations caused in *L. dispar* L. very far reaching changes in wing coloration (Table I.). It follows therefore, that preparations scarcely active in *V. urticae* L. may be very active in *L. dispar* and conversely.

There are however differences in susceptility amongst the sexes. The light ground wing colour of normal females darkens in consequence of injection, whilst the wings of males become more or less lighter.

In both compared species, tannine oxidated in ammonia proved to be active. The reaction in *Lymantria dispar* L. is expresed by darkening of wing pattern in females and lightening in males. (Table I.). The data of table I show that oxidated tannine causes darkening of wings in females in *L. dispar* L. whilst

TABLE I. Experiment on *Lymantria dispar* L.

The effect of oxidated tannine and the preparation I and II on *Lymantria dispar* L.

Prepar.	Number of pupae	Sex	Mortality	Without changes	Lightening males		Darkening females	
					Weakly changed	Strongly changed	Weakly changed	Strongly changed
Controle without injection	25	♀♀	0	24	—	—	1	0
Controle without injection	22	♂♂	0	20	2	0	—	—
Oxidated tannine 3%	30	♀♀	12	3	—	—	8	7
Oxidated tannine 3%	24	♂♂	15	5	4	0	—	—
Preparat. I.	54	♀♀	17	14	—	—	13	10
Preparat. I.	52	♂♂	16	20	12	4	—	—
Preparat. II.	85	♀♀	31	32	—	—	10	12
Preparat. II.	73	♂♂	27	21	13	12	—	—

preparation I and especially II do act in a much weaker fashion. Contrarily in males, preparations with polyfenoles especially II, produce strong lightening in control experiments females exhibit withe colour on the upper and lover part of the wing (Plate 9. fig. 1—3). The front wings show four weak bands, the hind wings exhibit a slight marginal dark band.

No females show light ground colour and a more intensive wing pattern. Control males of *Lymantria dispar* have dark grey or brown wings with a fully developed pattern (Plate 9, fig. 15—17). Two individuals had light grey wings.

The experimental forms were classed as strongly or weakly changed males and females. Strongly changed females after

injection of oxidated tannine, show mostly a great darkening of the wing upper surface which becomes almost grey (Pl. 9 fig. 9—12). From the lower surface both pairs of wings showed mostly actual darkening especially on the tops of the front wings. Females strongly changed after injection of preparations I and II shoved a well marked increase of pattern elements on both wing pairs and a darkening of the ground colour (Plate 9, fig. 4—8) especially the medial and lateral band widened markedly.

The posterior pair of wings showed at times a stronger darkening of wing basis and a pronounced appearence of the marginal dark band, which did not appear in control material.

Weakly changed females have a slight darkening of the wing, especially the posterior pair.

Strongly changed males after treatment with preparation I ad II show especially on the anterior pair of wings a whitish colour with totaly wanishing pattern (Pl. 9, fig. 21—24). This whitening covers almost the whole surface of the anterior pair of wings. Dark groupings of wing scales remain only in the region of the brachial vein and the basis of the anterior pair of wings. The posterior wings often also lighten. In strongly affected males, the scales on the anterior and posterior surface of the front wings are almost entirely lacking. The scales remain in the chitinous exuvium of the pupa and have a white creamish colour.

During the experiments a number of *Lymantria dispar* L. pupae were injected at the age of over 24 hours. The emerging females were lacking almost all scales on both pairs of wings (Pl. 9, fig. 13). In some cases the scales were lacking only on the front wings (Fig. 14). There seems to exist in females of *Lymantria dispar* L. asensible period for the disapearence of scales which follows the sensibility period for colour darkening.

No changes in the structure of the antennae in males and females were observed as well as abnormalities in the formation of scales. The changes seem to be confined, to pigment formation in the wing scales in *Lymantria dispar* L.

Our experiments show the biologically active preparation and the optimal condition produce changes in 37% females and 30% males of *Lymantria dispar* L., in *Vanessa urticae* L. certain preparations give change in all individuals. In no experiment dark grey females similar to normal males were obtained. Strongly

changed males do however, show great similarity of coloration to normal females of *Lymantria dispar* L.

The biological agens in relation to the chemical stimulus is entirely different in males and in females. The males become lighter, the females darker. One cannot therefore maintain, that changes in coloration are caused by the increase of black pigment in the wings scales. The chemical substances entering the hemolymph act not as a propigment which is deposited in the epidermal cells of the wings scales of *Lymantria dispar* L.

In the injection experiments on *Lymantria dispar* L., the relation between the degree of pattern changes and the sensibility period was not studied. Therefore, the modification obtained in both sexes are not the last the degree of the experimental possibility in this respect as far as *Lymantria dispar* L. is concerned.

Zaćwilichowski stated that the red pigment coccinine caused darkening of wings in both sexes of *Lymantria dispar* L. (6). I did inject a number of pupae of various age of a *Lymantria dispar* L. and *Euproctis chryssorhorea* L. with a semisaturated solution of the pigment.

The emerging specimens of *E. chryssorhorea* shoved an uniform pink coloring on both pairs of wings. The greatest intensity of pink coloring showed the wing bases. The females of *L. dispar* showed a weak coloring of both wing pairs. Here again, the greatest intensity of pink colour was found an the wing basis. In males especially strong pink colour showed the trunk, antennae and extremites. No melanistic action which after Zaćwilichowski (6) was observed in his genetic experiments, nor changes in wing pattern could be observed. An analogous action showed in *Lymantria dispar* L. methylene blue.

The last set of experiments prove, that in both *L. dispar* L. i *E. chryssorhorea* L. neither coccinine nor methylene blue can modify the wing pattern nor do they form propigments for the black colour. These pigments do simply mechanically enter the tissues.

Discussion

The comparison of the activity of chemicals which modify wing pattern of *Lymantria* and *Vanessa*, show such great differences in behaviour of both species, so that we may ask whether

the obtained results may altogether be compared. Perhaps the effects obtained in *L. dispar* L. should not be compared with the changes in wing pattern which in *V. urticae* are connected with the increased sensibility for thermal stimuli in the first 48 hours of pupal life, but rather with disturbances in pigment production in the sensibility period immediately before emergence of the imago (Merrifield 1893 (3), Feldotto 1933) (1).

The experimets of Hirschler (2) on larvae of *Lymantria dispar* L. gave similar results as mine on pupae. The chemical agents acted in both cases on different developmental stages. The results was exhibited later on in pigment production of the adult animal. The internal chemical processes in pupae which cause the external characters of sexual dimorfizm in coloration, can be disturbed through the introduction of an external agens into the hemolymph.

The pigments methylene blue and coccinine physiologically neutral, are deposited directly in the wing scales of *Lymantria dispar* L. One could suggest, that the same occurs also with preparations used in this work. The chemicals mentioned being easily oxidated may serve as propigments for wings scales. The existence of light males and dark females excludes however the possibility of direct pigment deposition in the scales of the artificially introduced chromogenes. The formation of dark pigment in the wings of females in *Lymantria dispar* L. can not be the result of direct mechanical deposition of chemical substances, artificially introduced into the hemolymph of the pupae.

The changes obtained in *Lymantria dispar* L. as a result of the injection of chemicals, I regard as the biological effect of a specific disturbance of the development caused by the action of most likely chinonon substances, artificially introduced into pupae of *Lymantria dispar* L. I wish to express my sincere gratitude to Prof. Hugo Kowarzyk M. D. for valuable help and assistance during the preparation of this paper.

Summary

Les produits de l'oxidation d'un mélange de tannin et des composés hydroxyaromatiques, étant introduit dans l'hémolymphe des jeunes nymphes de *Lymantria dispar* L. produisent chez les

femelles un assombrissement des ailes, chez les mâles une partielle perte des écailles et éclaircissement du reste des ailes.

Cette différence dûe au sexe n'a pas été mise en évidence dans expériences de Zaćwilichowski sur l'hérédité de la coloration des ailes chez les générations de *Lymantria* modifiées sous l'action des agents chimiques.

Il est probable que ces phénomènes sont dûs aux composés de quinone.

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Explanations of plate 9.

Fig. 1—3. Control females of Lymantria dispar L.

Fig. 5—6. Strongly changed females after injection of preparation II.

Fig. 4, 7—8. Strongly changed females after injection of preparation I.

Fig. 9—12. Strongly changed females after treatment with oxidated tannine.

Fig. 13. Female totally lacking scales after preparation I.

Fig. 14. Female partially devoid of scales after treatment with preparation I.

Fig. 15—17. Control males of Lymantria dispar L.

Fig. 18. Weakly changed male after treatment with oxidated tannine.

Fig. 19—20. Weakly changed males after treatment with preparation I.

Fig. 21. Strongly changed male with partially lacking scales on front wings after treatment with preparation I.

Fig. 22. Strongly changed male partly lacking scales on front wings after treatment with preparation II.

Fig. 23—24. Strongly changed males almost totally lacking scales on front wings after treatment with preparation II.

J. Rymar

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