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REPRODUCTION AND ONTOGENETIC DEVELOPMENT IN *TRACHELIPUS BALTICUS* VERH. 1907

BY

VASILE GH. RADU and N. TOMESCU

In this paper the authors present the results of their investigations on the reproduction and evolution of somatic and sexual morphological characters during the postembryonal ontogenetic development in *Trachelipus balticus* (= *Tracheoniscus balticus*), a terrestrial isopode species.

This paper is concerned with some aspects of the reproduction and evolution of postembryonal ontogenetic characters — particularly of those having a taxonomic value — in the terrestrial isopode species *Trachelipus Tracheoniscus balticus*, a widely spread species in our country. Other authors, too, [1] [5] [9] undertook similar researches relating to inferior terrestrial isopode species. The knowledge of the evolution of postembryonal ontogenetic characters is more and more essential for more precise delimitation of the species and for the establishing of phylogenetic ties.

MATERIAL AND METHOD

We used larvae of gestant females, collected from the ground, which were grown in the laboratory during the years 1967, 1968 and 1969. We observed the gestation period, the number of larvae laid by a female and the ontogenetic development. In order to study the evolution of postembryonal ontogenetic characters, we took samples resulted from the first egg-laying, every 4 days, beginning with the first day after the laying down of larvae (their liberation from the marsupium), until their sexual maturity. Comparatively, we also studied the material collected monthly (from Galcer hill, near Cluj) during the years 1967, 1968, 1969 — in a number of above 300 individuals.

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RESULTS

1. *Reproduction period.* The investigations on the spot showed that *Trachelipus balticus* resumes its biological activity in spring, in April. In this species, the fecundation of females takes place towards the end of this month and at the beginning of May, and the gestation period lasts from May till August. The greatest number of gestant females is in June.

Verhoef (1917) and Meinertz (1944, 1950) showed that in isopode species the gestation period is very long, but they do not analyse this phenomenon. Based on our researches in the laboratory, confronted with the observations on the spot, we found that the larvae laying takes place at various periods, according to the females' age. In the case of one year females it takes place in July, and only once during their first year of life. The two and three years females have two egg-layings in a year, subsequent to one fecundation which occurs in spring, in April. The larvae resulted from the first egg-laying are laid at the end of June and those of the second laying at the end of August. It may be stated therefore that the gestation period is really long (May-August) but that the individual duration of gestation is much shorter and it varies, depending on the age and on the order number of the egg-laying. The greater number of gestant females in June is due to the fact that during this month there are both one year egg-carrying females and two-three years ones. The embryonal development stage is different however, being more advanced in the older females who are probably fecundated earlier and whose larvae are laid between the 20th and the 30th of June. The one year females' larvae are laid—between the 10th and the 20th of July. A similar explanation may be given concerning the number of existing eggs in the gestant females' marsupium. Meinertz (1944) shows that the number of eggs greatly varies in the same species; in *Porcellio scabe*, for instance, it is of 13—119. The author does not explain this phenomenon.

We found that in *Trachelipus balticus* this individual variation is also due to the age and to the period when the egg-laying takes place. The one year females who are at their first egg-laying lay down 11—18 eggs, the 2—3 years ones 80—90 eggs, and at their second egg-laying only 25—30 eggs. We also found that only 1—3 of the great number of eggs existing in the marsupium are not embryonated. They are wholly resorbed before the disappearance of oostegites.

The conclusion which follows is that the *Trachelipus balticus* females are very prolific. However, the number of adults found on the spot is small in comparison with that of hatched larvae. We consider this high prolificness a result of the adaptation of the individuals of this species to the numerical adjustment process of the populations, in the oecological conditions where they live.

2. *Ontogenetic development.* Describing the postembryonal characters development, we tried to establish the growing and development stages of *Trachelipus balticus*, accepting to a great extent their delimiting criteria as well as the nomenclature used by Vandel (1950). In every stage we separately observed the principal morphological characters used in taxonomy. Based on these characters, we identified in *Trachelipus balticus* — from the laying of larvae until the adult stage — ten moultings and seven development stages, during a period of about 330 days, that is 11 months : 4 larval stages, the immature stage, the juvenile stage and the adult stage.

Stage I (*primary larva*). According to Vandel (1950) this stage begins when the larva is free in the marsupium and is able to move, and lasts till the first moulting which takes place after the liberation from the marsupium. During this stage the *Trachelipus balticus* larvae's body is of 1.6—1.7/0.8 mm. (length/breadth). The tegument pigmentation is slight, hardly visible at stereomicroscope. Through the transparent tegument the hepatic tubes and the posterior intestine may be seen. The last article of the antenna whip is much longer than the penultimate one (Fig. 2). The lateral lobes of the head are hardly outlined and the median lobe is more developed and pointed in the middle (Fig. 1). The eyes consist of four

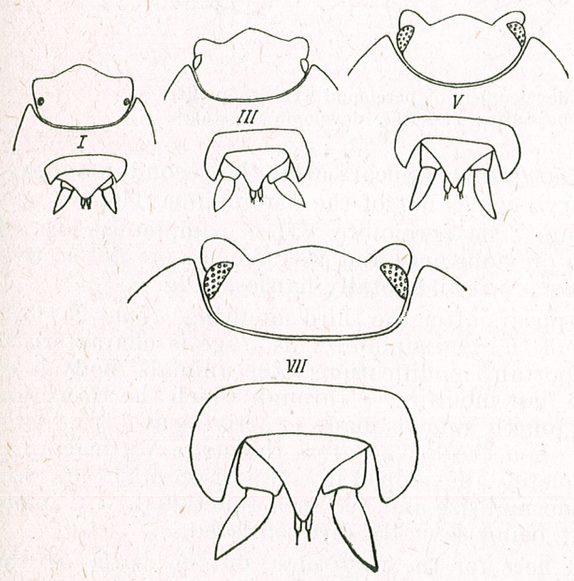

Fig. 1. — Stage development of cephalic lobes, of pleotelson and of uropods during the ontogenesis of *Trachelipus balticus*. *I, III, V, VII,* development stages.

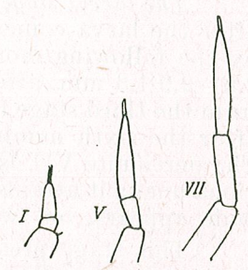

Fig. 2.—Lengthwise growth of antennary whip articles during ontogenesis. *I, V, VII,* development stages.

omatidia. The pleonepimeres are slightly developed. The VIIth thoracic segment is not completely developed and it is similar to the abdominal segments 1 and 2. The VIIth pereiopode is missing, as well as the first pair of pleopodes. Immediately after its liberation from the marsupium the larva has a free and active life, feeding itself intensely.

Larval stage II (*secondary larva*). It begins after the first moulting, about 6—7 days after the larva comes out of the marsupium. The larvae's body size is of 2.1/1 mm. The lateral cephalic lobes are more prominent (Fig. 1). The VIIth pereionite is incompletely developed. Pereiopode VII appears like a very thin filament, consisting of almost equal articles (Fig. 3). It is folded on the ventral side of the thoracic segment VII. Vandel (1950) shows that in *Halophthalmus danicus* the pereiopode VII appears only in the third stage.

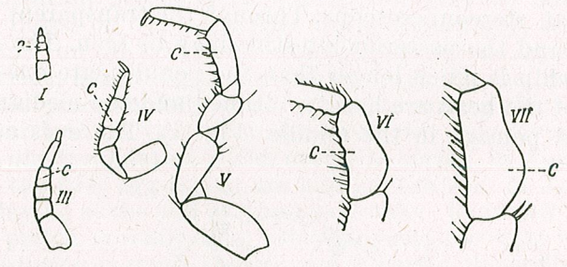

Fig. 3. — Stage development of pereiopod VII carpopodite in males. *c*, carpopodite; *II—VII*, development stages.

Larval stage III (*tertiary larva*) appears after the second moulting, about 14 days after the larva comes out of the marsupium. The larvae's body is of 2.3—2.4/1.1. mm. The pereionite VII is completely formed but it is narrower than the previous ones and its epimeres are not so well developed; it is slightly developed and totally hairless (Fig. 3).

The larval stage IV appears after the third moulting, about 25 days after the larva comes out of the marsupium. This stage is characterized by the following more important modifications: the animals' body is of 2.8—2.9/1.3 mm. after the first moulting — through which the transition from the third stage to the fourth stage is made — arriving at 5.3/2.5 mm. after the sixth moulting, when the larva enters the stage V (imaturus). The pereionite VII is completely developed in comparison with the previous ones; it attains the normal size and becomes functional. The pleopode appears, but without being sexually differentiated.

This stage presented here for the first time, on the basis of the observations on *Trachelipus balticus*, lasts about 40 days; when two more moultings occur and only after the third one (the 6th on the whole) the larva enters stage V. It is characterized by an intense growth, in the detriment of the development, since no differentiation of secondary sexual characters occurs during this period.

Stage V (imaturus) appears subsequently to the 6th moulting, about 64 days after the larvae come out of the marsupium. Now, the first secondary sexual differentiations — through the exopodite and endopodite of pleopode I modification (Figs 4 and 5) — appear, but they have not a final form yet, which is characteristic to the species. The pereiopode VII is not different from that of the female (the crest of capopodite does not

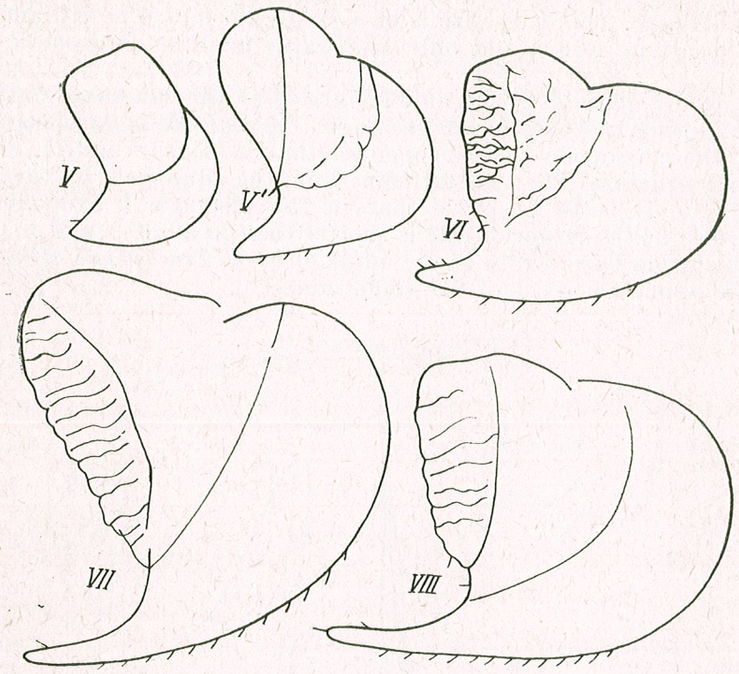

Fig. 4. — Stage development of pleopod I exopodite in males, from the beginning of sexual differentiation till the adult stage. *V—VII*, development stages; *VIII*, 13 months adult.

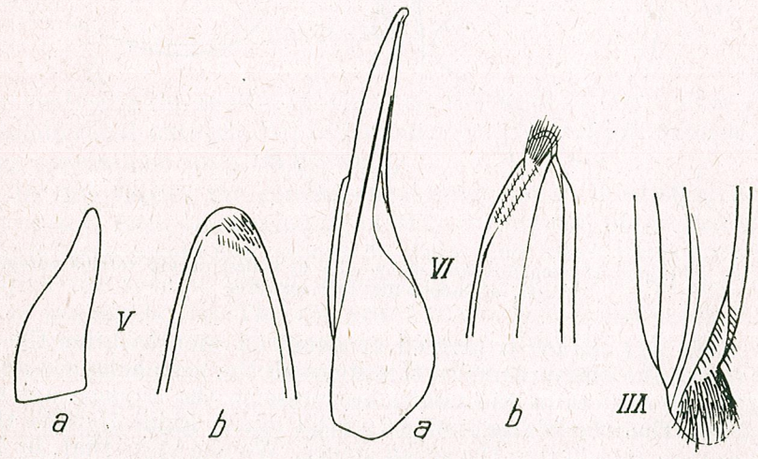

Fig. 5. — Stage development of pleopod I endopodite in males. *a*, endopodite, drawn at small objective; *b*, endopodite extremity, much enlarged; *V—VII*, development stages.

appear) (Fig. 3). The body size is of 5.3—5.4/2.5 mm. The 7th and then the 8th moulting follow and only afterwards another ontogenetic stage begins.

Stage VI (*juvenile*). The animals arrive at this stage only after the eighth moulting, at about 110 days of age. The cephalic lobes, the antenna articles, the pereiopode VII carpopodite, the pleopodes I and II, acquire their final structure. They are different from the adult only by their sizes (Figs. 1—6). It is to be noted that in the pleopode I exopodite the horn formed at the posterior side is shorter than in adults and is similar to the pleopode I exopodite of the adult form of *Trachelipus affinis* species. The animals'body is of 6.5—6.8/3 mm.

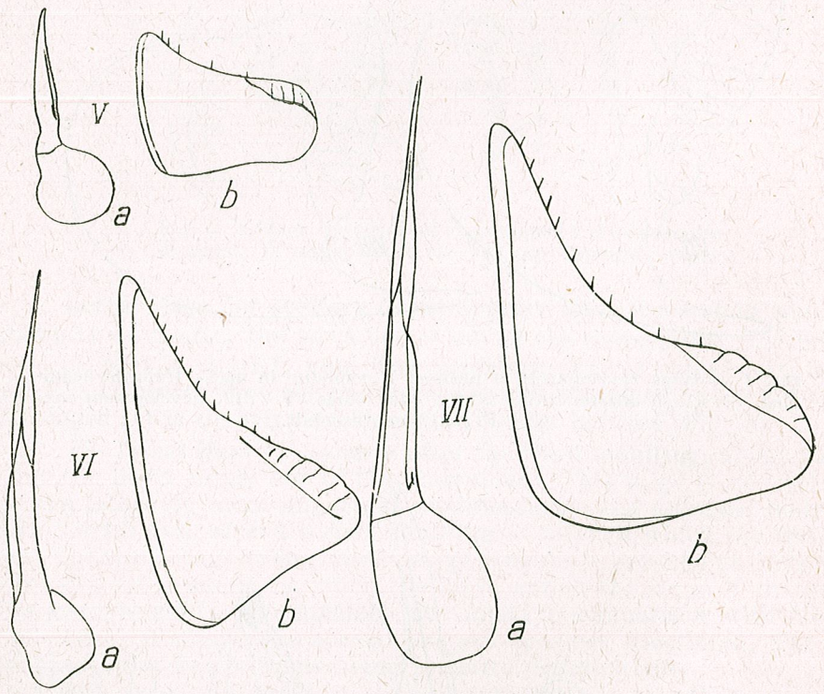

Fig. 6. — Pleopod II exopodite and endopodite in males, during various ontogenetic stages. *a*, endopodite; *b*, exopodite.

Stage VII (*adult*) is arrived at about 11 months after the larvae come out of the marsupium. In this stage all the morphological and physiological characters of the adults are present, the animals are able to reproduce. Their body size is of 8.2—8.4/4 mm. Figue 7 shows the animals' growth rate during the seven stages. We can state that, in *Trachelipus balticus*, after only about 11 months of ontogenetic development, the specific morphological characters are stabilized and the animals may by determined very accurately.

The figures included in this paper comparatively show the characters of a 2—3 years adult, the maximum age which some individuals of this species may arrive at. In fig. 1 we present the growth of cephalic lobes, of uropodes and of telsone, during the seven stages, and in fig. 2 the relation between the last and the penultimate antenna article.

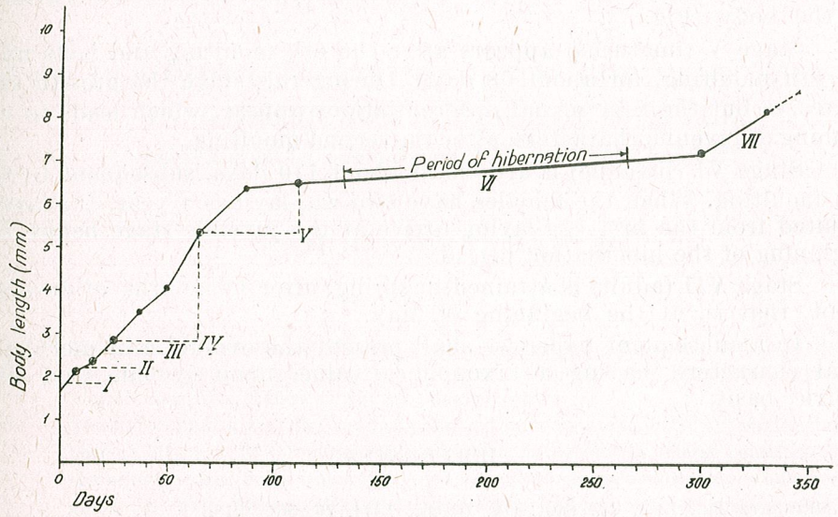

Fig. 7. — Growth rate of individuals during the ontogenetic development stages. ⊙, stage moultings; ●, simple moultings.

CONCLUSIONS

By analysing the results obtained on the basis of our studies upon the postembryonal ontogenetic development in *Trachelipus balticus*, the following conclusions may be drawn:

1. In this species the general incubation period is situated in the warmest season, from the beginning of May till the end of August. Individually, it lasts much less (about 50 days) and takes place earlier or later during the specific period, depending on the age or on the order number of the egg-laying. The one year females have a single egg-laying and the larvae leave the marsupium between July 10—20. The 2—3 years females have two successive egg-layings in a year. The larvae of the first egg-laying, 80—90 in number, leave the marsupium between June 20—30, and those of the second egg-laying, 25—30 in number, between August 10—25.

2. The postembryonal ontogenetic development takes place in stages. We identified a number of 7 stages against 6 stages identified by

the previous authors. The duration of the stages is different, its total being of about 11 months. During the whole ontogenetic development, until the adult stage, about 10—11 stages take place, at various periods of time. Stages I, II, III are short, each of them appearing after a unique moulting and being characterized by the evident modification of some somatic characters. Stage IV appears after the third moulting and lasts until the 6th one, about 39 days, and it is characterized by a marked growth of the body (Fig. 7).

Stage V (imaturus) appears after the 6th moulting and lasts until the 8th moulting, for about 68 days. During this stage the growth rate decreases but the first sexual differentiations appear, which leads to the forming of juvenile characters, after the second moulting.

Stage VI (juvenile) is arrived at about 110 days, subsequent to the 8th moulting. When the females have two egg-layings a year, the larvae resulted from the first egg-laying arrive at the juvenile stage before the beginning of the hibernation period.

Stage VII (adult) is attained in spring, after 11 months of development, that is, at the beginning of May.

In a subsequent paper we shall present the evolution of morphological characters, having a taxonomical value, upon graphic and biometric basis.

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CYTOPLASMIC CONSTITUENTS IN SPERMATOGENESIS IN *GRAPHOSOMA ITALICUM* MÜLL. (*HETEROPTERA—PENTATOMIDAE*)

BY

VIORICA TRANDABURU

According to observations effected mostly at the light microscope and partially also at the electrone microscope, in the species *Graphosoma italicum* Müll., cytoplasmic constituents show during spermatogenesis.

The mitochondria and dictyosomes polarized in spermatogonia, in spermatocytes become dispersed in cytoplasm. In spermatids, mitochondria merge constituting the mitochondrial body which is divided up, and the mitochondrial cords advance along the axial filament. The dictyosomes form the acroblast. This secretes the acrosome, which in spermatid extends on one side of the nucleus, a fact clearly likewise ascertained in electronmicroscopic preparations.

The results obtained are discussed in connection with researches effected in other species of insects as well.

The behaviour of cytoplasmic constituents in Heteroptera during spermatogenesis, constituted the object of numerous light and electron microscopy researches.

In the present work we are bringing new contributions concerning cytoplasmic constituents, during spermatogenesis, in the species *Graphosoma italicum* Müll., by researches with the light microscope combined with certain data of electron microscopy.

MATERIAL AND METHODS

Adult specimens of *Graphosoma italicum* Müll., collected from the Işalniţa— Craiova vegetable station, were dissected under binocular and the testes were removed. For the study at the light microscope, pieces were fixed in Bouin, Bouin-Hollande, Carnoy, Champy, Flemming,

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Kolacev, fixing solutions and stained with hemalun -erythrosin, Azan, Feulgen, ferric-hematoxylin and acid anilinated fuchsin. Drawings were executed in camera lucida with a 90 immersion objective and a 10 ocular. For electron-microscopic studies, fragments of less than 1 mm^3, were fixed in glutaraldehyde-osmium and embedded into Vestopal W. The fine sections were obtained in a LKB ultramicrotome, contrasted with lead citrate (Reynolds, 1963), and examined in a Jem 7 (80 kv) electron microscope.

RESULTS

From observations effected at the light microscope it is found that the *Graphosoma italicum* Müll. testis is made up of 5 seminiferous tubes in which sexual cells are disposed in isogenous groups forming separate cysts by conjunctive walls (Fig. 1). Seminiferous cyst wall is formed of nutritional cell prolongation. All sexual cells evolution stages are discerned along the seminiferous duct. Thus, in the apical part of the testis are spermatogonia, provided with a spherical nucleus in which either the nucleole with chromosome X attached to it, or spermatogonia intercepted in mitoses are observed. In cytoplasm, at one pole of the nucleus a more intensely coloured region is discerned, in which mitochondria as small filaments, 1—3 dictyosomes and the centriole are situated (Fig. 8, *1*). Next to the normal cysts with spermatogonia follows a zone comprising cells with pycnotic nuclei, positive Feulgen and then spermatocytes of order I, of greater size. In these spermatocytes both mitochondria and dictyosomes are diffusely spread throughout the cytoplasm. In this study, mitochondria appear as sinuous homogenous filaments (Fig. 8, *2*, *4*) while dictyosomes present one chromophilic part and a chromophobe one (Fig. 8, *3*). Some dictyosomes are in division (Plate 8, *4*). At the end of the first maturation division prophase, when its division spindle had been organized, filamentous mitochondria are disposed all around the latter. Centrioles appear under the form of 2 rods disposed in V at the poles of the division spindle (Fig. 8, *5*). At the second maturation division, in spermatocytes of the order II, one single rodlike centriole is discerned at each spindle pole (Fig. 8, *7*). In these cells mitochondria and dictyosomes have the same aspect as in the previous stage (Fig. 8, *8*).

As it is observed in electron-microscopic images (Figs. 5 and 6), in the spermatid mitochondria are grouping together and increase in size to form the mitochondrial body ("Nebenkern"). At first this is of a spherical shape and, as observed at the light microscope, presents one internal chromophilic and one external chromophobe part (Fig. 8, *9*). Dictyosomes merge by their edges constituting the acroblast situated at the place where the nucleus and the Nebenkern come into contact (Fig. 8, *9*). Inside the acroblast, one small granule is noticed and stuck to the nucleus membrane another larger one, whose significance is unknown (Fig. 8, *9*, *10*).

Already from the spermatid stage, the chromatic material agglomerated at the periphery is observed in the nucleus, forming in its fore-half a positive Feulgen layer. The mitochondrial body begins to elongate, and intensely chromophilous concentric layers, may be discorned in its

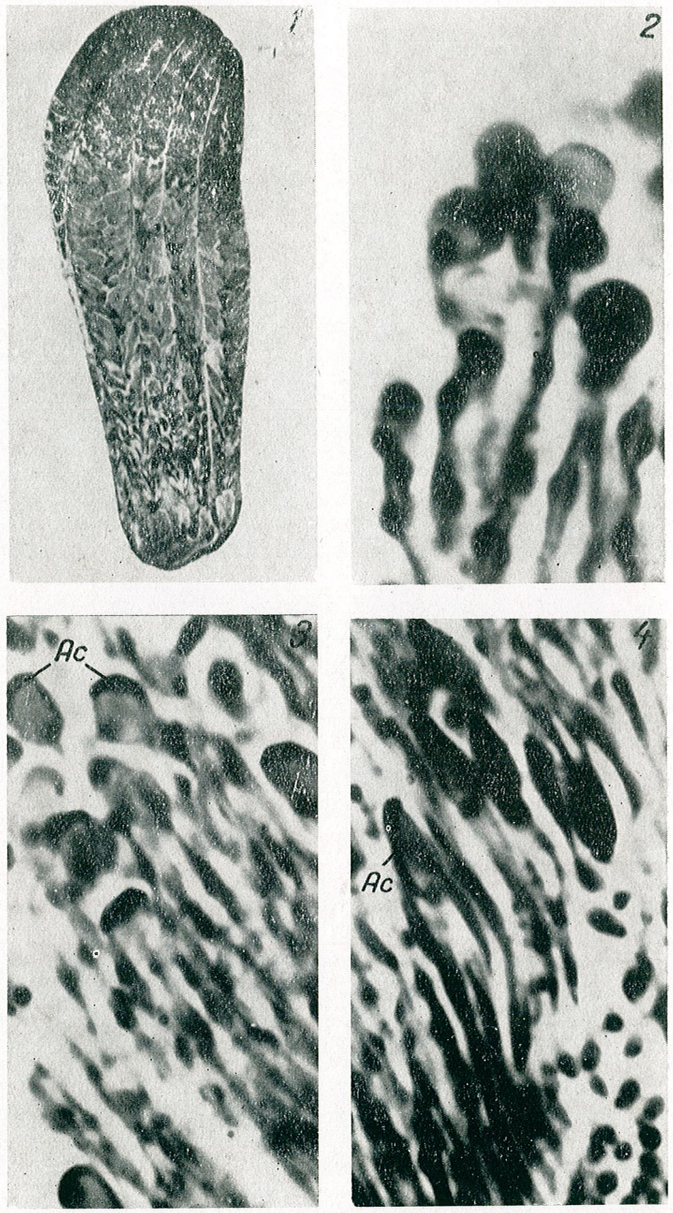
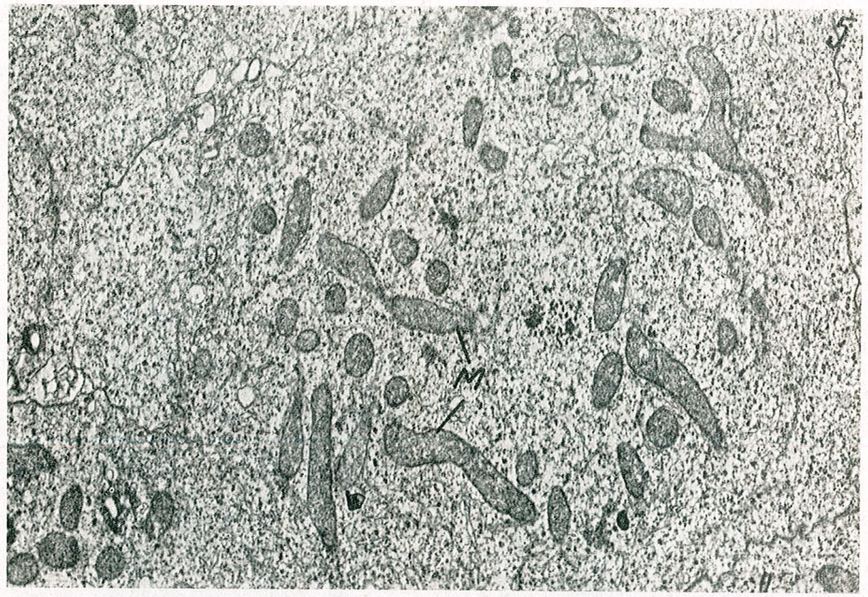


Fig. 1. — General view of *Graphosoma italicum* testis.
Fig. 2. — Section through spermatids with spherical nucleus. Presence of bleb-like swellings on tails.
Fig. 3. — Section through more advanced spermatids. Helmet-like acrosome (*Ac*) in the fore-part of the nucleus.
Fig. 4. — Section through advanced spermatids. The acrosome (*Ac*) attached on one side of the nucleus.



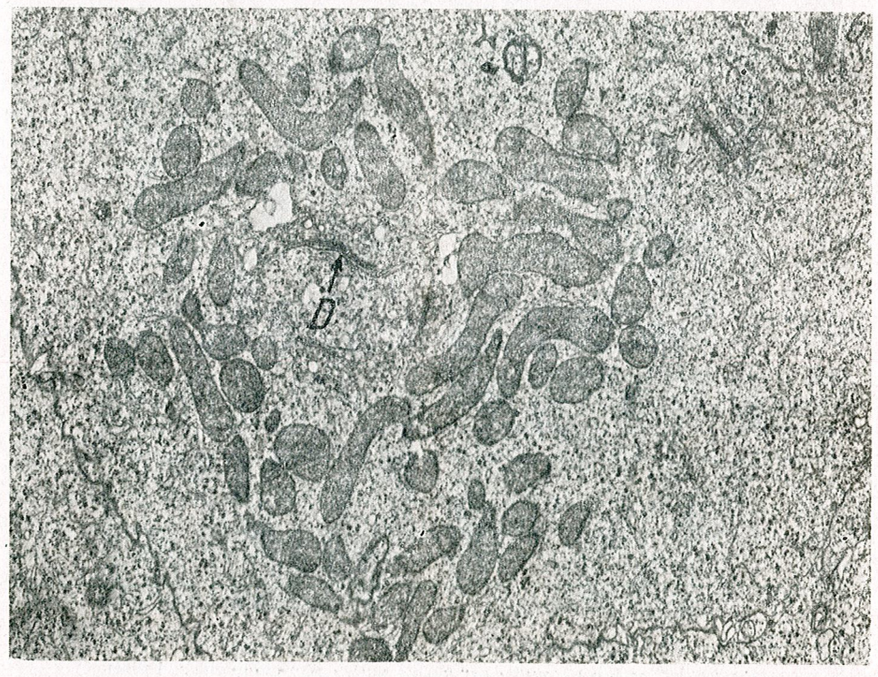


Fig. 5. — Beginning of mitochondria (*M*) grouping in young spermatids. × 12,000.
Fig. 6. — Precursory phase of the nebenkern formation; mitochondria more pronounced agglomeration and the increase of their volume. A dictyosome (*D*) is present in their centre. × 12,000.

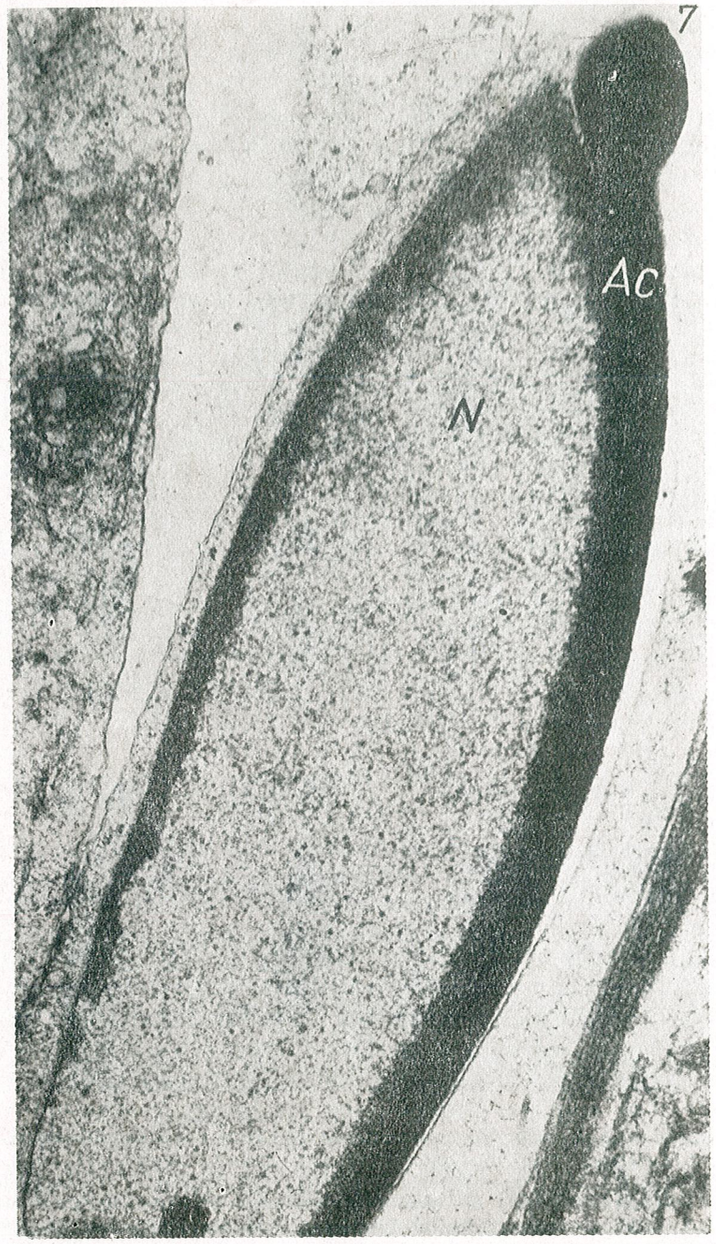


Fig. 7. — Head of advanced spermatid having the acrosome (*Ac*) disposed on one side of the nucleus (*N*). × 22,500.

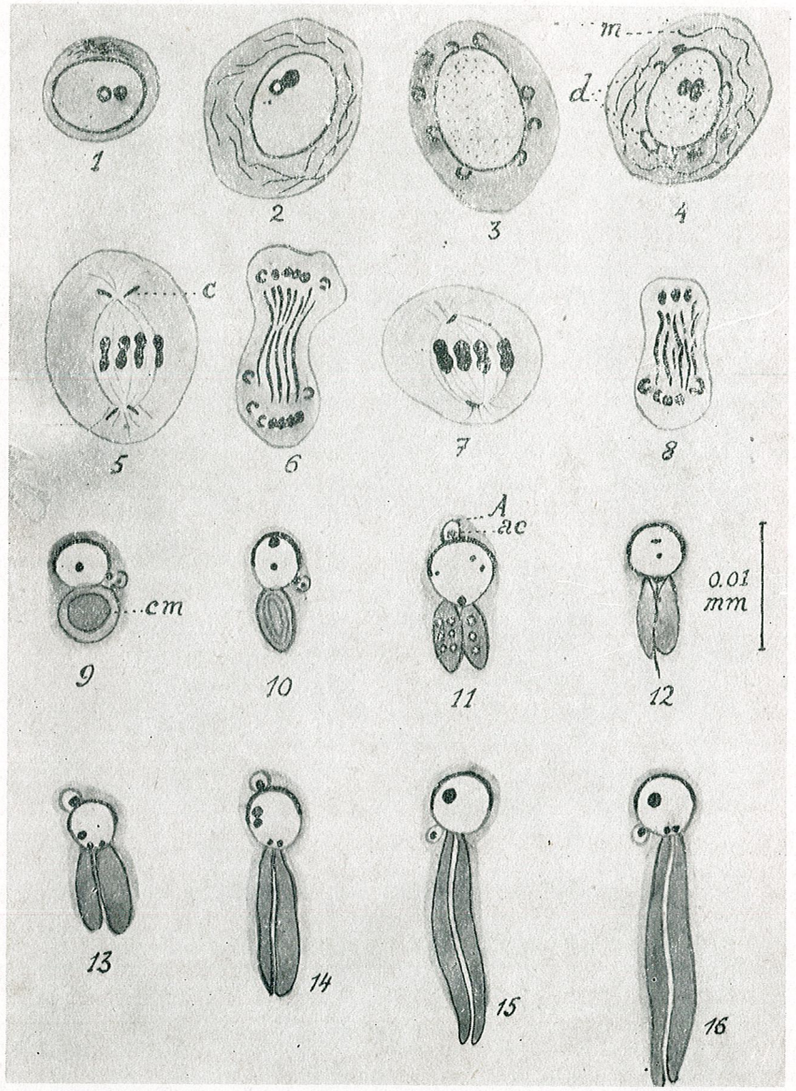


Fig. 8. — Different spermatogenesis stages :
1 — spermatogonia ; *2—4* — spermatocytes of order I ; *5*, *6* — spermatocytes of order I in division ; *7*, *8* — spermatocytes of order II in division ; *9—16* — sections through spermatids ; *m* — mitochondria ; *d* — dictyosomes ; *c* — centrioles ; *c*, *m* — mitochondrial body *A* — acroblast ; *ac* — acrosome. (The drawings were effected as follows : *1*, *2*, *4*, *6*, *8* after Flemming fixed preparations, stained with ferric hematoxylin ; *3* — after preparations fixed in Kolacev ; *5*, *7* — after preparations fixed in Bouin stained with ferric hematoxylin ; *9—16* —after preparations fixed in Champy, stained with ferric hematoxylin and acid anilinated fuchsin).

mass, alternating with layers more weakly stained with hematoxylin (Fig. 8, *10*). In the following stages, the mitochondrial body is elongated still more and is divided into two mitochondrial bodies in which, at the light microscope, small vesicles can sometimes be discerned, disposed in one or two rows (Fig. 8, *11*). Mitochondrial bodies continue to elongate along the axial filament which proceeds from the centriole (Fig. 8, *12*). Inside these mitochondrial bodies no further structure is evidenced at the light microscope, they appearing intensely stained in black with ferric hematoxylin (Fig. 8, *13—16*). When the two mitochondrial cords reach their maximum length, they twist around the axial filament, and a series of bleb-like swellings are recorded along them (Fig. 2). The acroblast together with the acrosome shift towards the fore-part of the nucleus ; it does not remain, however, in this position ; and in the following stages it is again observed in the contact place between nucleus and elongated, mitochondrial bodies. When the spermatid nucleus starts becoming ovoid, the helmet-like acrosome is seen at the fore-pole of the nucleus (Fig. 3). The acroblast is eliminated simultaneously with the other cytoplasmic constituents at the tail level. Then the acrosome begins to elongate only on one side of the nucleus (Fig. 4). This is clearly discerned also in the electronmicroscopic photographs, in which the acrosome appears intensely osmiophilous (Fig. 7). The fact is also to be pointed to that in preparations for light microscopy, the nucleole is observed in the spermatid nucleus (Fig. 8, *9*, *10*, *15*, *16*), and attached to this, the chromosome X (Fig. 8, *14*) ; in some cases two small bodies, intensely stained with hematoxylin, are also discerned in the nucleus at its hind-pole, whence the two mitochondrial bodies seem to proceed.

DISCUSSIONS

Our results achieved in light microscopy on cytoplasmic constituents, in spermatogenesis, in *Graphosoma italicum*, agrees to a large extent with those recorded in other groups of insects, and particularly in Heteroptera.

Thus in spermatogonia both mitochondria, as well as dictyosomes are localized at one pole of the nucleus, around the centrosome, as it is likewise mentioned in the species *Belostoma flumineum*, *Lethocerus americanus* (Chickering [4]), *Gelastocoris oculatus* (Payne [10] [11]), *Gerris* and *Velia* (Poisson [14]), *Pyrrhocoris apterus* (Steopoe [17]).

In spermatocytes, the diffuse spreading of mitochondria and dictyosomes throughout cytoplasm was likewise recorded by Bowen [2] in *Murgantia histrionica* and *Euschistus*, by Gupta and co-workers [5] in *Dysdercus cingulatus*, by Payne [10] in *Gelastocoris oculatus*, by Poisson (14) in *Gerris* and *Velia*, by Steopoe [19] in *Pyrrhocoris apterus*, a.o. At the end of the first maturation division prophase, the long mitochondrial filaments are disposed around the division spindle, and simultaneously with the accentuation of the plasmodieresis groove their fragmentation

is produced at this level (Poisson [14]) in *Gerris* and *Velia* and Steopoe [19] in *Gryllotalpa vulgaris*.

In the second maturation division, the localization and distribution of mitochondrial filaments is achieved similarly as in the first division, as was also described by Poisson [14] in the species *Gerris* and *Velia*.

In spermatids the merging of mitochondria and the formation of mitochondrial body (made up of one chromophilic and one chromophobe part) observed by us in *Graphosoma italicum* were likewise described in other species of insects by Bowen [3], Chickering [4], Gupta and co-workers [5] [6], Nath [8], Pollister [15] and others. Bowen [3] considers that the chromophilic substance of the mitochondrial body is disposed in a variety of forms producing the so-called "Nebenkern patterns" which are represented by the "blackberry, hemisected onion, and smooth or beaded cords form". These patterns are of two types : the "spirem" one characteristic of Lepidoptera, and the "plate-work" type characteristic of Heteropters. In the species *Graphosoma italicum* we recorded the "hemisected onion and beaded cords" type.

The division of the mitochondrial body, the elongation and twisting of the two mitochondrial cords, as well as the existence on these of bleb-like swellings observed on *Graphosoma italicum* were likewise recorded by Bowen [3], Nath [8], Poisson [14], Pollister [15] and others. According to us, the spiral disposition of the mitochondrial bodies around the axial filament may be due either to the contractile protein of the mitochondria, or to the fact that of the microtubules, disposed in a single row around the mitochondrial bodies those on one side of the mitochondrial body are first contracted and subsequently the others (Trandaburu [20]).

As regards acrosome formation, we consider that this is secreted by the acroblast resulting from the junction of dictyosomes, as was also shown by Bawa [1], Bowen [2], Gupta and co-workers [5], Nath [9], Steopoe [17]. In *Graphosoma italicum* we point to the fact that in the spermatid stage with elongated nucleus, the acrosome extends only on one side of the nucleus. A similar disposition was recorded by Payne (10) in *Gelastocoris oculatus*.

In the spermatid stage there is, as was already shown, a chromatin peripheric layer, which comprises only the fore-half of the nucleus. Gupta and co-workers [6] described in the young spermatids of *Lacotrephes maculata* the so-called "chromatin-plate" in the fore-half of the nucleus. The authors maintain, however, that in preparations stained with ferric hematoxylin and in the live cells examined in phase contrast, this chromatin layer and the condensed acrosome cannot be discerned as separate from one another. In the case of the species *Graphosoma italicum*, the preparations examined in the electron microscope have clearly shown that the acrosome is intensely osmiophilous and densely applied only on one side of the nucleus, while chromatin appears less dense and feebly osmiophilous.

CONCLUSIONS

1. In spermatogonia, mitochondria and dictyosomes are agglomerated at one pole of the nucleus.

2. In spermatocytes, the dispersion of mitochondria and dictyosomes in the entire cytoplasm mass is recorded.

3. In spermatids, mitochondria merge and form the mitochondrial body, which presents one chromophilic and one chromophobe parts. This differentiation into the two zones, as well as the existence of some small vesicles inside the mitochondrial bodies subsist until the two mitochondrial cords elongate along the axial filament, when they become homogenous.

4. In spermatids dictyosomes merge and form the acroblast which secretes the acrosome. In advanced spermatids, the acrosome extends only on one side of the nucleus, a fact clearly observed in electronmicroscopic preparations.

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Faculty of Biology
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REVISION OF THE GENUS *NEMATABRAMIS* (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

A large number of specimens, including the holo-, para- and syntypes of all species and subspecies of *Nematabramis* were examined; lectotypes are designed for *N. everetti* and *N. steindachneri*. *N. steindachneri* and *N. alestes borneensis* are considered subspecies of *N. everetti*. The values of *everetti* overlap with those of *steindachneri* and of *borneensis*, those of *borneensis* with those of *alestes*, while *everetti* and *alestes* occur sympatrically in NE Kalimantan without hybridization. It is suggested that *borneensis* formerly lived within the range of *everetti* from where it colonized Palawan and Mindanao; *everetti* may be a recent form which replaced *borneensis* in north-eastern Kalimantan.

Nematrabamis Boulenger 1894 (= *Mearnsella* Seale & Bean, 1907) is a genus of small to medium-sized minnows, whose range is restricted to the northern half of Kalimantan (= Borneo) Island and to Palawan and Mindanao islands, Philippines. Because of the presence of a ventral keel between anal fin and throat, the genus was included by Weber a. De Beaufort [6] within the "Abramidinae" (as accepted at present, the East- and South-East genera of so-called abramidin minnows actually represent a distinct subfamily, Cultrinae). Yet the presence of a very long rostral barbel; of breeding tubercles and of well-developed coloured stripes, as well as the strong resemblance with the South-Asian genus *Esomus* demonstrate that this genus belongs to the Danioninae.

Three species and one subspecies are at present ascribed to this genus: *N. everetti* Boulenger 1894 [1], *N. steindachneri* Popta 1905 [3], *N. alestes* (Seale & Bean, 1907) [5] and *N. alestes borneensis* Inger & Chin, 1962 [2]. (A fourth nominal species, *N. verrecundus* Herre, 1924 proved long ago to be a synonym of *alestes*). *N. everetti* and *N. steindachneri* were described after several syntypes and no lectotypes were designed. No critical comparison of the four nominal species and subspecies was hitherto published.

REV. ROUM. BIOL.-ZOOLOGIE, TOME 16, Nº 2, P. 103–111, BUCAREST, 1971

Material

Some 300 specimens, including all types and paratypes were examined ; they belong to the following collections : British Museum, Natural History, London (B.M.N.H.), Field Museum of Natural History, Chicago (F.M.N.H.), Rijksmuseum van Naturlijke Historie, Leiden (R.M.N.H.), United States National Museum, Washington (U.S.M.N.) and Zoölogisch Museum, Amsterdam (Z.M.A.). Tables I—III show the result of the comaparison of the available specimens.

SYSTEMATIC ACCOUNT

1. **Nematabramis everetti everetti** Boulenger, 1894

Specimens examined :

Syntypes of *N. everetti* : B.M.N.H. 1893. 5. 30. 61—62, Bongon R. northern extremity of Kalimantan Isl., 2 specimens, 89.0 and 93.0 mm ; the smallest one (1893. 5. 30, 61), whose pharyngeal bones were removed (quite probably by Boulenger when describing the species) is here declared lectotype, the largest is paralectotype (Two other syntypes proved to belong to the subspecies *N. ev. borneensis*).

U.S.N.M. 138 365, Tawao R., NE Kalimantan, 42 spec., 39.0—78.0 mm st. length (10 measured : 69.0—85.2 mm)

U.S.N.M. 138 366, Silimpopon R., NE Kalimantan, 218 spec., (10 measured : 57.2—73.8 mm).

B.M.N.H. 1938, 12. 1. 39—42, Balung R., Tawao, NE Kalimantan, 4 spec., 70.0—78.0 mm.

B.M.N.H. 1938. 12. 1. 43—36, Javan R., North Kalimantan, 4 spec., 6 3.0—77.5 mm.

B.M.N.H. 1938. 12.1.36—38, Kimbutan R., North Kalimantan, 3 spec., 70.0—78 mm.

B.M.N.H. 1938. 12. 1. 47—48, Kabili R., North Kalimantan, 2 spec., 69 and 84 mm.

Z.M.A. 110—112, Kalimantan (no locality !), 6 spec., 36.5—60.5 mm.

D 3/(9) 10—13 ; A 2/(15) 15—17 (18) ; L. lat. 36 $\frac{7-8}{1}$ 39.

The body is deeper than in the other forms of the genus (Table III and Fig. 5) ; as in most species, the body depth has a positive allometry and it is evident, from Fig. 5, that *ev. everetti* specimens are statistically deeper than the specimens of the same size belonging to the three other taxa. *N. everetti everetti* has the longest barbel within the genus (Table III : barbels 30—45%, rarely 22.6—28 or up to 52.5% of st. length, as against 24.1—26.4% in *steindachneri*, 23—27, rarely up to 43.5% in *borneensis* and 13—24% in *alestes*) ; the barbels usually reach well beyond middle of pectoral fin. The number of dorsal rays is greater than in the other subspecies and species (Table I).

A few irregular, more or less vertical blotches are present on the anterior part of body sides in most specimens ; a vague longitudinal stripe is usually present in the posterior body half, in a few specimens also anteriorly.

The range of this subspecies includes the northern and north-eastern part of Kalîmantan (rivers flowing into Sulu and Celebes seas), from Bongon R. to Tawao R. and probably slightly further south.

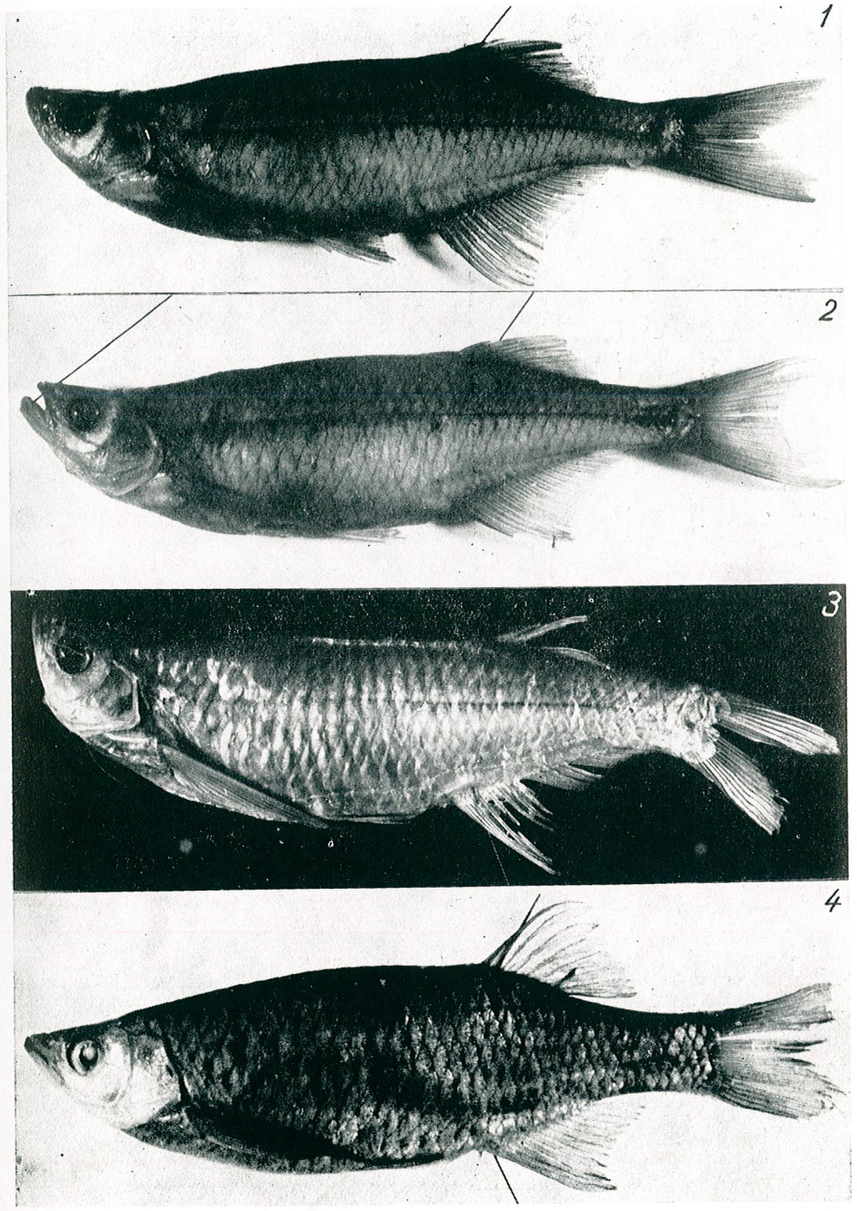

Fig. 1. — *Nematabramis everetti steindachneri* Popta. Lectotype, R.M.N.H. 7628. Kajan R.
Fig. 2. — *N. everetti steindachneri* Popta. Paralectotype. Kajan R.
Fig. 3. — *N. everetti borneensis* INGER & CHIN, R.M.N.H. 10986. Balingian R., Sarawak.
Fig. 4. — *Nematabramis alestes* (SEALE & BEAN). U.S.N.M. 190109. Iwahing R., Palawan.

2. **Nematabramis everetti steindachneri** Popta, 1905, Fig. 1, 2.

Specimens examined : syntypes of *N. steindachneri*, R.M.N.H. 7628, Kahan R., eastern slope of central Kalimantan, 4 spec., 102.0—112.0 mm ; one of them, 108.0 mm (Fig. 1) is here declared lectotype ; it retained its original catalogue number.

D 3/9—10 ; A 2/16—17 ; L. lat. 37 $\frac{6^1/_2}{1}$ 39.

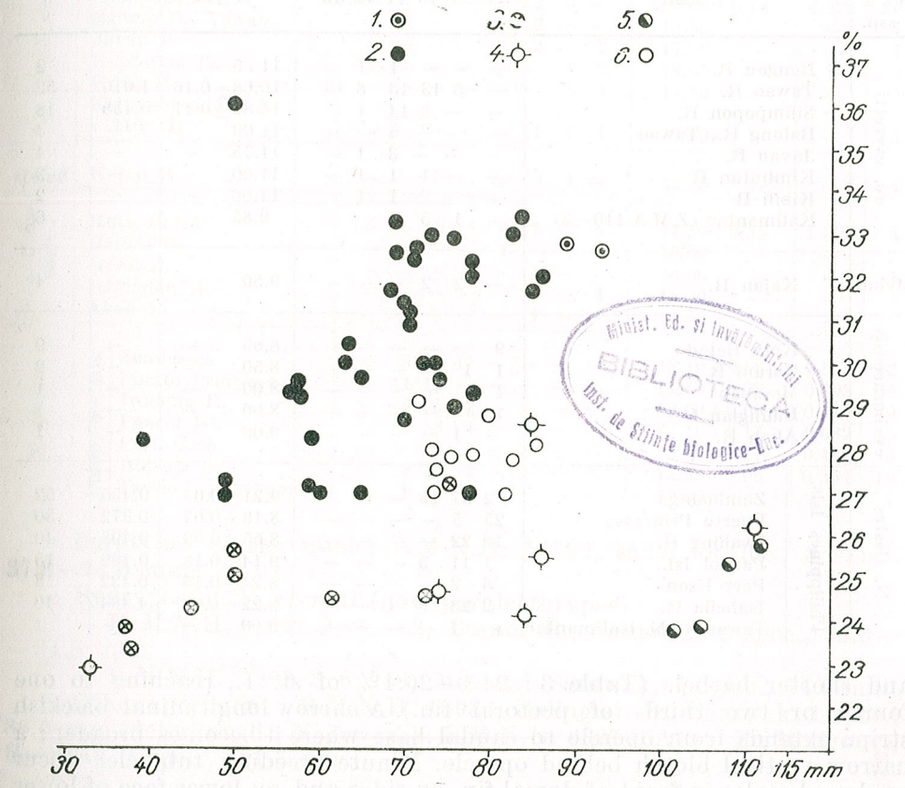

Fig. 5. — Value of body depth in *Nematabramis* in correlation with standard length. *1. N. everetti everetti*, Bongon R. ; *2. N. ev. everetti*, other localities ; *3. N. everetti borneensis*, holo- and paratypes ; *4. N. everetti borneensis*, other specimens (including Barah and Merabah paralectotypes of *N. everetti*) ; *5. N. everetti steindachneri*. *6. N. alestes*, Zamboanga, Mindanao.

In comparing *steindachneri* with *everetti*, Popta [4] mentions following characters of the first named : a lower body, a different coloration, the relative position of the dorsal and anal fins, and only 6 $\frac{1}{2}$ scales between dorsal origin and lateral line. Actually the anal fin is in all *Nematabramis* opposite to the dorsal, its origin lying slightly before, slightly behind or exactly under vertical from dorsal origin. It is quite evident from figure 5 that the body is much lower in *steindachneri* than in all other

Nematabramis. N. e. steindachneri differs from the nominal subspecies also in having fewer dorsal rays (in this character it is intermediate between *N. ev. everetti* and *N. ev. borneensis*, approaching rather *N. alestes* (Table 1)

Table 1

Number of branched dorsal rays in *Nematabramis*

Sp. & ssp.	Locality	8	9	10	11	12	13	M±m	σ	n
ev. everetti	Bongon R.	—	—	—	1	1	—	11 : 5 —		2
	Tawao R.	—	5	13	13	8	13	10.68±0.16	1.010	52
	Silimpopon R.	—	—	3	14	1	—	10.89±0.11	0.458	18
	Balung R., Tawao	—	—	—	4	—	—	11.00	—	4
	Javan R.	—	—	—	3	1	—	11.75 —	—	4
	Kimbutan R	—	—	1	1	1	—	11.00 —	—	3
	Kibili R	—	—	—	1	1	—	11.50 —	—	2
	Kalimantan (Z.M.A.110—2)	—	1	5	—	—	—	9.85 —	—	6
steind	Kajan R.	—	2	2	—	—	—	9.50 —	—	4
ev. borneensis	Kota Belud	9	—	—	—	—	—	8.00 —	—	9
	Baram R.	1	1	—	—	—	—	8.50 —	—	2
	Merabah	1	—	—	—	—	—	8.00 —	—	1
	Balingian R.	1	—	—	—	—	—	8.00 —	—	1
	Akah R.	—	1	—	—	—	—	9.00 —	—	1
N. alestes, Philippines Isls.	Zamboanga	2	37	13	—	—	—	9.21±0.07	0.495	52
	Puerto Princessa	25	5	—	—	—	—	8.16±0.07	0.372	30
	Iwahing R.	18	22	—	—	—	—	8.55±0.08	0.498	40
	Pancol Isl.	1	11	3	—	—	—	9.14±0.13	0.499	15
	Port Uson	6	2	—	—	—	—	8.24±0.15	0.432	8
	Isabella R.	2	28	9	1	—	—	9.22±0.08	4.494	40
	Tawao R., N. Kalimantan	—	1	—	—	—	—	9.00 —	—	1

and shorter barbels (Table 3 : 24.1—26.4% of st. 1., reaching to one fourth or two thirds of pectoral fin.). A narrow longitudinal blackish stripe extends from opercle to caudal base where it becomes broader ; a narrow vertical blotch behind opercle. Minute breeding tubercles occur on dorsal scales in front of dorsal fin, on sides and on lower face of lower jaw and on the base of first dorsal rays. Breeding tubercles were not recorded in other forms of *Nematabramis*, but may occur in all.

Range : Kajan R., perhaps also other river drainages on the eastern slope of Central Kalimantan.

3. **Nematabramis everetti borneensis** Inger & Chin, 1962, Fig. 3.

Specimens examined :

Holotype of *N. alestes borneensis*, F.M.N.H. 44791, Kota Belud, western slope of North Kalimantan, 75.5 mm. st. length.

Table 2

Number of branched anal rays in *Nematabramis*

Sp. & ssp.	Locality	11	12	13	14	15	16	17	18	M±m	σ	n
N. ev. everetti	Bongon R.	—	—	—	—	—	1	—	1	17.00 —	—	2
	Tawao R.	—	—	—	1	7	12	16	4	16.37±0.15	0.966	40
	Silimpopon R.	—	—	—	—	3	12	3	—	16.00±0.14	0.575	17
	Balung R., Tawao	—	—	—	—	2	2	—	—	15.50 —	—	4
	Javan R.	—	—	—	—	—	2	—	2	17.00 —	—	4
	Kimbutan R.	—	—	—	—	—	—	1	2	17.67 —	—	3
	Kibili R.	—	—	—	—	—	—	1	1	17.50 —	—	2
	Kalimantan (Z.M.A. 110—2)	—	—	—	—	—	1	4	1	17.00 —	—	9
steind	Kajan R.	—	—	—	—	—	3	1	—	16.25 —	—	4
N. e. borneensis	Kota Belud	—	—	2	1	4	1	—	—	14.50±0.35	1.000	8
	Baram R.	—	—	—	—	1	—	1	—	16.00 —	—	2
	Merabah	—	—	—	—	—	1	—	—	16.00 —	—	1
	Balingian R.	—	—	—	—	—	—	—	1	18.00 —	—	1
	Akah R.	—	—	—	—	1	—	—		16.00 —	—	1
N. alestes, Philippines Isls.	Zamboaga	—	5	36	11	—	—	—	—	13.10±0.08	0.543	52
	Puerto Princessa	—	11	19	—	—	—	—	—	12.63±0.08	0.493	30
	Iwahing R.	—	3	35	2	—	—	—	—	12.59±0.08	0.515	40
	Pancol Isl.	—	3	8	4	—	—	—	—	13.10±0.17	0.682	15
	Port Uson	—	—	5	3	—	—	—	—	13.40±0.17	0.486	8
	Isabella R.	—	12	35	2	—	—	—	—	12.98±0.06	0.354	19
	Tawao R., Kalimantan	—	1	—	—	—	—	—	—	12.00 —	—	1

Paratypes of the same : F.M.N.H. 14792, same locality, 8 spec., 37.8—74.0 mm.

Syntypes of *N. everetti* (now paralectotypes) :

— B. M.N.H. 1892. 2.41—42, Baram R., Sarawak, 2 spec., 33.0—84.8 mm.

— B.M.N.H. 1893. 3. 6. 246, Merabah,Sarawak, 1 spec., 74.0 mm.

Other specimens : R.M.N.H. 10986, Balingian R., Sarawak, 1 spec.. 86.0 mm (determined : *N. everetti*) ; F.M.N.H. 68277, Akah R., Meligong,, Sarawak, 1 spec., 101.5 mm.

D 3/8(9) ; A 2/13—17 (18) ; L. lat. 36 $\frac{6^1/_2-7}{1^{1/2}}$ 39 (40).

Inger & Chin [2] described a new subspecies of *N. alestes* from the western slope of North Kalimantan characterized, according to them, by : D 3/8 ; A 2/13—16, L. lat. 34—36 (these figures proved wrong : I found 36—37 scales in the holotype, 37—39 in the paratypes), barbels longer than in the Philippines *N. alestes* yet shorter than in *everetti*.

The few other available specimens from western North Kalimantan and from Sarawak agree in number of dorsal and anal rays (Tables 1 and 2) and in body depth (Fig. 5) with the types of *borneensis ;* the barbel is, in the specimen from Balingian as short as in the types, in that from Akah R, longer (31.4% of st. length), in those from Baran R. and Merabah still

Table 3

Body proportions in *Nematabramis*

Subsp.	Locality	st. l. mm	n	in % of standard length							
				depth	least depth	caudal ped.	predor-sal	head	barbel	snout	eye
everetti	Bongon R.	89—93	2	32.6—32.8	10.7—11.2	16.3—18.0	64.0—66.0	23.8—24.2	22.6—30.2	7.3—7.6	5.6—5.9
	Tawao R.	69—86	10	27.0—32.8 (30.36)	9.2—10.6 (10.08)	15.1—17.7 (16.75)	63.5—69.0 (65.92)	23.8—26.0 (24.51)	36.0—44.8 (38.75)	6.6—8.2 (7.43)	5.3—5.9 (5.52)
	Silimpopon R.	57.2—73.8	10	28.8—32.6 (30.24)	9.5—10.6 (10.03)	15.4—17.6 (16.45)	64.0—68.0 (66.5)	24.7—26.9 (26.19)	33.0—44.5 (38.6)	7.6—8.6 (8.03)	5.7—6.9 (6.42)
	others	49—78	19	23.3—36.0	9.0.—10.7	14.7—17.4	63.0—66.0	22.2—27.6	28.0—52.0	6.6—8.5	5.7—5.7
steindachneri		102—112	4	23.9—25.8	8.1—8.8	16.4—17.8	61.0—64.0	22.3—23.6	24.1—26.4	6.7—7.4	4.8—5.6
borne-ensis	Kota Belud	62—76	3	24.4—27.2	9.4—9.6	18.6—19.3	64.0—66.0	23.2—24.8	20.9—24.3	6.8—8.1	5.7—6.3
	others	74—102	4	25.6—28.6	9.5—10.8	15.7—18.7	63.0—67.0	22.5—24.8	26.8—43.5	6.4—8.0	5.2—5.9
alestes	Zamboanga	72—89	10	27.0—29.1 (27.91)	9.5—11.1 (10.65)	15.2—19.5 (18.36)	63.5—67.0 (65.0)	21.7—24.6 (23.26)	13.5—19.2 (16.31)	6.0—7.4 (6.71)	5.1—5.8 5.(31)
	Iwahing R.	80—101	10	24.4—30.6 (27.11)	9.6—11.3 (10.66)	18.3—20.5 (19.40)	62.2—65.9 (64.07)	23.4—25.6 (24.93)	13.7—24.3 (16.41)	7.7—8.3 (8.54)	5.3—6.2 (5.68)
	Puerto Princessa	69—88	10	24.8—30.0 (26.40)	10.0—11.2 (10.35)	17.4—20.5 (18.90)	62.5—66.0 (64.57)	23.8—27.0 (25.41)	13.2—21.0 (17.37)	7.6—9.0 (7.04)	4.9—5.9 (5.62)
	Pancol Isl.	67.82	10	26.9—31.2 (28.71)	10.0—11.1 (10.43)	17.9—20.8 (19.04)	63.0—65.5 (64.31)	24.3—26.0 (25.22)	— —	6.6—7.8 (7.26)	5.2—6.1 (5.65)
	Tawao R.	46	1	28.6	10.9	18.5	64.0	27.8	20.0	8.7	7.2

longer (38.0 and 43.5% of st. length, e.g. as in *ev. everetti*), reaching beyond middle of pectoral fin.

Short blackish vertical stripes exist on anterior part of body sides in all specimens; a longitudinal stripe only in the type specimens.

Comparatively to the other forms of the genus, *borneensis* has the smallest number of dorsal rays; in number of anal rays it is intermediate between *everetti* and *steindachneri* on the one hand, *alestes* on the other (Table 2); the body depth has intermediate values between those of *steindachneri* and of *everetti* and the same values as *alestes* (Fig. 5).

This subspecies inhabits the western (South China sea) slope of North Kalimantan and Sarawak, probably not reaching to southern Sarawak.

4. **Nematabramis alestes** (Seale & Bean, 1907). Fig. 4.

Specimens examined :

From Philippines Islands :

Holotype of *Mearnsella alestes* : U.S.N.M. 57841, Zamboanga, western extremity of Mindanao, Philippines Isls., 50.0 mm st. length.

Paratype of the same : U.S.N.M. 61151, same locality, 42.8 mm.

U.S.N.M. 190112, same locality, 50 spec. (10 measured : 73—89 mm).

U.S.N.M. 190113. Malagto and Canina rivers, Puerto Princessa, eastern slope of Central Palawan Isl., Philippines, 70 spec., 35—88 mm.

U.S.N.M. 190109, Iwahing R., eastern slope of central Palawan Isl., 70 spec. (10 measured : 80—101 mm).

U.S.N.M. 190110, Malina R., Mantaquin Bay, eastern Palawan, 2 spec., 85.0 and 88.0 mm.

U.S.N.M. 190116, Pancol Isl., Palawan, 15 spec., 67.0—80.2 mm.

Examined summarily : U.S.N.M. 190114, Port Uson, Philippines Isls., 8 spec., U.S.N.M. 190111, Caiholo R., Ulugan Bay, Palawan; U.S.N.M. 190115, Isabella R. (apparently Basilan Isl., west. of Mindanao), 40 sp.

From Kalimantan : out of U.S.N.M. 13865, Tawao, NE Kalimantan, 1 spec., 46.0 mm.

D 3/8—10 (11); A 2/11—14; L. lat. (34—) 35 $\frac{6—7}{1}$ 38

The number of dorsal rays is slightly greater than in *borneensis* (and greater in the *alestes* specimens from Isabella R., Zamboanga and Pancol Isl. —M = 9.14—9.22 — than in those from Palawan — M = = 8.16—8.55); that of anal rays is smaller than in all three subspecies of *N. everetti* (M = 12.6—13.1, as against 14.5 in *borneensis*, etc.); see Table II). The body depth is practically the same as in *borneensis* (Fig. 1);

the barbels are shorter than in three subspecies of *everetti* (their length being 13.2—24.3% of st. length) and do not reach the end of opercle.

The colour pattern is rather variable. In some populations, such as those from Iwahing R., Puerto Princessa and Isabella R. there are well marked anterior vertical bars (spots) and no, or only quite slight lateral stripes; in Caiholo R. and Port Calton populations the lateral stripes are well marked, the vertical bars being reduced, while both stripes and vertical bars are well developed in Zamboanga and Malina R. populations.

N. alestes was hitherto known only from Philippines Islands (Pawalawan and western part of Mindanao, probably Basilan Island too); yet I identified one specimen also from Tawao, NE Kalimantan, among a series of *N. ev. everetti.*

GENERAL CONSIDERATIONS AND CONCLUSIONS

The four taxa of *Namatabramis* have mainly representative ranges; only in Tawao, NE Kalimantan, *everetti* and *alestes* occur sympatrically. The differences between the three Kalimantan forms being only statistical, with overlap of extreme values (and probably intergradation at the limit of ranges), I consider them only as subspecies. In some characters, such as body depth, *borneensis* is intermediate between *everetti* and *steindachneri;* in other characters (number of rays) *steindachneri* is intermediate. The Philippines *N. alestes* is evidently closer to *borneensis;* yet I consider both as specifically distinct, because of the local sympatrical occurrence of *everetti* and *alestes.* A strong salt water barrier prevents at present a gene flow between *borneensis* and *alestes,* while such a gene flow probably exists between the first-named and *everetti.*

Nematabramis consists in a group of representative forms with partially circular distribution and overlap of the extreme forms without hybridization. It is difficult to explain the dispersal and speciation within this genus. Like all minnows and other continental Asian animals, *Nematabramis* reached the Philippines from Kalimantan. Palawan and Mindanao were colonized independently (there is no possibility of direct dispersal between both islands for primary freshwater animals) and both from the northern and north-eastern corner of Kalimantan, e.g. from the present range of *ev. everetti.* Yet from all three subspecies of *everetti, borneensis* is evidently closer to *alestes, ev. everetti* being on the contrary morphologically the most distant. One possible explanation is that the northern corner of Kalimantan was formerly inhabited by a *borneensis*-like form, which colonized (probably during an interglacial period) Palawan and Mindanao and was replaced later on by the more recent *ev. everetti.*

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L'OVOGENÈSE CHEZ LES POISSONS ACIPENSÉRIDÉS. LA MORPHOGENÈSE ET LA CONSTITUTION HISTOCHIMIQUE DES MEMBRANES EXTERNES

PAR

MARIA CALOIANU-IORDĂCHEL

The histochemical structure and composition of external membranes of the ovocyte in sturgeons are presented. The young ovocyte, still devoid of spare inclusions is surrounded by a pellucid area, by the membrane of follicular cells, by the perifollicular membrane, the internal and the external sheath. These are progressively modified during vitellogenesis. The histochemical study reveals that the totality of the pellucid substance is formed of neutral mucopolysaccharides linked to protides. Protides become gradually dominant marking the difference between the two membranes of the pellucid area. The perifollicular membrane has a rich and constant content of neutral mucopolysaccharides.

Les membranes de l'œuf présentent une importance considérable pour son développement, la réalisation de la fécondation et la formation du nouvel organisme. L'étude des membranes et spécialement de la zone pellucide a été abordée presque chez tous les groupes de vertébrés, mais particulièrement chez les Cyclostomes, les Téléostéens, les Amphibiens et les Mammifères. En ce qui concerne les Acipenséridés, on mentionne l'existence de plusieurs membranes externes dans une série de travaux consacrés à l'étude des étapes de maturation des gonades [4], [13], [15], [17], [18], [23], [24]. Nous avons décrit dans une communication récente [5] quelques caractéristiques structurales de la zone pellucide (zona radiata). Le présent travail comprend des observations plus déstaillées ainsi qu'une série de nouvelles données concernant les membranes des ovocytes et leur constitution chimique.

MATÉRIEL ET MÉTHODE

Les recherches ont été effectuées sur des exemplaires d'Acipenséridés anadromes : le huiron-grand esturgeon (*Huso-huso* L.), l'esturgeon (*Acipenser guldenstädti* Brandt) et une autre espèce d'esturgeon (*Acipenser stellatus* Pallas), qui migrent de la mer Noire dans les eaux du Danube pour la période du frais, ainsi que sur l'espèce rhéophile *Acipenser ruthenus*. L. pêchée dans le chenal du Danube. L'ovaire étant caractérisé par un

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développement synchrone des ovocytes (Pl. I, 1), il a été nécessaire d'effectuer des fixations périodiques afin d'obtenir toutes les phases. Pour les études histologiques et histochimiques on a utilisé les fixateurs : Bouin, Halmi, Carnoy, Sussa, Zenker, formol neutre 10%, Champy. Les colorations d'ensemble ont été effectuées avec « Azan » d'après Heidenhain, picro-indigo-carmine et trichromique Masson. Les réactions cytochimiques réalisées en vue de mettre en évidence les formations glucidiques et protidiques des membranes des ovocytes aux différents phases de développement sont groupées dans le tableau 1.

RÉSULTATS

Nous présentons ci-dessous le processus de formation et la structure des membranes externes et spécialement de la zone pellucide. Vu la grande diversité d'opinions concernant la définition et le dénombrement des étapes de développement des ovocytes [13], [15], [17], [18], [23], [24], nous utiliserons pour la description la périodisation suivante :

— la prévitellogenèse (Stade A_0) pendant laquelle se produit dans l'ovocyte : la migration des organites cellulaires, des modifications structurales corticales, l'apparition de la substance pellucide sous forme de plages discontinues, la formation autour de l'ovocyte de deux membranes cellulaires et d'une membrane hyaline ;

— le début de la vitellogenèse (Stade A_1), marqué par : l'apparition des premières plaquettes vitellines, zone pellucide impaire, des modifications qui apparaissent au niveau de la membrane folliculaire ;

— la vitellogenèse intense (Stade A_2) pendant laquelle ont lieu les processus de synthèse les plus intenses : la zone pellucide complètement différenciée se sépare en deux couches parallèles, la membrane hyaline secondaire est très développée et avec un double aspect ;

— fin de la période de vitellogenèse (Stade A_3), le repos avant l'ovulation, quand les synthèses vitellines sont très ralenties.

1. La différenciation et la structure des membranes externes

Chez toutes les espèces d'esturgeons de même que chez d'autres groupes de vertébrés les jeunes ovocytes qui se trouvent dans le stroma de l'ovaire sont limités à l'extérieur seulement par le plasmalemme. Autour des ovocytes se groupent graduellement des cellules folliculaires qui tendent à former une membrane externe continue (Pl. I, 2). La substance pellucide apparaît sous forme de plages de substance amorphe qui s'applique étroitement contre les cellules folliculaires (Pl. I, 2, 3). Ces plages se rejoignent et forment une zone continue (Pl. II, 5, 6,) qui entoure la cellule germinale. A la surface des cellules folliculaires (Pl. I, 2, 3,) se dispose en même temps une autre membrane sans structure, à aspect hyalin, et qui donne une réaction APS intense. Extérieurement à cette membrane, que nous allons dénommer — en fonction de sa position — membrane périfolliculaire, se dispose aussi la thèque interne (Pl. I, 2, 3, 4,), ainsi que des éléments encore discontinus de la thèque externe fibroconjonctive.

Avec l'accroissement de l'ovocyte, la zone pellucide se développe aussi. Sa limite avec la surface de l'ovocyte est marquée par une membrane irrégulière, intensément colorée par l'azocarmin. Les autres membranes

sont également mieux délimitées. On distingue ainsi à la fin de la période de prévitellogenèse (A_0) une membrane APS +, la zone pellucide impaire, les cellules folliculaires, la membrane périfolliculaire, la thèque interne et la thèque externe.

Au moment de l'apparition des granules vitellins dans le cytoplasme de l'ovocyte (A_1) on observe des modifications importantes au niveau des membranes externes, à l'exception de la membrane périfolliculaire qui garde la même structure et réactivité chimique. La thèque externe devient plus fortement vascularisée et les cellules manifestent des réactions positives aux glucides et aux protides. Le volume des cellules folliculaires augmente. La substance pellucide devient dense mais d'une épaisseur égale sur toute la surface de l'ovocyte.

En plein processus de vitellogenèse (A_2) la zone pellucide fortement développée apparaît au microscope optique avec des striations radiaires [5]. Ce n'est que vers la fin de la période de vitellogenèse que l'aspect et le rapport dimensionnel entre toutes les membranes changent. La zone pellucide se sépare formant une couche interne et une couche externe (Pl. II, 7). Apparemment homogènes, peu à peu, la couche externe se différencie apparaissant d'une consistance plus dense tandis que la couche interne de la zone pellucide continue à présenter une striation dans la portion basale [5]. Le volume des cellules folliculaires diminue parallèlement au ralentissement du processus de vitellogenèse. Nous assistons par contre à un développement accentué de la membrane périfolliculaire. La consistance de la membrane est elle aussi différente, apparaissant plus dense dans la zone externe. Par la coloration à l'Azan, le bleu clair de la zone interne devient un bleu intense dans la zone externe. A l'extérieur de cette membrane, la thèque interne formée à présent de petites cellules aplaties est séparée d'une membrane APS +, la membrane basale de la thèque externe.

Au moment du ralentissement des processus de synthèses vitellines dans l'ovocyte développé qui a atteint les dimensions finales (A_3), on remarque quelques modifications (Pl. II, 8). A la surface de la zone du cytoplasme cortical libre d'inclusions et pigments, se dispose une fine membrane APS +. L'aspect des couches de la zone pellucide se ressemble, mais elles ne sont pas égales sur toute la surface de l'œuf. Beaucoup plus épaisses au pôle végétatif, elles se rétrécissent progressivement vers le pôle animal. La membrane des cellules folliculaires, beaucoup plus réduite, apparaît sur les coupes histologiques plutôt comme une raie fine limitant la zone pellucide de la membrane amorphe périfolliculaire (Pl. II, 8), qui, au moment du détachement de l'œuf du stroma de l'ovaire viendra directement en contact avec le milieu environnant.

2. CONSTITUTION HISTOCHIMIQUE

Les réactions histochimiques utilisées pour obtenir des renseignements sur la constitution chimique des membranes des ocvocytes ont montré des variations importantes au cours de leur développement. Nous présentons dans le tableau 1 les modifications au niveau de la zone pellucide et de la membrane périfolliculaire, celles-ci restant comme couches protectrices de l'œuf mûr.

Les modifications des cellules folliculaires seront présentées séparément dans une étude consacrée spécialement à ce sujet. Les réactions utilisées nous indiquent la présence d'un composant mucopolysaccharidien et d'un composant protéique (tableau 1).

Dès le commencement de leur développement, la substance pellucide et la membrane périfolliculaire donnent une réaction APS +, ce qui indique la présence des polysaccharides. Les mucopolysaccharides neutres forment le composant commun. La valeur de la réaction sur les coupes soumises

Tableau 1

Réactivité histochimique de la zone pellucide et de la membrane périfolliculaire.

	A 0		A 1		A 2			A 3		
	Zp imp	Mpf	Zp imp	Mpf	Zp ci	Zp ce	Mpf	Zp ci	Zp ce	Mpf
A.P.S.	++	+++	++	+++	+	+(+)	+++	±	±	+++
A.P.S. après maltase	+	++	+	+++	+	+	+++	±	±	++
Mucicarmin	±	+	±	+	±	±	++	0	0	++
Bleu alcian	0	±	0	±	0	0	0	0	0	+
Bleu de toluidine pH 1	0	±	0	±	0	0	±	0	0	0
pH 3	±	±	0	+	0	0	0	0	0	0
pH 4	±	±	±	+	0	0	0	0	0	0
Bleu de toluidine après méthylation pH 1	0	±	0	0	0	0	0	0	0	0
pH 3	±	±	0	0	0	0	0	0	0	0
pH 4	±	+	±	+	0	0	0	0	0	0
Bleu toluidine après méthylation et saponification pH 1	0	±	0	0	0	0	0	0	0	0
pH 3	0	0	0	0	0	0	0	0	0	0
pH 4	0	±	±	+	±	±	0	0	0	0
Bleu de toluidine après sulfatation pH 1	++	++	+	+++	++	++	+++	±	±	+++
pH 3	++	++	±	+++	++	+++	++	±	±	++
pH 4	+	+++	+	+++	+	+	+++	+	++	++++
Alloxane-Schiff	±	0	+	±	++	++	0	+	±	0
Shiff sans oxydation	0	0	0	0	0	+	++	0	0	0
Bleu de bromophénol	+	±	+++	+	+++	+++	+	+++	++	0
Bleu de bromophénolmercurique	±	±	++	+++	+++	++	+	++	+++	+
Millon trichloracétique	++	+	+++	++	++	+	0	±	±	0
Millon sulfurique	+	+	+	+	++	+	±	±	±	0
Dinitroflouorobenzène	±	±	+	±	+	+	±	±	±	0
Azoréaction	0	0	±	0	+	±	0	+	0	0
R S R										
— SH (seuls)	0	0	0	0	0	0	0	0	0	0
— SH (bloqués)	0	±	0	0	±	0	0	±	0	0
— SS (seuls)	0	0	+	0	+	0	0	±	0	0
— SS (bloqués)	±	±	±	0	0	0	0	0	0	0

Zp – zone pellucide
ci – couche interne
ce – couche externe
Zp imp – zone pellucide impaire
Mpf – membrane périfolliculaire

antérieurement à l'action de la trypsine démontre aussi l'existence des protéines dans la substance pellucide. Donc dès le stade A_0, la substance pellucide comprend deux composants.

La réaction APS au niveau de la zone pellucide marque un enrichissement en mucopolysaccharides neutres au cours du stade A_1, en diminuant ensuite progressivement jusqu'à la fin du stade A_3 (Pl. III, 9, 10 ; Pl. IV, 11). On observe également une différence entre les deux couches de la zone pellucide, la couche interne ayant une réaction plus intense que celle de la couche externe. Par contre, la membrane périfolliculaire accuse une réaction intensément positive à l'APS pendant toute la période du développement. A côté des mucopolysaccharides neutres qui constituent le composant principal, on observe aussi de faibles traces de mucopolysaccharides sulfatés (Pl. IV, 12, 13).

La membrane basale de la thèque interne présente également une réaction APS positive.

En analysant les indications données par les réactions aux protéines, on observe que la zone pellucide marque un accroissement progressif de la quantité de protéines au cours du développement de l'ovocyte (tableau 1). En même temps les réactions sont plus intenses dans la couche interne de la zone pellucide que dans la zone impaire et la couche externe. Cette différence entre les deux couches de la zone pellucide diminue à la fin de la période de vitellogenèse et l'intensité des réactions est semblable (Pl. V, 14, 15, 16, 17). Le supplément protéique de la membrane interne de la zone pellucide paraît être donné par une protéine à groupements disulfures et à groupement phénol. Contrairement à la zone pellucide, la membrane périfolliculaire est pauvre en protéines. A la fin de la période de prévitellogenèse et au début de la vitellogenèse elle manifeste une légère réactivité, qui baisse ensuite progressivement jusqu'au stade A_3.

Au stade A_2 la membrane périfolliculaire et la couche externe de la zone pellucide marquent encore la présence des aldéhydes libres.

Par conséquent la substance pellucide réagit, dès sa formation, comme une mucoprotéine. Au début du processus de vitellogenèse le composant protéique devient plus accusé, ainsi que la différence d'intensité de la réaction entre les deux couches de la zone pellucide. La couche interne semble contenir une mucoprotéine à groupement disulfure tandis qu'au niveau de la couche externe apparaissent des aldéhydes libres. Dans la constitution chimique de la membrane périfolliculaire le composant principal est un mucopolysaccharide neutre et une très petite quantité de mucopolysaccharides sulfatés.

DISCUSSIONS

Les membranes des ovocytes chez toutes les espèces d'esturgeons étudiées démontrent les mêmes caractéristiques. On observe seulement des différences dimensionnelles. Leur formation parcourt les étapes généralement connues chez les animaux, présentant en même temps quelques caractéristiques propres au groupe.

Quant à l'apparition de la zone pellucide comme une membrane sans structure autour de l'ovocyte, Olschvang [15] émet l'hypothèse que celle-ci

se réalise chez les esturgeons après la formation du follicule, tandis que Molchànova [13] soutient, au contraire, qu'elle a lieu jusqu'au moment de sa formation. Nos données confirment le fait que les plages de substance pellucide apparaissent précocement en s'insinuant entre la surface de l'ovocyte et la surface des cellules folliculaires qui n'ont pas encore formé une membrane bien délimitée.

Au cours de la vitellogenèse la substance pellucide devient très abondante, fait comparable seulement aux Cyclostomes [3] et aux Téléostéens [2], [8], [13], [27], [28], etc. Mais chez les esturgeons la substance pellucide est nettement différente de celle citée pour les Cyclostomes [3], les Téléostéens [2], [8], [14], [21] et les Mammifères (9), ressemblant plutôt à celle décrite chez Urodeles [26]. L'aspect semblable des couches de la zone pellucide sur les coupes histologiques a déterminé quelques auteurs [13], [15], [23] à considérer que celles-ci ont une structure identique. De nos données ainsi que de quelques observations au microscope électronique encore non publiées, il ressort que la structure des couches n'est pas identique chez les esturgeons.

Toujours dans la période de vitellogenèse intense, quand la zone pellucide a des striations radiaires, quelques auteurs [13], [15], [17], [18], [23], [24] mentionnent la formation d'une nouvelle membrane à la surface de la zone pellucide. En effet, cette membrane présente une intensité maximum de son développement vers la fin de la période de vitellogenèse, mais elle apparaît déjà dès le début du stade de prévitellogenèse (A_0), (Pl. I, II). Nous l'avons dénommée la membrane périfolliculaire d'après sa position à la surface des cellules folliculaires et afin de ne pas être confondue avec la zone pellucide impaire. Ainsi qu'il ressort clairement des figures, la membrane folliculaire est formée à ce stade de cellules fortement aplaties comme suite de la pression exercée par l'ovocyte. C'est pour cette raison qu'elle peut passer inaperçue et qu'on peut considérer le rapport direct entre la surface de l'ovocyte et la membrane hyaline fortement APS+, qui est en réalité la membrane périfolliculaire. La confusion est d'autant plus aisée du fait qu'extérieurement à cette membrane se trouve la thèque interne, qu'on peut facilement considérer la membrane des cellules folliculaires. A notre avis, c'est pour cette raison qu'on parle si tard de l'apparition et du développement de la membrane périfolliculaire.

Parallèlement au développement de l'ovocyte, autour de lui se forment trois membranes cellulaires (la membrane des cellules folliculaires, la thèque interne et la thèque externe) et deux membranes acellulaires (la zone pellucide et la zone périfolliculaire).

Les mucopolysaccharides et les protéines de la substance pellucide chez les esturgeons représentent une caractéristique généralement rencontrée chez les animaux : Tuniciers [14], Equinodermes [1], Cyclostomes [3], [28], Téléostéens [1], [6], [11], [19], [21], [22], [29], etc., Amphibiens [7], [16], [20], [25], Mammifères. Contrairement à la majorité des groupes étudiés où sont mises en évidence les mucopolysaccharides acides, chez les esturgeons la zone pellucide contient des mucopolysaccharides neutres. Ce n'est que la membrane périfolliculaire qui contient aussi des mucopolysaccharides sulfatés. La présence des protéines à groupement disulfure qui n'ont été mentionnées avec précision que chez quelques groupes d'animaux [1], [3], [25], [29], est également caractéristique pour les esturgeons. La différence entre la constitution chimique des couches de la zone pellucide est donnée par les protéines.

Il est intéressant à noter que la différence du contenu protéique dans les couches de la zone pellucide a été décrite seulement chez *Lampetra japonica* [28] et *Lampetra planeri* [3].

Nos observations sur la constitution chimique des membranes des ovocytes et spécialement de la zone pellucide chez les esturgeons mettent en évidence pour la première fois des caractéristiques qui sont générales aussi pour d'autres vertébrés, ce qui conduit à supposer une fonction semblable chez ces formations. En même temps sont mises en évidence quelques particularités spécifiques :

— la présence des mucopolysaccharides neutres et l'absence des mucopolysaccharides acides dans les couches de la zone pellucide ;

— la présence des protéines à groupement disulfure et à groupement phénol dans la couche interne et des aldéhydes libres dans la couche externe de la zone pellucide ;

— l'abondance des mucopolysaccharides neutres dans la membrane périfolliculaire ainsi que l'existence des mucopolysaccharides sulfatés.

Etant donné qu'une partie de ce travail fut réalisé à la Faculté des Sciences de Paris, dans les Laboratoires d'Anatomie Comparée, conduits par Monsieur le Professeur Charles Devillèrs, j'exprime à cette occasion mes chaleureux remerciements. Pour l'aide et les conseils accordés, je remercie tout spécialement Monsieur le Professeur Roland Bauchot. Pour la collaboration amicale et les nombreuses discussions instructives que nous avons eues, j'exprime également ma gratitude au Dr Sylvie Busson-Mabillot.

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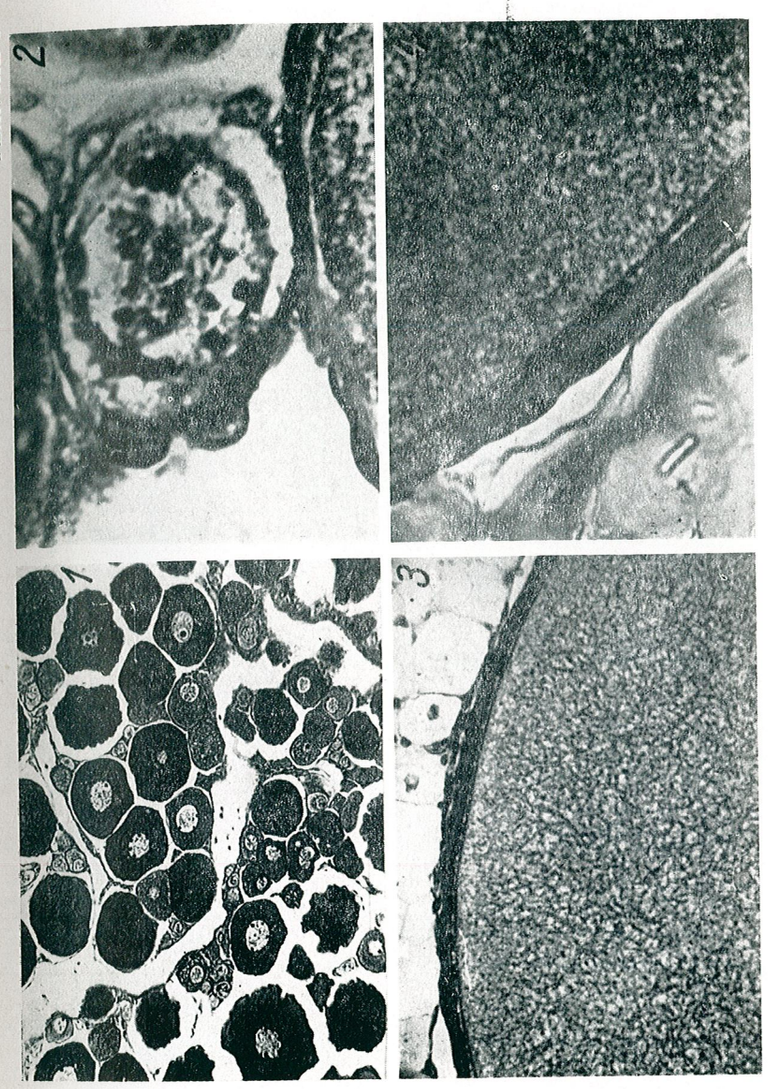
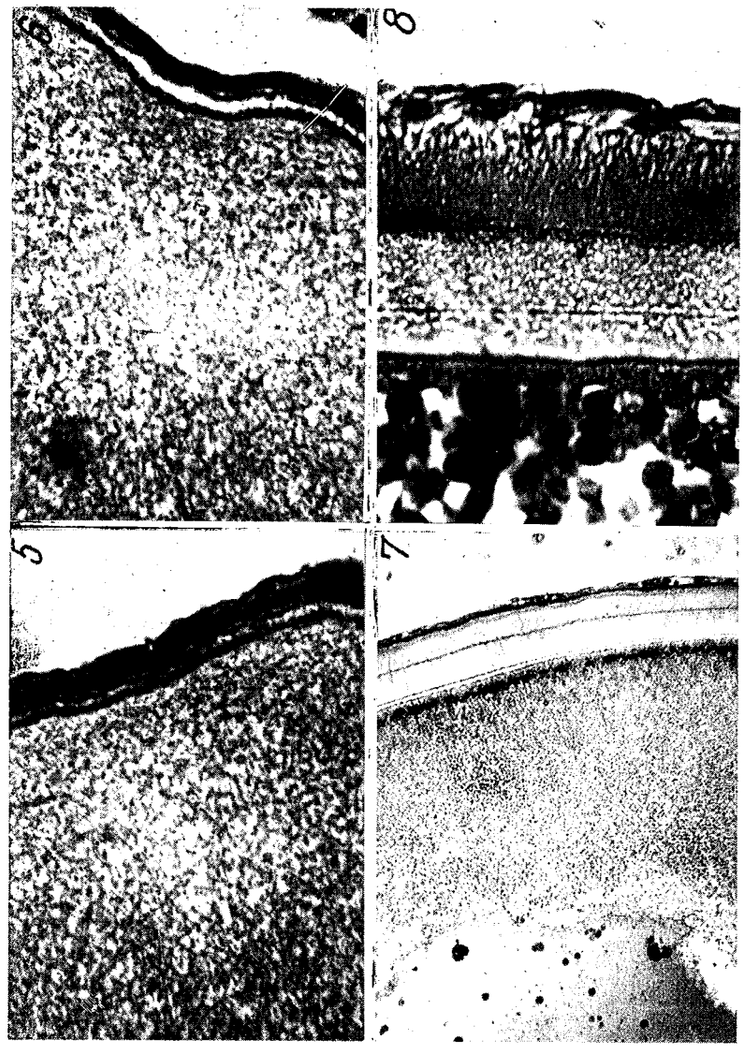
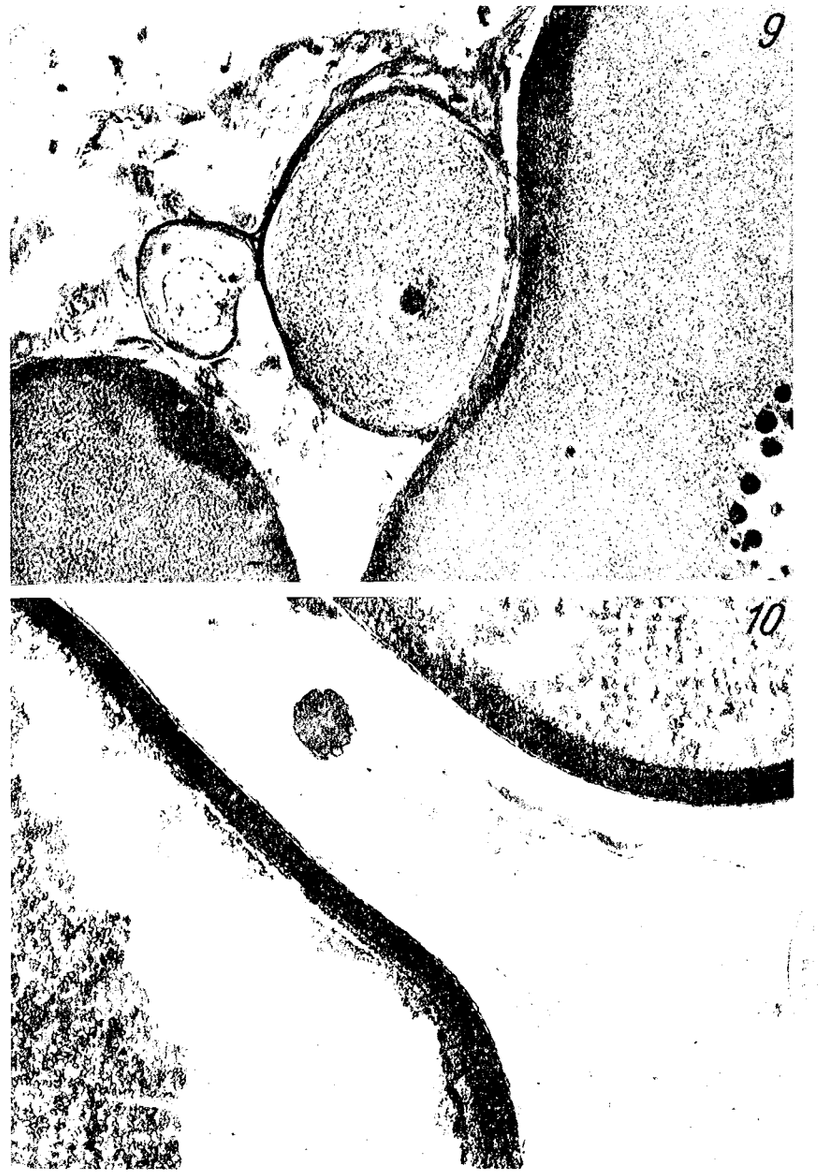
PLANCHE I



— La période de prévitellogenèse.
1. L'aspect général de l'ovaire (Ao) chez l'*Acipenser ruthenus* L. Trichromique Masson, oc. 6, ob. 6, 3. 2. Le début de la formation de la membrane folliculaire autour du jeune ovocyte. « Azan » oc. 8 immersion, n, les noyaux des cellules folliculaires, n', le noyau de la cellule folliculaire à un stade de développement plus avancé de l'ovocyte. 3. Les membranes formées autour de l'ovocyte; m, membrane APS +; s, la substance pelluside; f, les noyaux des cellules folliculaires; mpf, la membrane périfolliculaire; ti, thèque interne; te, thèque externe. « Azan », oc. 6, ob. 40. 4. La structure des membranes, « Azan », oc. 8, immersion.



— Phases successives du développement de la substance pelluciде et de la zone pelluciде impaire.
5. *Acipenser ruthenus*, « Azan », oc. 6 immersion. zp, la zone pelluciде, f, cellule folliculaire, mpf, la membrane périfolliculaire. 6. *Acipenser ruthenus*, « Azan », oc. 6 immersion. 7, 8, La formation définitive des membranes externes. *Acip. ruth.*, « Azan », oc. 6, ob 25 et oc. 6 immersion; p, granules pigmentaires, vitellus.



— La réactivité des membranes aux polysaccharides.
9. Le huiron, APS picro-indigo-carmin, oc. 8, ob. 40, la période de prévitellogenèse. 10. Le huiron, APS picro-indigo-carmin, oc. 10, ob. 6,3. la période de la vitellogenèse.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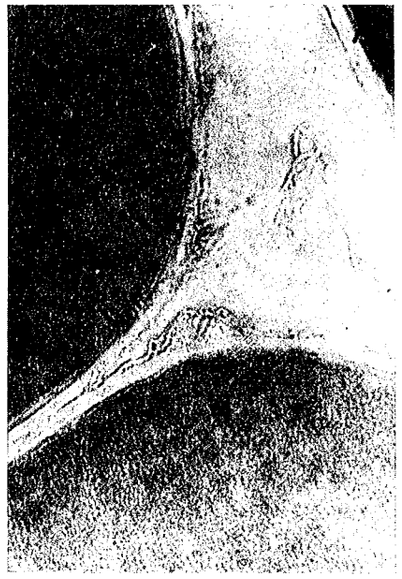

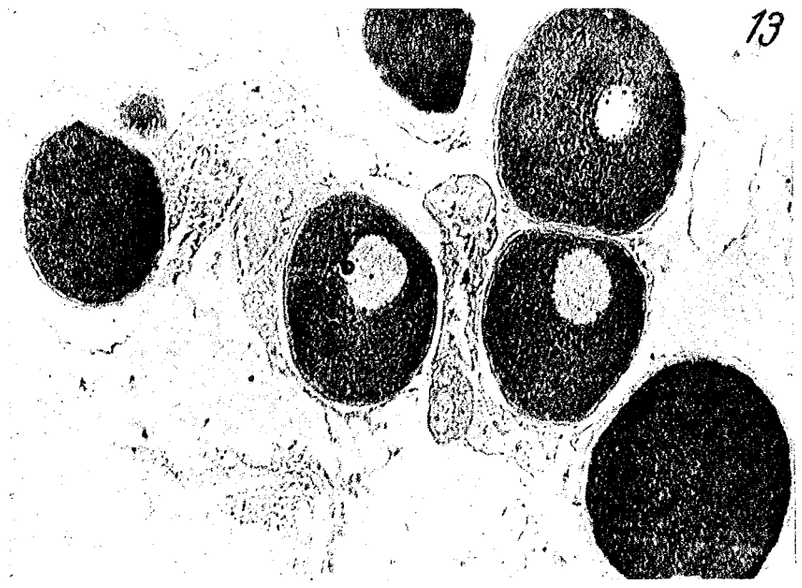
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11. Le huiron. APS picro-indigo-carmin, oc. 8, ob. 40. 12. Le huiron bleu de toluidine, oc. 8, ob. 40. 13. Le huiron bleu de toluidine, oc. 8, ob. 6, 3.


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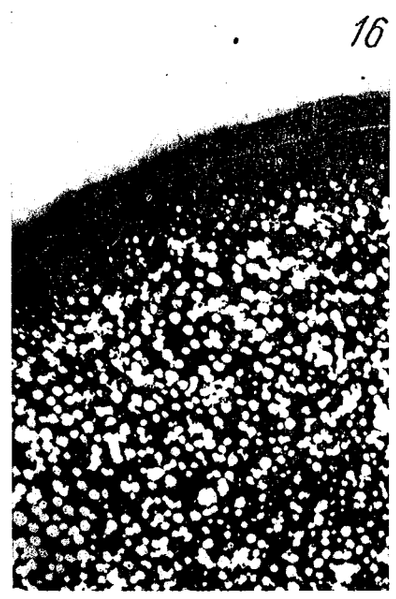
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— La réactivité des membranes aux protéines.
14. Le huiron. alloxane — Schiff. oc. 8, ob. 6,3. 15. Le huiron dinitrofluorobenzène, oc. 8, ob. 6,3. 16. Le huiron bleu de bromophénol. ob. 6, 3, oc. 8. 17. Le huiron bleu de bromophénol mercurique, oc. 8, ob. 6, 3.

CHANGES IN ORGANS AND SYSTEMS DURING COLD ADAPTATION IN ANIMALS OF DIFFERENT ECOLOGICAL SPECIALIZATION

BY

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The increased tolerance of organism to cold is observed as the result of a repeated or long-term exposure of laboratory animals (rats) at low temperatures (+4—6°C) and is accompanied by a decrease in electrical activity of skeletal muscles (EA), increase in general metabolism (GM), changes in visceral organs metabolism (VOM), increased metabolic reaction to nor-epinephrine injection as well as by increased thyroid activity and an increase in corticosteroid secretion during the initial period of adaptation [22] [25] [27], (Ivanov and Rashevskaya, 1969 [4] [9] [13], and many others).

Quite different results are obtained in studying the natural adaptation to cold in animals kept out of doors or in those captured outdoor in winter. Here, along with a decrease in EA, high GM, absence of thyroid reaction, high rate of corticosteroid secretion, slight increase in VOM, and an increase in weight of kidneys and brain are observed [9—11] [19] and others).

Extensive material collected by different authors permits the general conclusion to be made that the physiologic changes during cold adaptation can cover a number of systems (skeletal muscles, visceral organs) or take place within one system. This finding is particularly essential for understanding the phenomenon of substitution (heat production compensation) in a whole organism not only during muscular exercise (Bazhenov, 1967, [1] [2] [3] Hart, 1950; [8]; Jansky, 1955, [16] [18], but also for maintaining the heat production at rest [24] [26].

This communication is devoted to the comparison of data obtained as a result of individual (4—6 weeks of keeping at 0— +2°C) cold adaptation of species of different ecological specialization— boreal, of vast areal, polar and southern. The main material was collected in white rats

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— in the species which, as is well known, can adapt to cold very well. Fine changes in skeletal muscles where a comparison of electrical activity shifts with changes in chemical dynamics is possible, are best studied in these animals. The investigation carried out by Deribas and Philipchenko have shown these changes in SDH activity. The decrease in EA of muscles is attained by an increase in succinatdehydrogenasal (SDH) activity determined histochemically and biochemically. The greatest changes in SDH activity are observed in the muscles of hind extremities (48%), taking part in additional heat production only under essential cooling (Slonim [23], 1965). Changes in SDH activity occur mainly at the cost of red fibres of muscles; in the postural-tonic musculature (m. masseter, m. trapezius) these changes are absent. Unlike m. trapezius constantly involved in thermoregulatory activity, femoral muscles seem to be reserve organs increasing heat production as a result of cold adaptation.

Tumakova has shown that the extremity muscles of rodents (rats) heterogeneous in their structure and chemical features take different parts both in the reaction to cold exposure and in adaptation of the organism to cold. The highest EA is observed in the deep parts of muscles containing red muscular fibres. In summer the increment of EA under cooling (+6—8°C) equals to 51.6±1.3 mcv in red fibres and 7.9±0.4 mcv in white ones, in winter 18.7±3.0 mcv and 3.9±0.3 mcv, respectively. Red fibres are characterized by higher respiration of homogenates in Warburg device. A decrease in electrical activity of skeletal muscles in cold adapted animals is accompanied, however, by an increase in respiration of muscle homogenates in vitro investigated in the same device.

In the investigations carried out by Khaskin it was shown that the increase of respiratory activity of skeletal muscle homogenates is accompanied by a marked decrease in O_{10} of tissue respiration. These changes are also more pronounced in hind extremities muscles than in cervical ones. The decrease in Q_{10} is connected with the reduction in the energy of oxidative enzymes activation, SDA in particular. Weakening of temperature dependence of tissue enzymes contributes to maintenance of high functional activity. But thus far the question on the origin of the so-called non-shivering thermogenesis is rather disputable. Aside from an increase in thermogenesis in visceral organs which will be discussed later, the study of dynamics of heat production connected with electrical activity manifesting in the course of adaptation is of a great significance. These investigations have been carried out in our laboratory by Ivanov, Tkachenko and Yakimenko.

Correlation and regression analyses of experimental data on general gas metabolism and EA of muscles carried out by Tkachenko show that during cold adaptation the thermic effect in the whole organism falling within a unit of EA becomes 3—4 times higher than in control animals. At the same time the increase in gas metabolism of muscles in situ under cold exposure is connected essentially with their shivering activity. Cold adaptation leads to an increased oxygen consumption by muscles if calculated within a unit of EA [28].

In the studies carried out by Ivanov, Tkachenko and Yakimenko with the help of simultaneous recording EA and cervical muscles temperature in rats correlation relationship was found between the integral

of EA burst (S mcv. sec) and maximal increase in muscular temperature (t°C), the correlation coefficient being 0.8. The equations of regression are:

$$t = (77.4 + 1.43\ S)\ 10^{-4}$$ for control animals.

$$t = (41.3 + 2.25\ S)\ 10^{-4}$$ for cold adapted ones.

The comparison of angular coefficients in these equations show higher thermic effect of muscular contraction to be in cold adapted animals.

Significant changes are found in heat production in visceral organs. An essential increase in tissue respiration of kidney (10.05—12.15), liver (6.38—8.22), heart (10.19—12.41), diaphragm (4.76—6.75 Q_{O_2} mcl/mgr dry weight/hour) takes place in white rats.

The muscular system reacts by lesser increase in homogenate respiration *in vitro* (m. rec. femoris 4.46—3.03; triceps surae 2.57—2.53). A certain part in the increase in the non-shivering share of thermogenesis is played by an increased VOM. Quite different results are observed in tropical and southern species (*Nesokia indica* and *Rhombomis opimus*). Cold exposure in these animals leads to an increase in metabolic rate in muscular system and to a decrease in body temperature. GM in visceral organs is decreased [21] [25]. In a northern species (*Lemnus obensis*) high metabolic rate and very small E A of skeletal muscles are observed.

This fact brings together the picture of experimental cold adaptation in rats coming after 4—6 weeks of exposure at 0— +2°C and the phenomena of genetic adaptation of polar organisms — lemmings. Comparison of insulation values in different animals shows the following picture. Highest insulation was observed in lemmings, then in cold adapted rats; in adapted rats trained to muscular activity in the treadmill and in heat adapted rats. The least insulation was found in Middle-Asian hedgehog (*Hemiechinus auritus*). In this animal insulation is replaced by an increased heat production of dorsal circular muscle (m. orbicularis dorsi) which is reflected in sharp increase in its electrical activity at cold exposure. Thus, in experimentally cold adapted white rat the "muscular" type prevails, the one with changes in chemical dynamics of skeletal muscles inherent to boreal organisms as well as to organisms of vast areal. The type of adaptation inherent to southern species is connected with a decrease in thermogenesis in visceral organs and an increase in that in skeletal muscles. These phenomena are the basis of physiological mechanisms specifying the increase in cold tolerance of homeothermal organisms of a vast areal and of species limited in their expansion to the North.

The comparison of the facts obtained make it possible to draw the following conclusions:

1. Cold adaptation is accompanied by significant changes in chemical dynamics of skeletal muscles. They lead to a decrease in efficiency of muscles and to increase in thermogenesis under muscular contraction, thermoregulatory tone and shivering.

2. The phenomena of individual "muscular" adaptation are inherent to boreal species and those of vast areal.

3. Correlations between shivering and non-shivering thermogenesis similar to those in experimentally adapted rats are observed in polar species (*Lemnus obensis*).

4. "Visceral" type of adaptation with maintaining high electrical activity of muscles and with a significant decrease in thermogenesis in visceral organs is inherent to southern species (*Nesokia indica*) sharply limited in its expansion to the North.

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STUDY ON THE INTERACTION OF THE BIOFIELD AND THE EM ARTIFICIAL FIELD

BY

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The authors present the results of myelogram modifications in the rabbit, as a consequence of interaction of the EM biofield and of an artificial EM field. The modifications of each cellular type and the relation granulocytes/erythroblasts in comparison with control animals are given.

I. MYELOGRAM OF THE NORMAL RABBIT

The aim of the first stage of our investigations was to establish the normal myelogram in control animals. Hence, the myelogram was made in control animals. The experimental way we adopted was in keeping with the specific character of our research, namely : the high variability, both morphological and numerical, of the cellular elements of the hematopoetic marrow.

In the first stage of the experiment a group of animals were taken as controls providing the material for the count of leukocytic elements and for the morphological study of specimens stained by employing the May-Grünwald-Giemsa method. The experiment was pursued with the same group of animals where each animal was to become its own control. We implied that in this way the difficulties of interpretation resulting from the high variability and sensibility of the hematopoetic tissue of the marrow in animals could be avoided to a great extent.

Our group of animals included 30 rabbits. After making a selection, we considered accurate only the results obtained in 15 animals.

Numerical myelogram

The count of the nucleated elements of the marrow was done with the aid of an ordinary method, using Thoma's plate. An average number of 79,633 cells/mmc in the range of 46,000—125,000 cells/mmc was obtained. Table records these data.

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Table 1

Number of nucleated elements/mmc

Rabbit	Control animal
1	46,000
2	110,000
3	75,000
4	82,500
5	98,000
6	81,000
7	58,000
8	125,000
9	64,000
10	98,000
11	68,000
12	56,000
13	65,000
14	75,000
15	92,500

Morphological myelogram

The percentage myelogram was established on 26 cytological elements. After the average number of nucleated cells/mmc was counted, the numerical figure/mmc for each cell on the myelogram could be obtained.

Table 2 shows both the percentage myelogram as well as the numerical one. They point out that the relation G/E (granulocytes/erythrocytes) in the control animals is of 361 — an ordinary relation in human hematophysiology, by the way.

The development of this relation will be dealt with in due time.

Table 2

Normal myelogram in the rabbit

No.	Medullar cell/type of myelogram	%	No/mmc
1	Proerythroblasts	2.32	1,847
2	Polychromatophilic erythroblasts	2.25	1,791
3	Ortochromatophilic erythroblasts	5.74	4,570
4	Ortochromatophilic normoblasts	4.37	3,479
5	Myeloblasts	2.34	1,863
6	Promyelocytes	3.60	2,866
7	Neutrophilic myelocytes	5.87	4,674
8	Eosinophilic myelocytes	1.08	860
9	Basophilic myelocytes	2.43	1,935
10	Neutrophilic metamyelocytes	8.61	6,856
11	Eosinophilic metamyelocytes	0.68	541
12	Basophilic metamyelocytes	0.95	756
13	Nonsegmented neutrophilic leukocytes	13.38	10,654
14	Nonsegmented eosinophilic leukocytes	0.36	296
15	Nonsegmented basophilic leukocytes	0.32	254
16	Segmented neutrophilic leukocytes	12.83	10,216
17	Segmented eosinophilic leukocytes	0.20	159
18	Segmented basophilic leukocytes	0.37	294
19	Lymphoblasts and small lymphocytes	2.82	2,245
20	Mean and small lymphocytes	9.21	7,334
21	Monocytes	10.70	8,520
22	Megakaryocytes	2.34	1,863
23	Reticulo-histiocytic cells	5.22	4,156
24	Plasmocytes	0.68	541
25	Hemohistioblasts	0.07	55
26	Hemocytoblasts	0.94	748

II. MYELOGRAM MODIFICATION AS A RESULT OF INTERACTION OF THESE TWO EM FIELDS

Qualitative modifications

Table 3 gives an account of the myelograms established in the course of the experiment. The percentage calculation was effected taking 26 cellular elements against 100 of the total myelogram. It should be pointed

Table 3

Influence of interaction between biological and artificial CEM of the myelogram in rabbits

No.	Cell	%			σ			e. Ma		
		Control	After 5 exp.	After 10 exp.	Control	After 5 exp.	After 10exp.	Control	After 5 exp.	After 10exp.
1	Proerythroblasts	2.32	3.20	2.75	1.3	2.23	1.41	0.34	0.57	0.36
2	Polychromatophilic erythroblasts	2.25	3.14	3.49	1.23	1.25	2.00	0.31	0.32	0.51
3	Ortochromatophilic erythroblasts	5.74	5.40	5.68	3.46	2.64	1.00	0.23	0.68	0.21
4	Ortochromatophilic normoblasts	4.37	2.10	5.05	2.23	1.73	3.00	0.62	0.44	0.77
5	Myeloblasts	2.34	3.00	3.48	1.41	1.41	2.00	0.36	0.36	0.51
6	Promyelocytes	3.60	4.70	3.32	2.23	2.23	1.73	0.62	0.57	0.44
7	Neutrophilic myelocytes	5.87	6.66	7.66	3.74	3.31	3.60	0.96	0.85	0.93
8	Eosinophilic myelocytes	1.08	2.50	1.86	0.15	1.21	0.22	0.03	0.31	0.06
9	Basophilic myelocytes	2.43	4.12	3.64	1.41	1.73	1.41	0.36	0.47	0.36
10	Neutrophilic metamyelocytes	8.61	7.10	5.70	4.79	2.64	2.64	1.23	0.68	0.65
11	Eosinophilic metamyelocytes	0.68	0.41	0.66	0.26	0.41	0.08	0.06	0.10	0.02
12	Basophilic metamyelocytes	0.95	0.71	1.25	1.07	0.40	0.24	0.27	0.10	0.06
13	Nonsegmented neutrophilic leukocytes	13.38	12.10	10.25	6.40	4.40	3.31	1.65	1.13	0.85
14	Nonsegmented eosinophilic leukocytes	0.36	2.12	0.52	0.15	0.02	0.10	0.04	0.005	0.02
15	Nonsegmented basophilic leukocytes	0.32	2.24	1.24	0.10	0.17	0.19	0.02	0.04	0.04
16	Segmented neutrophilic leukocytes	12.83	10.20	13.01	6.16	4.35	4.79	1.39	1.12	1.23
17	Segmented eosinophilic leukocytes	0.20	0.19	0.51	0.07	0.16	0.012	0.018	0.04	0.003
18	Segmented basophilic leukocytes	0.37	0.78	1.39	0.16	0.40	0.17	0.04	0.10	0.04
19	Lymphoblasts and small lymphocytes	2.82	3.10	3.78	1.44	1.41	1.14	0.37	0.36	0.29
20	Mean and small lymphocytes	9.21	7.60	7.23	5.29	3.74	2.44	1.36	0.96	0.63
21	Monocytes	10.70	8.80	8.95	3.46	2.82	1.42	0.89	0.72	0.36
22	Megakaryocytes	2.34	2.87	3.31	1.73	2.00	1.25	0.44	0.51	0.32
23	Reticulo-histiocytic cells	5.22	5.10	3.34	2.23	3.00	1.60	0.62	0.77	0.40
24	Plasmocytes	0.68	0.81	0.78	0.10	0.11	0.16	0.02	0.02	0.04
25	Hemohistioblasts	0.07	0.07	0.08	0.83	0.29	0.258	0.21	0.08	0.72
26	Hemocytoblasts	0.94	0.98	0.87	0.12	0.19	0.34	0.03	0.04	0.09

out that certain authors use different systems for establishing the myelogram.

The table also shows the standard deviation and the count Ma. The percentage variation of the cellular elements on the myelogram was calculated by taking the value 0 of the control animals as reference value.

Table 4 records the data obtained.

Table 4

No.	Cytological range	Controls	After 5 exp.	After 10 exp.
1	Erythroblastic range	0	− 6.41%	+15.59%
2	Granulocytic range	0	7.18%	2.77%
3	Lymphoblastic range	0	−11.06%	− 8.48%
4	Monocytic range	0	−17.76%	−16.36%
5	Megakaryocytic range	0	22.64%	41.54%
6	Reticulo-histiocytic cell	0	− 0.39%	−36.02%
7	Plasmocytic cell	0	19.11%	14.70%

Dynamics of cytological medullar maturation

Successive diagrams represent the maturation process taking place in every cellular range shown on the myelogram. On the abscise the evolutive stage of different cells is noted; on the ordinate — the related percentage. Each diagram represents three types of results, those obtained in regard to the control animals and those obtained after 5 and 10 experiments respectively.

Table 5

Variation of eosinophilic and basophilic granulocytic elements on the myelogram

Cell	Eosynophils			Basophils		
	M	5	10	M	5	10
Myelocytes	1.08	2.50	1.86	2.43	4.12	3.64
Metamyelocytes	0.68	0.41	0.66	0.95	0.71	1.25
Nonsegmented leukocytes	0.36	2.12	0.52	0.32	2.24	1.24
Segmented leukocytes	0.20	0.19	0.51	0.37	0.78	1.39

It is easy to see on the diagram that in the first evolutive stage there occurs a big increase of cellular elements, while in the last stage their number sharply decreases. That may provide a proof — according to certain hypotheses — of the cytobase being brought about by the maturation process itself as well as by the need of restoring the cytological equilibrium of the hematoforming marrow.

Dynamics of erythroblastic maturation

The dynamics of maturation in blood cells of the erythroblastic range takes place in three stages and is exactly alike the dynamics of maturation found in control animals after the last five experiments. However, in these experiments the dynamics were higher during the

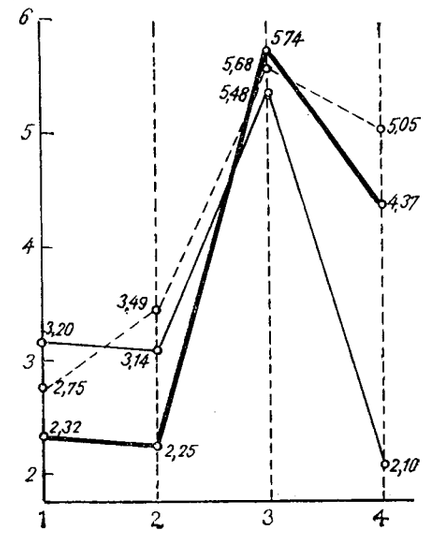

Fig. 1. — Dynamics of erythroblastic maturation. —— control animal; —— after 5 experiments; ····· after 10 experiments. *1*, Proerythroblasts; *2*, polychromatophilic erythroblasts; *3*, ortochromatophilic erythroblasts; *4*, ortochromatophilic normoblasts.

early two stages, while in the next stage, considered as representing the cytodiabase, it was lower. In respect only to the last stage, the diagram shows after five experiments that the cytodiabase is very pronounced and after 10 experiments it is less marked in relation to the curve of control animals.

Dynamics of granulocytic-neutrophilic maturation

The process of granulocytic maturation presents a specific diagram, since in the third phase, comprised between the myelocyte and the metamyelocyte, significant modifications occur. In this stage, the diagram shows in the control animals an ascending process which ends in the last stage with a decline to be ascribed to the medullar cytodiabase.

After five experiments, during the same cytological stage the curve shows a certain decline marked by a plateau. The variation then is only of 0.34%. In the last stage the decline is sharper, being a sign of slow maturation.

After ten experiments, during the third stage (myelocyte-metamyelocyte) there occurs a noticeable decrease (1.96%) of myelocytes; the same process starts again and in the last stage the decline ascribed to the cytodiabase does no longer take place. It is presumed that the decline is due to the fact that the cellular elements do not present the

required maturation enabling them to go through the respective membrane in circulation.

This way of establishing the diagram also raises the problem of the cytological chain of maturation and of the sensibility of its segments.

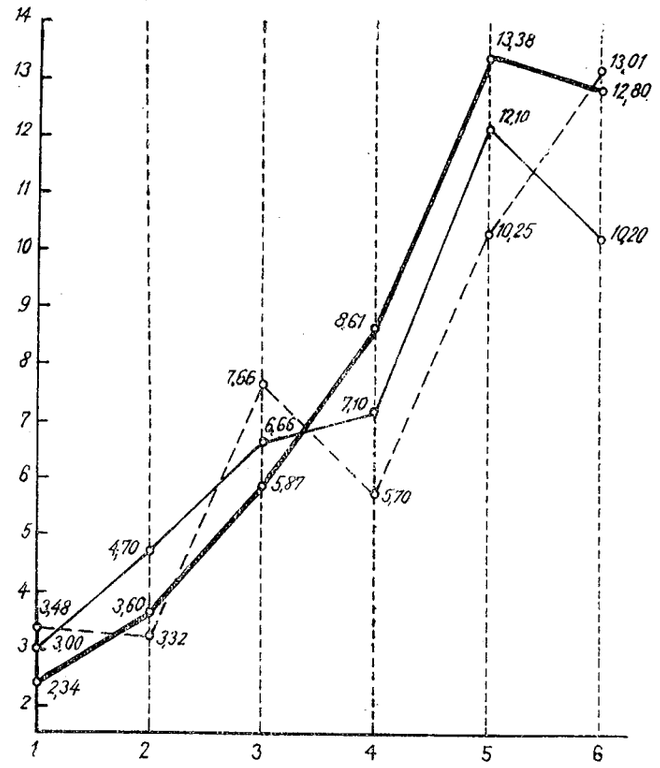

Fig. 2.—Dynamics of granulocytic-neutrophilic maturation. —— in control animal; —— after 5 experiments; ----- after 10 experiments. *1*, Myeloblast; *2*, promyelocyte; *3*, neutrophilic myelocyte; *4*, neutrophilic metamyelocyte; *5*, nonsegmented neutrophilic leukocyte; *6*, segmented neutrophilic leukocyte.

It is obvious enough that the stage comprised between the myelocytes and the metamyelocytes is mostly affected by the action and interaction of the electromagnetic radiation.

Naturally, further accurate research would provide more knowledge in this respect. It should however be pointed out that there is a difficulty which interferes with the carrying out of such research coming from the speed of evolution. For if the average life-span of a granulocyte, which is of 4—6 days, is taken into account, it should be admitted that the stages of its maturation are very swift and more numerous than those observed today by employing the morphological criterion. The diagram below confirms this assertion.

Dynamics of maturation of eosinophilic and basophilic maturation

For the sake of these hemomedullar elements the table of percentage variation of cells during the experiments is given first; then a diagram where the surface criterion was taken in order to show more clearly this variation.

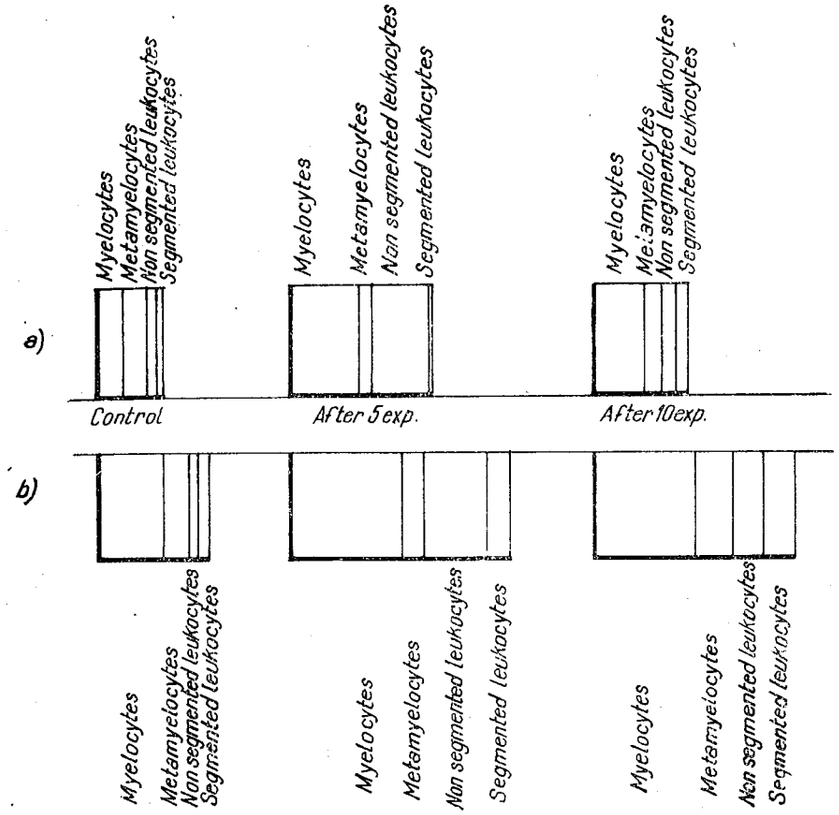

Fig. 3. — Dynamics of eosinophilic (*a*) and basophilic (*b*) granulocytic maturation.

It comes out from the diagram that in the control animals both the eosinophylic as well as the basophilic elements show similar dynamics; yet, after five experiments, the eosinophylic metamyelocytes and the basophylic metamyelocytes go through a noticeable decrease — similar to the decrease occurred in the neutrophylic granulocytes, thus raising again the problem of the sensibility shown by the cellular medulohemopoetic chain.

It should be noted that after ten experiments both diagrams become similar as recorded below.

Dynamics of cytological lymphocytic maturation

It was found necessary to establish a single evolutive stage in the lymphoidic range having at one of its ends the group formed of lymphoblasts and large lymphocytes and mean and small lymphocytes at the other end. This system was adopted since the passing of a type of cell to the next was difficult to observe, especially in the case of a lymphoblast passing to a prolymphocyte and then to a large lymphocyte, all of them

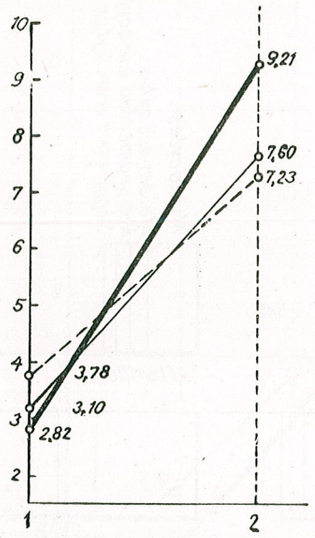

Fig. 4. — Dynamics of lymphocytic maturation. *1*, Large lymphoblasts; *2*, mean and small lymphocytes. —— Control; —— after 5 experiments; ----- after 10 experiments.

getting together into a single group. As for the mean and small lymphocytes, these do not in the least differ from the others, except for their size.

The action and interaction of electromagnetic variation is followed by a decline of the maturation dynamics. The diagram below shows this aspect.

Variation of reticulohistiocytes and monocytes during the experiments

This problem being still disputed, the present work has avoided dwelling upon the relation between reticulohistiocytes and monocytes. By taking a look on the myelogram at these two cellular categories one finds such aspects as may offer a good opportunity to further investigations to clarify the problem. One should use as a means of influencing the hematopoietic marrow the action and interaction of biological and electromagnetic fields.

Our findings bring out the following:

1) In the early part of the experiment, that is during the first five field sessions, it was noticed that while the number of monocytes obviously decreased, the reticulohistiocytes did not show any variation.

2) In the next part of the experiment, that is during the last five field sessions, while the monocytes stayed almost unchanged, the reticulohistiocytes obviously decreased.

It should be pointed out that the decrease of monocytes in the early part of the experiment was almost equal to the decrease of reticulohistiocytes in the next part of the experiment (1.9% for monocytes and 1.84%

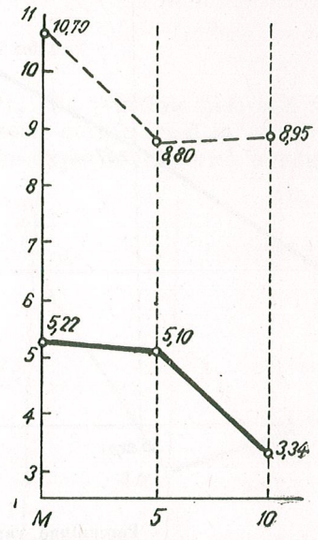

Fig. 5. — Variation of monocytes and reticulohistiocytes during the experiments. *M*, control; *5*, after 5 experiments; *10*, After 10 experiments. ----- Monocytes; —— reticulohistiocytes.

for reticulohistiocytes). At the same time the percentage of reticulohistiocytes varied only by 0.12% in the early stage of the experiment and the monocytes by 0.15% in the next one. To point out these aspects a joint diagram for monocytes and reticulohistiocytes is given above.

III. VARIATION OF MONOCYTES AND RETICULOHISTIOCYTES DURING THE EXPERIMENTS

Variation of megakaryocytes

The variation of megacaryocytic cells went up to a high percentage in a slow but constant rate. On the myelogram these cells were able to take but a small place. In the same way as for the other cells at least one more cytological category could be set off.

In the cytological variety involved in our myelogram the megacaryocyte whose cytoplasm contains in its structure the future thrombocytes was described. Under the same denomination younger elements, less frequent — megakaryoblasts and young megakaryocytes — were included. This denomination was adopted in connection with the purpose of the study aimed at pursuing the influence of action and interaction of electromagnetic radiations on the ranges constituting the structure

of the hematopoietic marrow, the more so that certain ranges contain a relatively small number of elements.

Further studies would go deeper into this matter, allowing for more accurate research involving each element.

The diagram below shows the percentage variation of elements in the megacaryocytic range during the experiment.

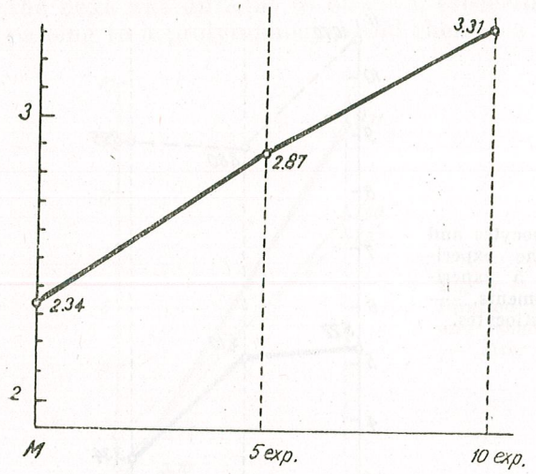

Fig. 6. — Dynamics of megakaryocytes variation. *M*, control; *5*, after 5 experiments; *10*, after 10 experiments.

Percentage variation of plasmocytes

Plasmocytes are found seldom enough on smears of sternal marrow, but they are found more often there than on human marrow. Morphologically these plasmocytes resemble the human ones; however the basophylic character of its cytoplasm is more obvious.

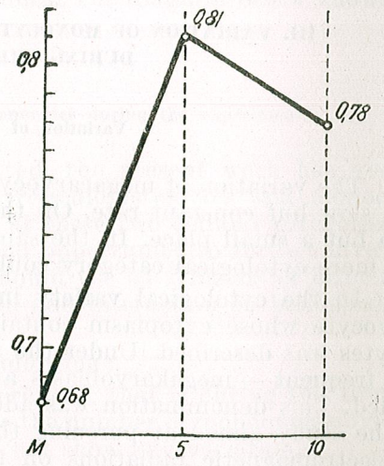

Fig. 7. Percentage variation of plasmocytes. *M*, control; *5*, after 5 experiments; *10*, after 10 experiments.

Percentage variation of plasmocytes is unnoticeable. For this reason the diagram below used the distance of 0.01 in order to point out the variation which, otherwise, could not be detected.

The diagram shows the percentage variation for three groups of experiments.

Dynamics of G/E relation

In the course of the experiments, the relation between the total number of granulocytes and erythroblastic cells varied in relation to the control animals. In the early part of the experiments with account taken

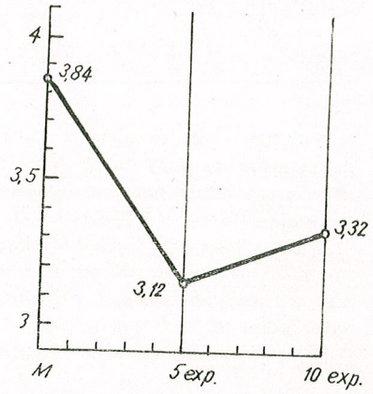

Fig. 8. — Dynamics of G/E relation. *M*, control; *5*, after 5 experiments; *10*, after 10 experiments.

of the conditions in which they were carried out, the radiations exerted a higher influence on erythroblastic cells than on granulocytes; a decline in this relation followed. During the next part of the experiments this action and interaction extended on both cytologycal ranges; a growth of this relation followed showing a tendency to a return to normal.

The respective diagram is given below.

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INFLUENCE OF ENCEPHALON EXCITATION ON GLYCEMIA IN *EMYS ORBICULARIS*

BY

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The changes of glycemia under the influence of a single or repeted excitations of encephalon in *Emys orbicularis* were investigated. In all cases an accentuated, long-term hyperglycemia was produced. Telencephalon and diencephalon excitation elicits a hyperglycemia with a peak value attained 5 hours after the excitation while the mesencephalon and metencephalon induces a hyperglycemia with a peak value after 3 hours. The repeated excitation of the encephalon maintains the hyperglycemic curve at a high level a relative long periode from the first excitation (48 hours). After 72 hours the value were higher than the initial level. The intervention of the encephalon in the regulation of glycemia in *Emys orbicularis* is very possible.

Generally, there is assumed that the nervous system plays an important role in glycemia regulation [1] [2][7—9], but the specific glycoregulation factors have not as yet been identified even in mammals, on whom a relative great number of investigations were performed [3—4], [6].

A more complex study performed by us on reptilians, concerning the factors which intervene in glycemia [1] [7 — 9], did not actually revealed any work regarding the influence of nervous system on the changes of blood sugar.

In the present work we expose the preliminary data of our investigations concerning the electrical excitation on glycemia of different encephalic segments of *Emys orbicularis*.

MATERIAL AND METHOD

The researches have been carried out on 30 *Emys orbicularis*, with an average body weight of 450 g.

An initial blood samples was taken off from the veins of the posterior leg, after which the animal was fixed in a stereotaxic device (prototype RSR [5]). The tegument of the dorsal

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1 — c. 2059

region of skull was removed. The muscles situated in the lateral parts of neurocranium have been ligaturated, in order to render evident the bones of brain pan from the dorsal part. In the level of encephalic formations, in which the electrodes ought to be inserted, holes with a diameter of 500 microns have been drilled with the aid of a hand cutter.

In each animal, was introduced in these holes a couple of NiCr electrodes, with a diameter of 50 microns each. The electrodes : have been isolated with Viniflex lac and sticked together with a caoutchouc solution, preserving a distance of 80—100 microns between them. The peak of both electrodes remains non-isolated on a portion of 0.5 mm. The external head of every electrode have been connected with one of the bornes of rectangular impulses generator of EMA type.

The duration of excitations was of 30 s, with a tension of 3 volts, at an intensity of 2 mA and a frequency of 80 cycles/s.

The blood samples have been taken off at 3,5 and 24 hours after application of a single excitation and at 1, 2, 3, 4, 5, 24, 48 and 72 hours after the application of the first excitation. This has been repeated successively at one hour intervals.

At the end of experiences the animals have been sacrificed and their brain has been submitted to a histological control, in order to localize the site of implantation of electrodes peak.

Glycemia has been determined by Hagedorn-Jensen's method.

RESULTS AND DISCUSSIONS

1. Changes of glycemia under the influence of a single excitation of encephalon

In figure 1 there is observed that a single excitation of different encephalon segments (telencephalon, diencephalon, mesencephalon and metencephalon) produces in all cases, an accentuated, long-term hyperglycemia. From the whole of these data, we can deduce the following :

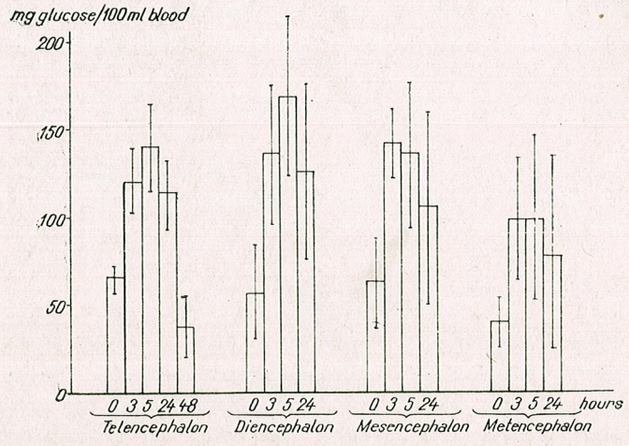

Fig. 1. — Changes of glycemia in *Emys orbicularis* under the influence of a single excitation applied at the level of different encephalon segments.

a) Telencephalon and diencephalon excitation elicits a hyperglycemia with a peak value attained 5 hours after the excitation while the mesencephalon and metencephalon induces a hyperglycemia with a peak value after 3 hours, a tendency to normal being recorded in all cases after 24 hours.

b) As to the intensity of hyperglycemia we mention that the peak effect was obtained after diencephalon excitation and the minimal effect — after metencephalon excitation.

2. Changes of glycemia under the influence of repeated excitation

In this series of experiences the influence of 5 excitations elicited at one hour intervals in the two segments of the central nervous system (telencephalon and mesencephalon) have been followed up, in order to observe if there are differences as regards the intensity and duration of induced hyperglycemia (in the case of these segments, in the first series of experiences there was not recorded notable differences).

The results of these experiences are reproduced in fig. 2. In cases of repeated excitation of telencephalon, there was observed a progressive increase of glycemia, the maximal level being attained after the Vth ex-

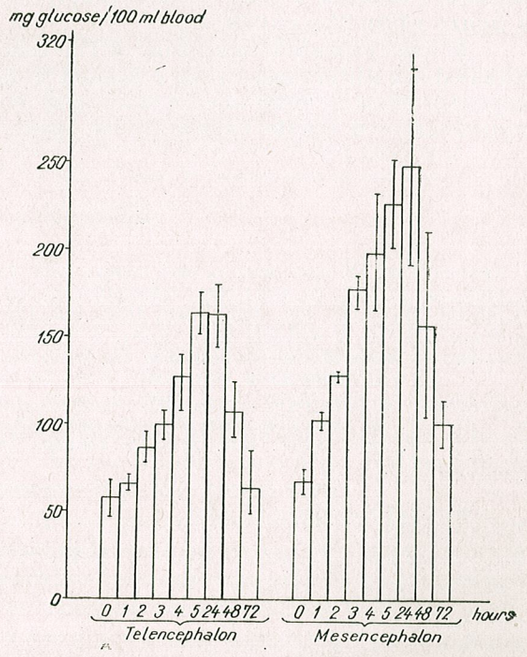

Fig. 2. — Changes of glycemia in *Emys orbicularis* under the repeated excitation applied at the level of tele- and mesencephalon.

citation (5 hours after the first excitation). The initial values are reached after 72 hours. Repeated excitation of mesencephalon induces an increase of glycemia with a peak value at 24 hours after the first excitation, the

recovery being slower and after 72 hours the values were higher than the initial level.

The intensity of obtained hyperglycemia by a single as well as by repeated excitation of different encephalon segments can be explained only as a consequence of adrenergic system participation. Only the presence of a relative great amount of adrenaline in the internal environment can explain the intensive increase of glycemia obtained as a result of excitation, as well as its maintaining at a high level after a relative long periode from the first excitation (3—5 hours). Certainly that this did not exclude the possibility of a parallel action of other systems, which in the last analysis, can totalize, explaining in this way the maintenance of hyperglycemia. This effect may be due to the inefficiency of insulinic compensatory system. An obvious explanation of this phenomenon can be found only after direct dosings of hormones in the internal environment, fact we did not consider in this first series of experiences.

CONCLUSIONS

1. A single excitation applied at the level of different encephalon segments induced a long-term hyperglycemia with a peak value at 3 to 5 hours after the excitation.

2. The repeated excitation of the telencephalon and mesencephalon revealed the differences in the obtained response, namely that the elicited hyperglycemia is more accentuated and of a greater duration as this recorded after a single excitation of these formations.

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REGULATION OF FOOD INTAKE IN FOWLS. THE EFFECT OF GLUCOSE AND ADRENALINE ON FOOD INTAKE IN *GALLUS DOMESTICUS* (L.)

BY

CONSTANȚA MATEI-VLĂDESCU

The effect of glucose (1 g/kg) and adrenaline (500 μg/kg), administered interperitoneally, on food intake and plasmatic glucose concentration was studied in Leghorn hens.

Both substances, in single, or in certain cases, repeated injections, induced in hens an evident anorexia, lasting 2—3 hours.

Anorexia induced by glucose coincided in time with a systemic hypoglycemia, while adrenaline anorexia seemed to be related to its glycogenolytic and respectively hyperglycemic effect. This suggests the existence in hens of liver glucoreceptors which send information about glucose content in hepatic cells to the central nervous system, affecting, in this way, feeding behaviour.

The considerable anorexigenic effects of glucose and adrenaline, injected intraperitoneally in dogs and cats, suggested to Russek [12], [13] the idea of the existence of certain hepatic glucoreceptors with an important role in the regulation of food intake in mammals.

Direct evidence in this sense was offered by Niijima [11] who recorded, both *in vitro*, and *in vivo* a decrease of afferent discharge rate at the level of the hepatic branch of the vagal nerve after the perfusing and respectively injecting of glucose into the portal vein.

By approaching lately the investigation of control mechanisms for food intake in fowls, a problem scarcely studied so far [1] [2][7—10] we raised the problem whether such glucoreceptors are present and intervene in food intake regulation in this class of vertebrated as well. To begin with, we tried to ascertain whether glucose and adrenaline, administered intraperitoneally in hens, yield anorexigenic effects and glycemic modifications similar to those recorded by Russek and coll. in mammals [13].

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MATERIAL AND METHODS

Experiments were performed on 18 hens of the Leghorn race, their weight varying between 1,200 and 1,900 kg. As food for fowls the following mixture was used :

	%
maize	65
sunflower groats	15
meat meal	10
fodder yeast	2.5
degreased powder milk	2
bone meal	1.5
fodder chalk	2.5
Premix	1.5

The hens, kept in separate cages, were submitted to a 22 hours' fast, after which, for 2—3 hours, were given food ad libitum. In this way the food consumed by hens in normal conditions, without a prior treatment, was measured, and after the intraperitoneal (i.p.) administration of the following substances : 5.5% NaCl, isoosmotic solution with a 30% glucose solution, and in an equal volume to that used in the case of the 1 g/kg b.w. glucose dose, respectively 3.3 ml/kg, 30% glucose in a 1 g/kg b.w. dose, and $1^0/_{00}$ adrenaline in a 500 µg/kg dose.

In some experiments plasma glucose was likewise assessed, before and at different time intervals after glucose and adrenaline administration. Plasmatic glucose in the blood samplings collected from the crest was dosed by the glucosoxidase method [3], using the Boehringer test with glucosoxidase. Readings were made at an universal V.S.U.1 spectrophotometer.

RESULTS

Intraperitoneal administration of the 5.5% NaCl solution did not affect food intake. Out of the food offered ad libitum, the hens consumed in two hours, 70 ± 7.9 g in normal conditions, and after 30 minutes from sodium chloride injection, 62 ± 9.1 g.

Responses to i.p. injecting with glucose and adrenaline presented a fairly high individual variability.

EFFECT OF GLUCOSE

Glucose administered intraperitoneally induced in some of the hens a certain diminution of the appetite, while in the others a refusal of food. Investigating in the latter, concomitantly glycemic modifications and appetite changes produced by i.p. glucose, we ascertained that anorexia did not occur when hens were hyperglycemic, but, on the contrary, when the glycemic level remained almost normal or became even hypoglycemic. This is illustrated comparatively by several examples in figure 1 *A*, *B*.

Anorexia occurred after 1/2—1 hr from the first injection or even in the first minutes after the second glucose injection, there being a

close dependence between the time of the occurrence of anorexia and the levelling or diminution tendency of glycemic level (Fig. 2). In only two

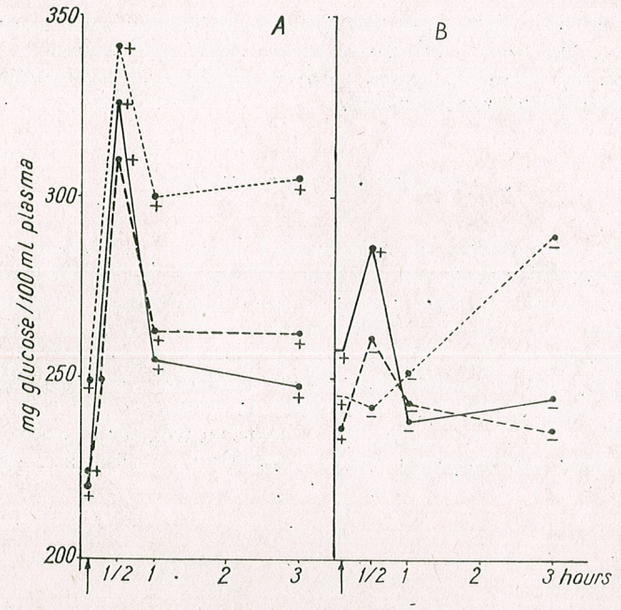

Fig. 1. — Effect of glucose administered intraperitoneally upon plasmatic glucose concentration and food intake in *Gallus domesticus* (L.) *A*, hens not presenting anorexia (+); *B*, hens presenting anorexia (—).

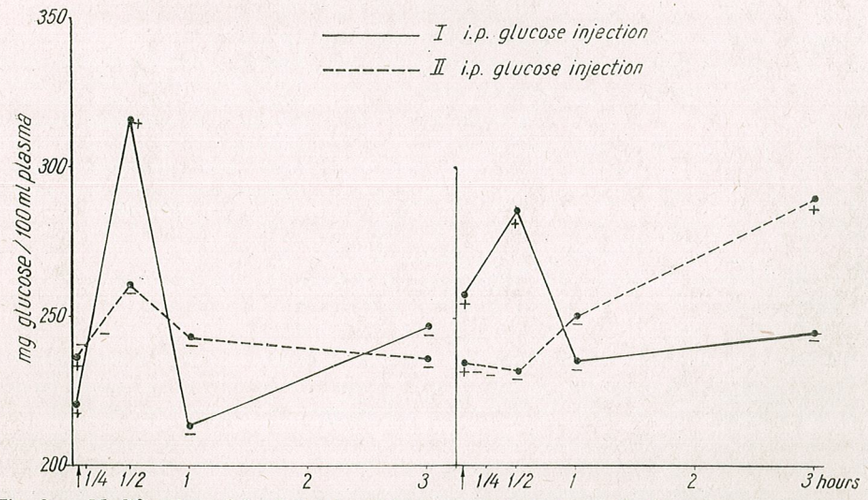

Fig. 2. — Modification of food intake and plasma glucose concentration after repeated i.p. glucose administration. To note : the occurrence of anorexia (—) in the first minutes after the 2nd glucose injection.

cases we recorded anorexia even in the first minutes after the first injection, and then the plasmatic glucose level was below the one before injection.

Noteworthy is that hens which refused to eat, were very calm and mostly fell asleep. Only after 2—3 hours they became active again, and began to eat. In some of them, appetite remained sometimes low likewise in the day following injection.

EFFECT OF ADRENALINE

The effect of adrenaline was tested in 6 hens. Table 1 shows that hens responded differently. In only two of the investigated hens, the first i.p. adrenaline injection (500 μg/kg) induced anorexia, already from the first hour (hen 1 and 2). In the other hens, after the first injection, only some diminuation of food intake was recorded (53 ± 6.6 g, as against 70 ± 7.9 g, in 2 hours), and only after repeated hormone injections, in the same day (hen 3 and 4), or in different days (hen 5) did they arrive at refusing food. Hen nr. 6 always ate after adrenaline injection. This hen presented a very low hyperglycemic reaction to adrenaline as compared with the other 5 hens (Fig. 3).

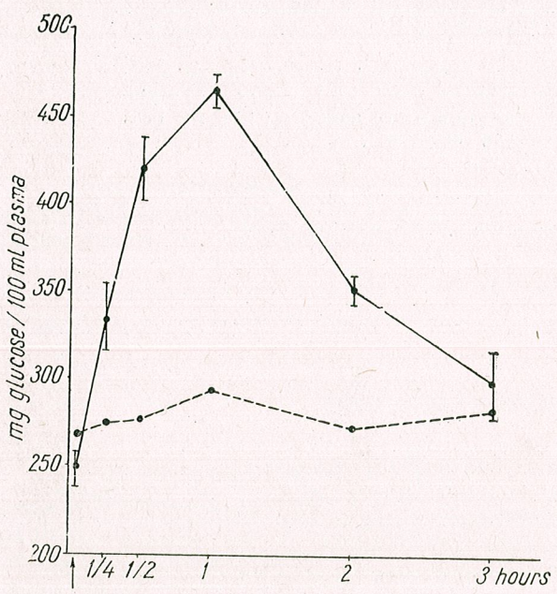

Fig. 3. — Action of 500 μg/kg adrenaline upon plasmatic glucose concentration. values of plasmatic glucose —— in hens which presented anorexia ; ---- in specimen which, even after repeated injections with adrenaline, did not present anorexia.

In addition, anorexic hens presented polypnea followed later by a state of somnolence. Sometimes their appetite was not fully restored even after 24 hours from injection.

Table 1

Food intake modification after i.p. injection of adrenaline, 500 μg/kg b.w.

Item	1st injection 5′ 30′ 1 2 3 hours	2nd injection 5′ 30′ 1 2 3 hours	3rd injection 5′ 30′ 1 2 3 hours	Observations
1	+ + − − −	+ + + + +		
2	+ − − − −			2 nd injection made 1hr after the 1st injection
3	+ + +	+ − − − −	− − − − −	,,
4	+ + +	− − − − −	− − − − −	
5	+ + + + +	+ + + + +	− − − − −	Repeated daily injections
6	+ + + + +	+ + + + +	+ + + + +	Daily injections slightly reduced appetite

\+ hen received food
− hen refused to eat even if the food in left is cage for hours.

DISCUSSION

From our results it ensues that in hens, just as in mammals [13], [14], anorexia can be determined by glucose or adrenaline i.p. administration.

Individual variability of responses is more accentuated than in mammals, while effective glucose or adrenaline doses are larger, in most cases a repetition of the injections with the two substances being necessary for inducing anorexia. The duration of anorexia, once appeared, was likewise greater than in cat and dog, of 2—3 hours, both after glucose, as well as after adrenaline.

The dependence of anorexia upon modifications of glucose concentration in hepatic cells is suggested by its coincidence in time with systemic hypoglycemia which appeared after glucose i.p. administration.

The same phenomenon was recorded by Russek and coll. in dogs [13] and cats [14], and the explanation offered might likewise be valid in the case of hens. As in the hypothalamus, in the liver, and in other tissues [4] [5] [6] there would exist glucoreceptors which would transmit information to the central nervous system about the disponibility of glucides at the level of cells, which would determine a modification of systemic glycemia and of feeding behaviour.

The diminution of afferent impulse discharges from tissue glucoreceptors in the case of intracellular glucose increase, a phenomenon already evidenced by Niijima [11]for hepatic glucoreceptors, diminishing the state of excitation of the "feeding centre" and releasing the "satiety centre", would determine the occurrence of anorexia.

The possibility of obtaining aphagia [2] and hyperphagia [1], [7] [9] by injuring or stimulating certain parts of the hypothalamus in certain species of birds shows that they have nervous centres similar to the "feeding centre" and "satiety centre" of mammals, though the localization of these centres does not seem as well delimited as in mammals [1],[9].

In our experiments, anorexia induced by adrenaline appeared dependent upon its glycogenolytic effect which, concomitantly with systemic hyperglycemia, probably likewise increases glucose concentration in hepatic cells. In the hen, in which no evident increase of plasmatic glucose was recorded, under the influence of adrenaline, we never obtained anorexia (Table 1 and figure 3).

CONCLUSIONS

1. Glucose and adrenaline injected intraperitoneally induce anorexia in hens.

2. The coincidence in time of anorexia induced by i.p. glucose with systemic hypoglycemia, as well as the dependence of adrenaline anorexia upon its glycogenolytic and hyeperglycemic effect, suggest the existence in hens of hepatic glucoreceptors, which send informations about glucose concentration in the hepatic cells to the central nervous system, being able to modify in this way the feeding behaviour.

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ANDRIANA DAMIAN-GEORGESCU, **Fauna Republicii Socialiste România** 114 Abb. Edit. Acad., Bukarest, 1970. Bd. IV, Heft 11, **Harpacticoida (forme de apă dulce)** (*Harpacticoida*— Süßwasserarten). 149 S.,

Dies ist das dritte und letzte Heft der Reihe „Fauna Rumäniens", in welchem Süßwasserkopepoden behandelt werden. Ihre Studien über die Ruderfüßerfauna in Rumänien (1952) hat die Verfasserin mit Harpacticoiden begonnen, hat aber zu erst das Heft über Cyclopiden (1963 erschienen), dann das über Süßwassercalanoiden (1967) veröffentlicht, um die artenreichste Gruppe zu letzt zu lassen.

Wie die übrigen Hefte der „Fauna Rumäniens", umfaßt der Band einen allgemeinen Teil (Geschichtliches, äußere und innere Morphologie, Ökologie usw) und einen speziellen (systematischen) Teil. Aus dem allgemeinen Teil sind besonders die Kapitel über äußere Morphologie (alle Abbildungen sind Originale) und über geographische Verbreitung zu erwähnen. Der systematische Teil umfaßt die Beschreibung von 68 Arten (und 4 Unterarten) die zu 24 Gattungen und 9 Familien gehören. Die artenreichste Familie ist die der *Canthocamptidae*; die artenreichste Gattungen sind *Bryocamptus*, *Elaphiodella* und, natürlich, *Parastenocaris*. Für jede Art ist die Hauptsynonymie, eine eher umfangreiche Beschreibung und kurze Angaben über Biotop (in seltenen Fällen über Ökologie) und über geographische Verbreitung (im allgemeinen und in Rumänien) gegeben. Mit einer einzigen Ausnahme (die 1969 beschriebene der Verfasserin nach gar zweifelhafte *Elaphoidella romanica*) sind alle Arten ausgebildet; die meisten Abbildungen sind Originale. Für etwa 25 Arten und Unterarten sind Abbildungen aus der Literatur angeführt; es handelt sich meistens um hypogäische Arten, für welche die Verfasserin kein Material zur Verfügung hatte (z. B. vier, von Chappuis 1925 aus dem Leitungswasser von Cluj beschriebene *Parastenocaris*-Arten, die niemals wiedergefunden wurden). Es ist zu bedauern, daß das reiche, aus unseren Höhlen gesammelte Material der Verfasserin nicht zugänglich war.

Die Harpacticidenfauna der Binnengewässer Rumäniens ist besonders reich; diese enthält u.a. 19 Endemiten (fast alle hypogäisch); eine Art, *Ectinosoma abrau* ist marinen Ursprungs; *Limnocletodes behningi* ist eine ponto-kaspische Süßwasserart.

Dieses Heft stellt, einen wichtigen Beitrag zur Kenntnis der Süßwasserkopepodenfauna Mittel- und Südosteuropas dar. Es wären aber zwei Einwände zu machen. Für vier Arten (*Campthocamptus staphyllinus*, *Bryocamptus zschokkei*, *Athyella wierzeiskyi*, *Elaphoidella gracilis*) sind für Rumänien je zwei Unterarten angegeben. Es handelt sich aber, in allen Fällen, nicht um vikarierenden Formen, da deren allgemeine Bereiche sich zum größten Teil decken, obwohl beide „Unterarten" an keinen Standort zusammen vorkommen (ökologische Trennung!).

Die betreffenden Formen können Geschwisterarten („sibling Species") sein. Außerdem ist die allgemeine Verbreitung der meisten Arten nach Ländern angegeben; es wäre viel besser gewesen, diese Verbreitung nach natürlichen geographischen Gebieten anzugeben.

Petru Bănărescu

B. KIS, C. NAGLER et C. MÎNDRU, **Fauna Republicii Socialiste România** (La Faune de la République Socialiste de Roumanie), vol. VIII, fasc. 6, *Neuroptera (Planipennia)*, Ed. Acad., Bucarest, 1970, 343 p., 165 fig.

La bibliographie entomologique roumaine et la série « La Faune de la République Socialiste de Roumanie » s'enrichissent d'un ouvrage particulièrement valeureux : une synthèse sur l'Ordre des Neuroptères (Planipennes) tel qu'il est représenté dans la faune de Roumanie ; cette synthèse constitue en même temps la première Monographie de l'Ordre pour un pays européen. Ajoutons que les 105 espèces (appartenant à 35 genres) signalées jusqu'à ce jour dans la faune de Roumanie représentent la majorité des espèces de la faune européenne de l'Ordre, et l'importance de l'ouvrage ressortira clairement.

La Monographie s'occupe essentiellement des adultes, mais on présente aussi des données sur les larves, dans la mesure, assez restreinte, dans laquelle celles-ci sont connues. L'Ordre se caractérise par la grande variété d'aspect des représentants des divers sous-ordres (*Coniopterygoidea, Osmyloidea, Mantispoidea, Hemerobioidea, Myrmeleonoidea*). Evidemment, la structure de l ouvrage est celle imposée par le standard de la « Faune ». Le texte est concis, rigoureux, clair. Les passages de la « Partie systématique » dédiés à la répartition géographique sont la preuve du travail assidu fourni par les auteurs pour collecter un très riche matériel sur l'ensemble du territoire du pays. Il faut mentionner que la bibliographie de l'Ordre pour la Roumanie se réduisait, avant que les auteurs n'aient commencé de s'en occuper. à quelques notes faunistiques qui mentionnaient moins de la moitié des espèces effectivement présentes. Les auteurs n'ont pas fourni des listes complètes de stations pour les espèces les plus largement répandues, probablement pour réaliser une économie d'espace mais une série de cartes de répartition vient combler cette lacune.

Une particularité importante du volume est constituée par l'abondance et l'excellence de l'illustration. On donne pour chaque espèce de bons dessins d'ailes et d'armatures génitales, suivant un standard presque toujours rigoureusement respecté le long de l'ouvrage. Ceci donne un total de plusieurs centaines de figures originales. Ce qui plus est, on présente une série d'admirables figures d'habitus, qui donnent la mesure de la beauté et de la variété de ces insectes, permettant aussi la rapide orientation de celui qui s'essaye à déterminer des neuroptères adultes.

Au point de vue graphique, le volume se présente bien ; on peut dire de certains dessins qu'ils auraient mérité un sort meilleur.

L. Botoșăneanu

EUGEN V. NICULESCU et FREDERIC KÖNIG, **Fauna Republicii Socialiste România.** *Insecta* (La Faune de la République Socialiste de Roumanie), Vol. XI, fasc. 10, *Lepidoptera* — Partea generală (*Lepidoptera* — Partie générale), 300 p., 129 fig., 29 pl. blanc-noir, Bucarest,1970.

Depuis longtemps s'imposait la parution d'un guide pour les entomologistes, et particulièrement pour les lépidoptéristes, tel celui qui vient d'être publié dans la collection : « Faune de la République Socialiste de Roumanie ».

Dans la littérature scientifique roumaine un tel guide manquait jusqu'à présent et la littérature mondiale n'en possède qu'un nombre très réduit (Jean Bourgogne in Grassé, W. Forster, A. Villiers), qui sont peu accessibles et moins complets et détaillés que le présent volume. C'est un fait bien connu par les spécialistes que dans les trois travaux mentionnés ci-dessus manquent totalement les chapitres traitant de l'historique, la phylogénie et l'importance économique, et le chapitre concernant la morphologie est tellement restreint, surtout dans les deux derniers ouvrages, qu'il présente peu d'intérêt pour les chercheurs.

« *Lepidoptera* — La partie générale » comprend un ensemble de données morphologiques, phylogénétiques, biologiques et zoogéographiques, formant un tout bien équilibré, exposé avec clarté, et constituant un guide utile pour les zoologistes de notre pays qui s'intéressent à l'étude des Insectes. Les auteurs se sont assumé une tâche très difficile qu'ils ont réussi à mener à bonne fin après un travail assidu de plusieurs années.

L'ordre des *Lepidoptera* est l'un des grands ordres d'Insectes. Il suit immédiatement celui des *Coleoptera* en ce qui concerne le nombre d'espèces (130 000). C'est le premier grand ordre d'Insectes dont la Partie générale paraît dans la collection « La Faune de la République Socialiste de Roumanie ».

Le chapitre I[er] présente l'historique de la Lépidoptérologie. C'est un chapitre original, le premier publié dans la littérature de spécialité des 60 dernières années. L'auteur de ce chapitre ne présente pas seulement une simple succession de noms propres, mais il nous montre l'évolution de la Lépidoptérologie depuis Linné jusqu'à nos jours, en soulignant les étapes les plus importantes qui ont constitué des tournants dans la voie ascendante de la science des Lépidoptères. On souligne le rôle de certains grands chercheurs, pionniers de la science, tels que Linné, Fabricius, Latreille, Boisduval, Rambur, Herrich-Schäffer, etc., dans l'essor continu de la Lépidoptérologie. Très utiles sont les références sur les chercheurs modernes, leurs conceptions et leurs préoccupations actuelles. Nous nous permettons la remarque que le chapitre « Historique des recherches en Roumanie » aurait dû avoir, à notre avis, une plus grande extension.

Le chapitre suivant, « la Morphologie », est le plus vaste et le plus important de tout l'ouvrage. Son auteur soutient que c'est seulement par la morphologie que l'on peut élaborer une systématique rationnelle, puisqu'elle se trouve à la base de la systématique et le systématicien moderne doit posséder d'amples connaissances morphologiques pour pouvoir décrire correctement une espèce et l'encadrer justement dans la systématique. C'est le chapitre le plus étendu et le plus complet sur la morphologie des Lépidoptères paru jusqu'à présent dans la littérature mondiale. Outre les données existant dans des travaux similaires, on y trouve de nombreuses données inédites, résultat des recherches de l'auteur, données nouvelles pour la science, ainsi qu'un paragraphe tout à fait nouveau : l'exosquelette, que l'auteur étudie depuis plusieurs années à des fins taxonomiques. Très intéressants sont le schéma du dessin présentant tous les termes morphologiques concernant le dessin de l'aile, la classification des types de dessins, dans la conception originale de l'auteur, ainsi que le dessin synthétique original, représentant les principaux éléments de l'armure génitale mâle.

Plus détaillée que chez l'imago est la présentation morphologique des premiers états surtout chez la chenille, très utile surtout aux chercheurs du domaine de l'entomologie appliquée, qui étaient jusqu'à présent privés d'un tel guide. L'auteur présente des données nouvelles pour la science, comme par exemple une nouvelle terminologie de la chêtotaxie, qui est plus commode et en même temps plus réelle, en tenant compte de la vraie position des soies sur le corps. Cette terminologie a déjà commencé à être adoptée à l'étranger dans quelques travaux récents.

Dans la chapitre de « Biologie » l'auteur présente, dans un style concis, les divers aspects biologiques de l'adulte et de la chenille. Ont été utilisées et systématisées toutes les informations classiques et celles plus récentes (Standfuss, M. Hering, P. Portier, Al. Klots, etc.) mais on remarque aussi l'apport personnel de l'auteur qui a présenté un nombre de données et de tableaux originaux. Le chapitre sur la biologie et l'écologie des chenilles est plus largement traité, pour les mêmes motifs invoqués à propos de la morphologie de la chenille. Il faut néanmoins mentionner que le paragraphe sur l'activité sexuelle et l'accouplement n'est pas bien placé dans le chapitre intitulé « Métamorphose ».

Le chapitre de « Phylogénie », entièrement original, revèle la compétence de l'auteur dans les questions de phylogénie, problème rarement abordé par les lépidoptéristes à cause de sa difficulté. C'est le plus ample et le plus documenté chapitre de tous ceux publiés jusqu'à présent dans la littérature. Son analyse, qui serait très utile, dépasse le cadre du présent compte rendu. Il faut toutefois signaler les nouvelles relations phylogénétiques établies par l'auteur à a suite de ses recherches concernant les superfamilles des *Eriocranioidea*, *Psychoidea*, *Zygaenoidea*, *Noctuaidea*, *Hesperioidea* et *Papilionoidea*, ainsi que le changement de la position systématique dans le système de classification de ces superfamilles.

Dans le chapitre « Distribution géographique » on présente, peut-être trop sommairement, les aspects zoogéographiques des Lépidoptères dans notre pays, sur la base des données bibliographiques. Il aurait été très utile que le chapitre comprenne aussi les biotopes de Roumanie avec leurs éléments caractéristiques. Si cette absence est justifiée par le manque d'espace, on aurait pu renoncer aux généralités zoogéographiques.

Le chapitre « Importance économique » clôt le travail et est suivi d'une liste bibliographique comprenant 252 titres de travaux, qui atteste une riche documentation. Il faut également mentionner les dessins très réussis qui illustrent ce travail remarquable, ainsi que les 29 planches photo, bien choisies et correctement exécutées. La plupart des dessins et des planches sont originaux.

Le travail sera d'un réel profit non seulement aux lépidoptéristes mais à tous les chercheurs entomologistes des Instituts, ainsi qu'aux cadres didactiques et aux étudiants des Facultés de Biologie et d'Agronomie.

Ion Nemeș
Suceava

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