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SOMMAIRE

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MYSIMENZIES HADALIS G.N., SP.N., A BENTHIC MYSID OF THE PERU TRENCH, FOUND DURING CRUISE XI/1965 OF R/V ANTON BRUUN (U.S.A.)

BY

MIHAI BĂCESCU

The author describes the genus *Mysimenzies* captured at about 6200 m, in the Milne Edwards depression of the Trench Peru, hence the name of *hadalis* given to the species. Primitive characters: the antennule structure, absence of eyes, the lateral notching of telson. The genus is placed in the Erythropini tribe, but its exact position will be established only when ♂ is known.

Having had the opportunity to participate in the exploration of the abyssal benthic fauna of the Milne Edwards Trench in South-East Pacific, I was able to establish among other Mysida of an archaic type found there (six new species of *Hansenomysis* e.g., **1**, & **2**), a new genus named in honour of Professor Robert MENZIES, chief Scientist of R/V Anton Bruun Cruise 11.

Occurrence 1 ♀ ad. Peru Trench, St. 197, 6146—6354 m depth (11° 29′5″ S; 79°24′5″ V). Holotype ♀ = 50 mm, No 74 in Coll. Crustacea of "Gr. Antipa" Museum of Natural History, Bucharest.

Description. *Carapace* smooth, translucent, moderately long, leaving only the last thoracic somite uncovered; anterior margin convex, with only a very short indication of a broadly rounded rostrum, beyond which the anterior margin of the head projects as an evenly rounded crescent. Eyes rudimentary, lacking all visual elements, represented by two large, acutely pointed, triangular processes, which forward almost to the level of the distal end of the first segment of the antennules (this condition can be seen in several species of the Erythropini — *Parerythrops rostrata* W.M.T. 1905, *Dactylerythrops dactylops* W.M.T. 1905, *D. bidigitata* W.M.T. 1907. Ocular processes cannot be confounded with

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"lappets" of the Tattersall organ of the *Hansenomysis* genus (*H. tropicalis* Băcescu f. i, **1**, p. 157).

In fact "lappets" is an intermediary piece between ocular lobes and the sensory fossette at the basis of the proximal segment of A_1. That piece, existing only in *Hansenomysis*, is absent here as well as the characteristic fossette at the A_1 basis; in the drawing of A_1 the black fossette which appears there is a deep depression in the proximal segment of A_1 and above it is the rudimentary eye reduced to a triangular flattened chitinous sac, without a trace of pigment or corneal structure.

Antennular peduncle very short, half shorter than antennal scale. Proximal joint of sympod of A_1 with chitinous *edge in middle of lower portion having triangular profile* (resembling a long ship keel) and which in certain bending movements probably rubs into the corresponding edge of the lower frontal part of the cephalic lobe.

Peduncle of A_2 somewhat longer than that of A_1, both of them reaching half length of enormous scale. It consists of a short terminal article, twice longer than medial one (both without phanera), and one minute basal joint, more or less fixedly articulated at sympod. Scale of antenna large, 4 times longer than broad with straight, bare, outer margin, terminating in small non articulated thorn not reaching the non articulated apex of lamella (fig. 1 F.). Apex with about 22 setae, which as the numerous setae of the inner edge, are broken, therefore they are exceptionally fragile.

Labrum obtuse triangular, posteriorly having two hairy bifid paragnatha (fig. 2 B) enclosing pars incisiva. The mandible is of a special type having masticatory part entirely hairy with gold coloured, weak, 3—4 dented pars incisiva; enormous masticatory part like a rasp with 2 rows of faint asperities, setae in upper portion and transverse incisions on internal face of large lobe. But in the place of the mobile lacinia — which is perhaps represented by strong curved serrated seta — there is a *large protuberance covered throughout with serrate setae* (fig. 1 D). Mandibular palp short (fig. 1 E).

Maxillule of the usual Mysidae type (Fig. 2 A).

Maxilla with much longer palp than exopod and large endite at basal joint, heaving marginal plumose setae (Fig. 1 G).

First thoracic appendages (first Maxilliped) (fig. 2 C) *without exopod* although with enormous outward expansion of basis articulated with epipod (*e*) and one endite having double row of setae extended medially. Remaining joints without thorns or hooks but with fine plumose setae.

Second thoracic appendages (Maxilliped II), (fig. 2G) *entirely pediform*; shape and length of its articles similarly as in pereiopods; without any kind of endites (so characteristic for the mouth parts) (cf SIEWING 1958). Dactyl foliaceous with tufts of plumose setae around and across the plate; propodus long, equal in length with carpus; merus and remaining two articles short, but abundantly provided with long plumose phanera. Exopod natatory with about 21 articles.

Last pereiopods are broken; only their bases were preserved with their oostegites, bordered with numerous plumose setae (fig. 2 F). Rudiment of third pair of oostegites at base of fourth pereiopod visible.

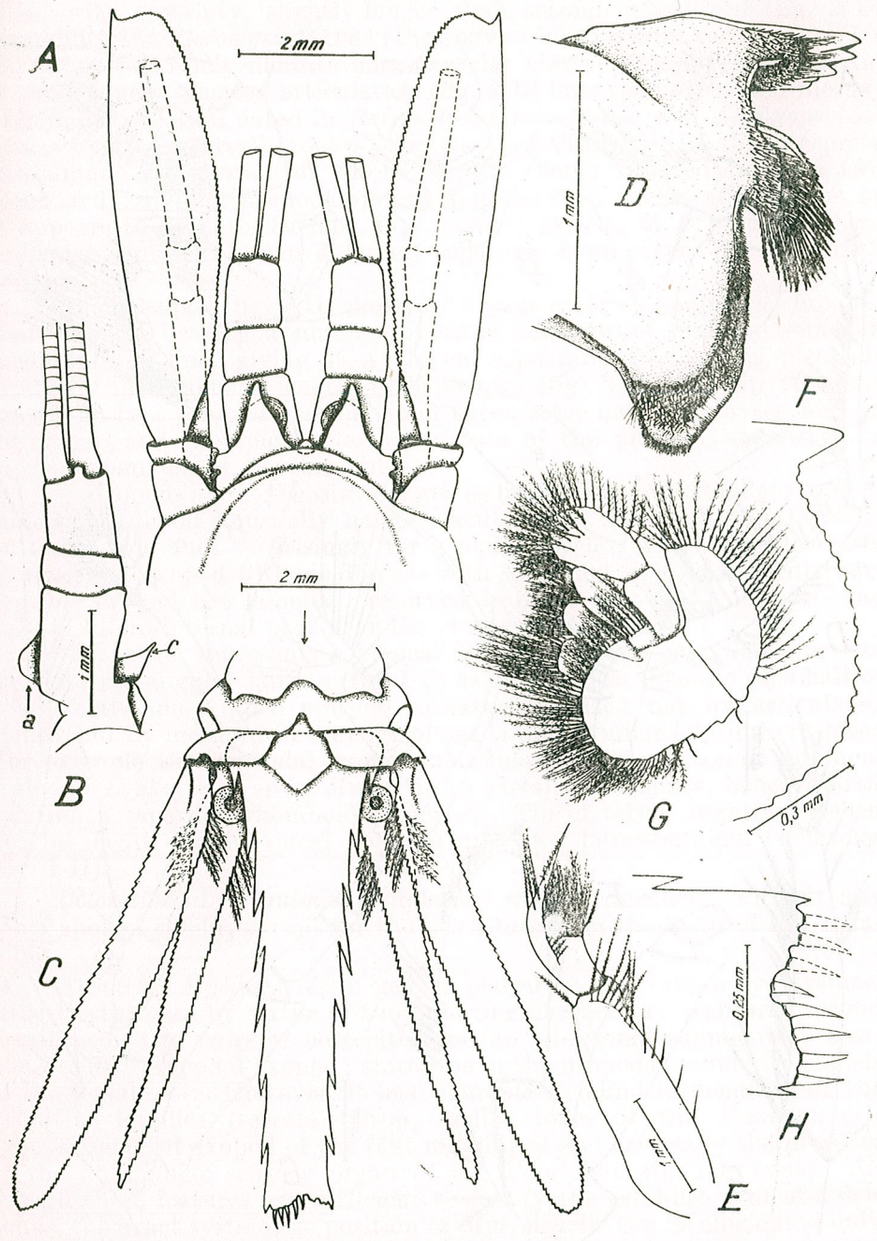

Fig. 1. — *Mysimenzies hadalis* n.g., n.sp., female. A, Cephalon, anterior part; B, Antennule, lateral view (a=lower carina; c=lappet); C, Telson and uropods (scale same as A); D, Masticatory part of left mandible; E, Mandibular palp; F, Apex of antennal scale; G, Maxilla with short exognathos (scale same as A); H, Tip of telson.

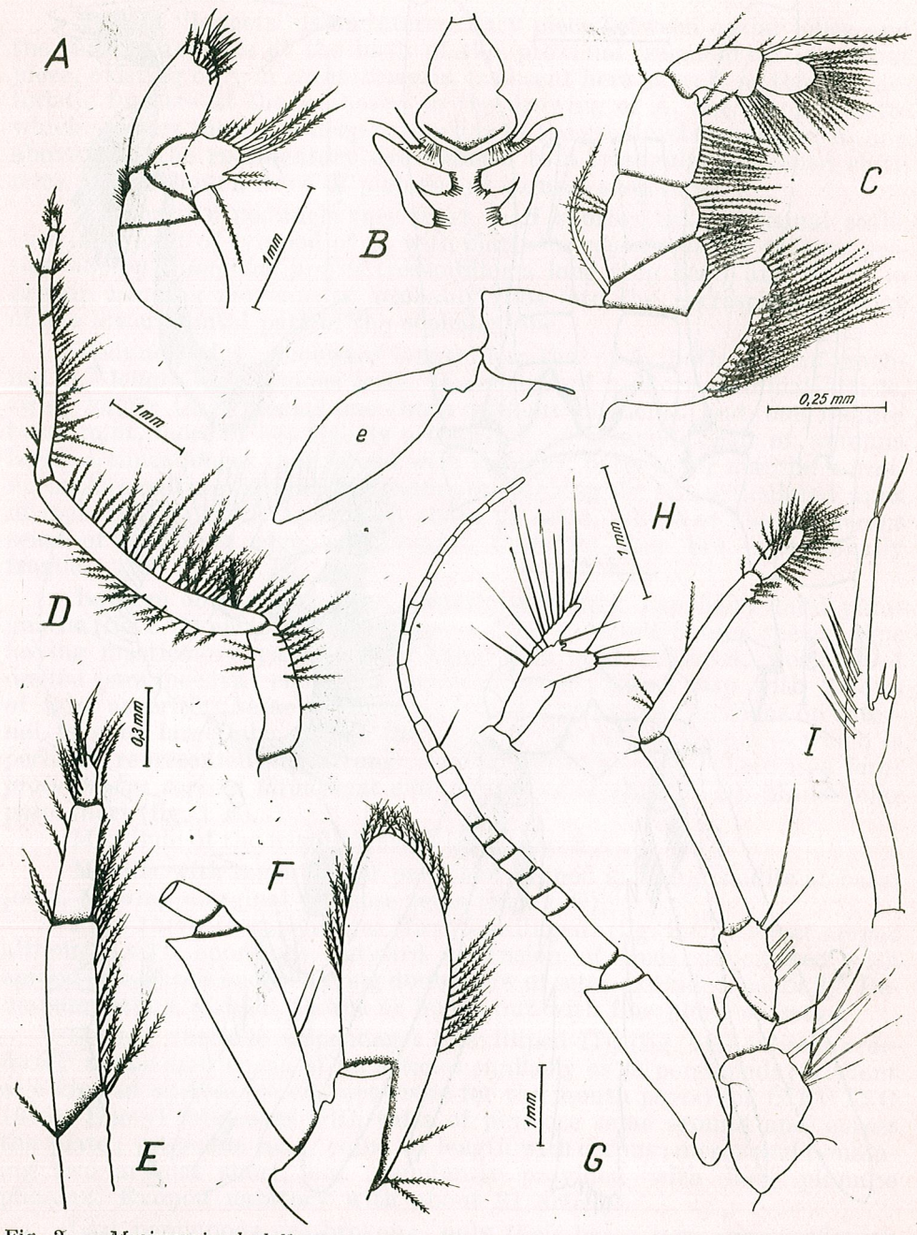

Fig. 2. — *Mysimenzies hadalis* n.g., n.sp., female. A, Maxillule; B, Labrum and paragnatha; C, First thoracic appendages (First maxilliped) (e=epipod). D, Anterior pereiopod; E, Anterior pereiopod, tip enlarged; F, Fragment of broken last pereiopod; G, Second thoracic appendages (scale same as F); H, First pleopod; I, Fifth pleopod.

Anterior pereiopods (only one was found) could not be identified with certainty, slightly longer than second maxilliped (fig. 2 D) resembling the *Pteromysis* Ii 1964 ; they have a long ischium, serrate dactylar claw and two finely dentate para-dactylar claws ; another characteristic is an obliquely situated articulation (fig. 2 E) between carpus and merus ; oblique articulation noted in *Paramblyops brevirostris* and *Amblyopsoides obtusa* by O. Tattersall (1955). However O. TATTERSALL (5) interprets the oblique articulation of *Paramblyops* as being situated between two secondarily divided propodal joints, not between merus and carpus as it appears to be here. In fact, in *Paramblyops* (e.g. in *P. rostrata*) these propodal joints may occur obliquely separated from carpus (O. Tattersall 1951).

First pleopod (fig. 2 H) short, with large external apophysis, broader than tip ; tip bearing a number of setae and row of plumose setae on medio-inferior line, giving it a cloven appearance ; remaining pleopods gradually increasing in length, fifth pleopod (fig. 2 I) with tip twice as long as in first pleopod bearing six or seven setae near apophyses and in lower part and two apical setae. Structure of the pleopods somewhat as in some members of Erythropini.

Uropods about the same length as telson, finely denticulate around lamella ; endopod unusually narrow, acute, with about 60 minute setae on either side and, surprisingly for a hadal species, with *typical statolith in statocyst*. Exopod without diaresis with some 200 frail setae hardly perceptible around the lamella, preserved in basal part only ; normal setae only in infero-internal part near the statocyst (fig. 1 C).

The telson represents a typical feature of the genus ; more or less elongate rectangular lamina (fig. 1 C) as long as last pleonite plus half of penultimate one ; quite unusual armature realized not by articulated spines, but by incisions in the edge of the lamina, similar to primitive forms (for example the uropodal exopod in *Chalaraspidum alatum* or antennal scales in *Lophogaster* or in *Meterythrops picta*). Proximally, beneath articulation, a separate, rhomboid projection. Tip of telson slightly notched bearing eight simple apical spinules and two laterotermínal denticles (fig. 1 H).

Coloration. Live animal completely white, translucid, without any other spot of colour, except for the brick-brown of the teeth of the mandible.

Remarks. Mysimenzies n. gen. is placed in the subfamily Mysinae, tribe Erythropini by virtue of the following characters : pediform second maxilliped ; two pairs of oostegites and an additional rudimentary one ; non-divided uropodal exopod ; statoliths in the uropods ; reduced pleopods in the female, etc. However, it has characters found in members of the primitive families (serrate telson, ocular lobes of the *Hansenomysis* type, absence of exopod of the first maxilliped and especially the presence of the antennular sensory organ of the Petalophthalmidae type). Although these features are sufficient to justify the establishment of a new genus, the exact systematic position of *Mysimenzies* can be elucidated only when the morphology of the male is known.

The type species was found at about 6,200 m depth in the most typical biotope of *Neopilina ewingi* Clarke and Menzies.

Although more than 35 species of benthic Amphipoda are known from the hadal zone (6,000—10,000 m) and more than 30 from the bathyhadal zone, and although there are more than 40 hadal Isopoda (Wolff 1960, Menzies 1962, Birstein 1969), this mysid is only the second one found in the ocean at depths below 6,000 m. Until now only the recently studied *Amblyops magna* B. & T. was dredged from the hadal zone at 7 260 m, and *A. aequispina* had been found at 5,760 m by Birstein and Tchindonova (1958). Mysids are probably even more abundant at hadal depths than is now known. The *Dactylamblyops tenella* (2) was also quoted in a proof from 6600 to 0 m depth, but the species was probably captured in pelagic zone at any depth as far as the hadal.

I thank Mrs O. Tattersall for the trouble she had in revising the text of this note and whose observations I took into account; many thanks also to Dr. Ed. Chin.

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FURTHER STUDIES ON THE SYSTEMATICS OF CULTRINAE WITH REIDENTIFICATION OF 44 TYPE SPECIMENS (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

The examination of holotypes and paratypes of 44 nominal species of Cultrinae confirmed some previous synonymizations and taxonomic assumptions and lead to some new ones: *Rasborinus taeniatus* is an *Ancherythroculter*; *A. brevianalis* is a synonym of *A. taeniatus*; *Rasborinus formosae* is a synonym of *Ischikauia macrolepis*; *Pseudolaubuca jouyi engraulis* cannot be maintained as valid subspecies; *Chela nicholsi* is a synonym of *Pseudolaubuca sinensis*; *Parachela williaminae* is an *Oxygaster*; *Oxygaster pointoni* is a subspecies of *Ox. anomalurus*. Six lectotypes are designed.

In a series of previous papers [3—8], I delimited the subfamily Cultrinae, revised some of its genera, established new synonymizations and described a few new taxa. Those papers were based on Eastand South-East Asian specimens borrowed from several museums in Europe and U.S.A., including types belonging to the museums in Paris, Berlin and Vienna; a few conclusions were based on photographs of holotypes received from some museums in the U.S.A.

Having recently the opportunity to visit some museums in the U.S.A. and the British Museum, I studied further specimens, including holotypes and paratypes of little-known species.

The specimens examined belong to following museums: American Museum of Natural History, New York (A.M.N.H.,), Academy of Natural Sciences in Philadelphia (A.N.S.P.) , British Museum, Natural History (B.M.N.H.), Field Museum of Natural History, Chicago (F.M.N.H.), United States National Museum, Washington (U.S.N.M.), Zoölogisch Museum, Amsterdam (Z.M.A.), Zoologisches Museum, Berlin (Z.M.B.), Zoologitscheskii Institut Akademii Nauk, Leningrad (Z.I.A.N.).

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SYSTEMATIC ACCOUNT

Genus **Erythroculter** Berg, 1909

Erythroculter illishaformis illishaformis (Bleeker)

Holotype of *Culter aokii* Oshima; F.M.N.H. 59110, Jitsugetautan (Lake Candidus), Taiwan; st. 1. 239 mm; A 2/23; L. lat. 87—88; Sp. br. 27; predorsal distance 51.5% of st. 1.; identical to Yangtze and Amur specimen of *Er. illishaeformis*.

Erythroculter illishaformis recurvirostris (Sauvage)

Holotype of *Er. pseudobrevicauda* Nichols & Pope: A.M.N.H. 8400, Nodoa, Hainan Isl., 173.0 mm st. 1.; A 3/23; L. lat. 69; sp. br 25.

Genus **Culter** Basilewski, 1855

Culter alburnus Basilewski, 1855

Syntypes of *Culter brevicauda* Günther, 1868: B.M.N.H. 1865.10.29. 29—31, Taiwan, 3 spec., 185.0, 156.5 and 151.0 mm st. l.; A 2/26—27; L. lat. 65—66; Sp. br. 26—29; a fourth specimen (B. M.N.H. 1865.10.29. 32, 101 mm proved to be a *Hemiculter leucisculus*. The largest specimen, 185.0 mm (B.M.N.H. 1865.10.29.29) is here designed lectotype.

Paratype of *Culter tientsinensis* Abbott, 1901: U.S.N.M. 49 550, Tientsin, Paiho River, 97.2 mm; A 2/26; L. lat. 64 — ± 68 (the holotype is, according to Abbott, S.U. 6297; st. 1. 120 mm.).

There are no differences between the specimens from North China (*alburnus*, syn. *tientsinensis*), Taiwan (*brevicauda*), Yangtze, etc.

Genus **Ancherythroculter** Yih & Wu, 1964

Ancherythroculter taeniatus (Nichols, 1943) (syn.: *A. brevianalis* Banarescu, 1968)

Holotype of *Rasborinus taeniatus* Nichols: A.M.N.H. 15 219, Loshan (Kiating), Szechwan; 87.0 mm; D III 7; A 2/17; L. lat. 55 $\frac{11}{5}$; Sp. br. 14. This species is not a *Rasborinus*, but an *Ancherythroculter*, the last simple dorsal ray being spinified. *A. brevianalis* is the same species.

Genus **Hemiculter** Bleeker, 1859

Hemiculter leucisculus (Basilewski, 1855)

Holotype of *Parapelecus eigenmanni* Jordan & Metz, 1913: F.M.N.H. 55 802, Suigen, Korea: 112.0 mm; D III 7; L. lat. 52—51; Sp. br. 17.

Holotype of *Cultriculus akoensis* Oshima, 1920: A.N.S.P. 49 953, (Fig. 1) Ako, Taiwan; 78.0 mm; L. lat. 49—50; Sp. br. 21. In both type specimens the lateral line is strongly decurved, the shape of pharyngeal teeth typical for *H. leucisculus*.

Hemiculter bleekeri Warpachowski, 1888

Paratype of *Toxabramis argentifer* Abbott, 1901: U.S.N.M. 49545, Tientsin, Paiho R.; 109.3 mm; L. lat. 46—47, slightly decurved; Sp. br. 22; pharyngeal bones removed. The holotype is S.U. 6299., 130 mm. st.

Holotype of *H. clupeoides* Nichols, 1925: A.M.N.H. 8433, Tungting Lake, Hunan; 127.5 mm; L. lat. ± 47, slightly decurved; Sp. br. 22. Nichols is wrong [13], [14] in indicating ±55 scales in lateral line.

Both specimens are typical *H. bleekeri*.

Hemiculter dispar hainanensis (Boulenger, 1899)

Holotype of *Barilius hainanensis* Boulenger: B.M.N.H. 1899. 11.30 22, Hainan Island; 119.0 mm; A. 2/13.; L. lat. 47 $\frac{7}{2}$ 45; Sp. br. 10.; lateral line abruptly bent; postventral keel.

Holotype of *Hemiculter hainanensis* Nichols & Pope, 1927: A.M.N.H. 8379, Nodoa, Hainan; 118.0 mm; A 2/15; L. lat. 56; agrees with the Nodoa specimens described previously [7], in which the number of lateral line scales ranges between 46 and 56.

This is a rare case of a species, described twice as new species under different generic but under the same specific name.

Hemiculter serratus (Koller, 1927)

Holotype of *Hemiculter serracanthus* Nichols & Pope, 1927: A.M.N.H., 8380, Nodoa, Hainan; 113.0 mm; A 2/14; L. lat. 52 $\frac{10}{2}$. strongly decurved; Sp. br. 9. Dorsal spine very long and serrated, its length 23.8% of st. 1. The specimens agrees perectly with the holotype of *Hainania serrata* (N.M.W. 10420) and with A.M.N.H. 10870 described previously [7]

Genus **Toxabramis** Günther, 1873

Toxabramis swinhonis Günther, 1873

Syntypes of T. *swinhonis*: B.M.N.H. 1873, 7.30.97, Shanghai, 8 specimens, 82.5, 80. 0, 79.0, 75.0, 66.2, 55.0. and 53.0 mm st. 1. The best preserved one, 66.2 mm, whose pharyngeal teeth were examined by Günther, is here declared lectotype. These specimens, as well as other lower Yangtze specimens (U.S.M.N. 130494, etc.) are characterized by: A 2/16—18; L. Lat. 57—59; Sp. br. 23—29.

Genus **Ischikauia** Jordan & Snyder, 1900

Ischikauia macrolepis macrolepis (Regan, 1908)

Holotype of *Rasborinus formosae* Oshima, 1920: A.N.S.P. 49952, Mantsa, Taiwan (Fig. 2); 67.9 mm; D 3/7; A 2/13; L. lat. 48—47; Sp. br. 16; Phar. t. 5.4.2—2.4.4. The species *R. formosae* was described after a single specimen and never found again; yet it continued to be listed in all comprehensive papers (Mori [12]; Wu & others [16]). The type proved to be an *I. macrolepis*: ventral profile more arched than the dorsal one, mouth almost vertical, dorsal fin slightly notched, lateral line gradually but rather strongly bent.

Ischikauia macrolepis hainanensis Nichols & Pope, 1927

Holotype of *I. hainanensis*: A.M.N.H. 8390, Nodoa; 73.0 mm; A 2/15; L. lat. 44; Sp. br. 19; depth 26%; head 26.3% of st. 1. Other Nodoa specimens (A.M.N.H. 10986, 10975, 25 in all) are characterized by: A 2/14—18 (M = 15.88 ± 0,18); Sp. br., 18—20, rarely 21 (M = = 18.84 ± 0.26); L. lat. 41—49 (M = 44.0 ± 0.28); depth 20.4 — 27.1%; caudal peduncle 18.4—22.7%; least depth 8.5—11.2%; head 26.2—29.0% of st. 1.

Genus **Hemiculterella** Warpachowski, 1887

Hemiculterella sauvagei Warpachowski, 1887

Holotype of *H. sauvagei*: Z.I.A.N. 4474, Szechwan; 98.0 mm; A 2/13; L. lat. 51; Sp. br. 9; Phar. teeth 4.4—3.5. This specimen agrees perfectly with the already [4] mentioned ones (M.N.H.N. 6285 & 6284), in which the teeth are in three rows, and with two others (A.M.N.H. 15287 &

15244, Szechwan). The holotype is unique in having the teeth in two rows. The species is characterized by: A 2/11—13, exceptionally 15; L. lat. 50—54 (57 in one specimen); Sp. br. 9—11, rarely 13; teeth usually in three rows; lateral line strongly bent but not ondulated; peritoneum blackish; colour briliant silvery with a dark longitudinal stripe in well preserved specimens.

Hemiculterella polylepis (Regan, 1904)

Holotype of *Barilius polylepis*: B.M.N.H. 1904. 1. 26—31, Yunnan-Fu, 120.0 mm.; A 2/13; L. lat. 77—83; Sp. br. 19. Other specimens: A 2/13, L. lat. 70—78; Sp. br. 18—24. Yih & Wu (Wu et al [16]) mentions somewhat different values: A 2/12—14; L. lat. 66—71, Sp. br. 25—28.

Hemiculterella andersoni (Regan, 1904)

Syntypes of *Barilius andersoni* Regan: B.M.N.H. 1904. 11.30.46, Yunnan Fu, two species, 114.0 & 88.0 mm, the larger one is declared lectotype. The lectotype, paratype and other specimens are characterized by: A 2/9—11; L. lat. 79—94; Sp. br. 15—18. Yih & Wu (loc. cit.) mention A 2/9—12; L. lat. 90—97; Sp. br. 19—22.

Hemiculterella alburnops (Regan, 1904)

Syntypes of *Barilius alburnops* Regan: B.M.N.H. 1914. 1.28, 11—20 Yunnan Fu, 15 specimens, the largest, 165.2 mm is here declared lectotype (B.M.N.H.1.28.11); the 14 paralectotypes have 129—155 mm.

Holotype of *Hemiculter andrewsi* Nichols: A.M.N.H. 7038, Kumming (Yunnan Fu), 138.0 mm; L. lat. 82; Sp. br. 47.

Other specimens examined, all from Yunan Fu: M.N.H.N. 4945, 4946, A.M.N.H. 7172, then N.M.W. 53188, labelled *Hemiculter schneideri*, probably intended by Steindachner to be described as new species.

The species is characterized by: A 2/12—14; (M = 13.2 ± 0.17), L. lat. 78—87; Sp. br. 41—49 (46.1 ± 0.67); Yih & Wu (loc. cit.) give: A 2/10.11, L. lat. 77—84, Sp. br. 20—24.

Hemiculterella grahami (Regan, 1908)

Syntypes of *Barilius grahami* Regan: B.M.N.H. 1908.2.24.27—32, six specimens, Chenkiang Lake, Yunnan, more or less dried; the largest (104.2 mm as against 92.8—980 mm in the 5 others), better preserved and whose pharyngeal teeth were examined by Regan is declared here lectotype. Other specimens: M.N.H.N. 4943, Kumming Lake, 2 spec., 113 &. 190 mm, not well preserved. The species is characterized by: A 2/9; L.

lat. ± 62—69; Sp. br. 33—37, body slender, snout long and pointed, mouth small, reaching under nostrils, lateral line but slightly bent.

Hemiculterella transmontana (Nichols, 1925)

Holotype of *Ischikauia transmontana* Nichols: A.M.N.H. 8441, Yunnan Fu, 102,0 mm; other specimen: A.M.N.H. 10985, Yunnan, 94.2 mm. A 2/8—9; L. lat. 57—61; Sp. br. 13. Pelvic origin slightly behind that of dorsal; this character does not justify the separation of this species in a distinct genus, *Rohanus* Chu, as admitted by Yih & Wu [16].

Genus **Pseudolaubuca** Bleeker, 1864

Pseudolaubuca jouyi (Jordan & Starks, 1905)

Holotype of *Parapelecus jouyi* Jord. & Starks: U.S.N.M. 51497, Chemulpo, Korea, 179.0 mm; A 2/21; L. lat. 45; Sp. br. 11; the original description mentions A 24 (2/22?); L. lat. 40.

Holotype of *Hemiculterella engraulis* Nichols, 1925: A.M.N.H. 8432, Tungting Lake, 149.0 mm; A 2/20; L. lat. 44—46; Sp. br. 14.

Other specimens from the lower Yangtze (Tungting L., Shanghai): A 2/18—20 [21]; L. lat. 43—50; Sp. br. [12] 13—14 [15]. It appears thus that there are no difference between Korean and Yangtze specimens; the Hwangho form, *Ps. tsinanensis*, known from a single specimen, apparently not preserved in any museum, is said to have 52 scales, but this value must be verified on more specimens. Contrary to my earlier assumption [3], I presently consider *tsinanensis* and *engraulis* not as distinct subspecies, but as full synonyms of *Ps. jouyi*. The poorly known *Ps. angularis* Kimura from Tsung Ming island, off Chekiang coast, decribed with 42—43 scales, may also not deserve subspecific rank.

Pseudolaubuca sinensis sinensis Beeker, 1864

Holotype of *Paralpelecus argenteus* Günther, B.M.N.H., 1889, 6.8. 104, Kiukiang, 207 mm; A 2/23; L. lat. 72—75; sp. br. 13.

Holotype of *Chela nicholsi* Fowler 1923: A.M.N.H. 8480, Ningkwo, Anhwei, 144.5 mm; A 2/26; L. lat. 72—74; Sp. br. 14; Fowler [9] wrongly indicates 64 + 4 scales. Other available Ningkwo specimens have 67—73 scales.

Pseudolaubuca sinensis machaerius (Abbott, 1091)

(syn. Parapelecus tungchowensis Tchang, 1932)

Paratype of *Parapelecus machaerius* Abbott: U.S.N.M., 49549 Paiho R., 108.0 mm; A 2/27; L. lat. 66—68; Abbott [1] mentions the same values for the holotype S.U. 6307.

The number of scales in the type and paratype of *machaerius* ranges within the limits of *Ps. sin. sinensis*; yet I accept *machaerius* as distinct subspecies, because the type of *P. tungchowensis*, described also from the Peiho R., has some 58 scales. The mean number for the Paiho specimens may be of about 61—63.

Pseudolaubuca sinensis fukiensis (Nichols, 1926)

Holotype of *Parapelecus fukiensis*: A.M.N.H. 8479, Fukien, 99.0 mm; A 2/21; L. lat. ±63; Sp. br. 13; depth 21.0%; lateral line abruptly decurved (the illustration by Nichols [14] is inadequate). Two other Fukien specimens have 62—66 scales (the 5 already [3] mentioned specimens have only 54—59 scales).

Summarizing the data on the available specimens of *Ps. sinensis*: lower Yangtze ones (*Ps. s. sinensis*): 67—75 scales, M = 71.0; upper Yangtze ones: 60—64 scales, M = 62.5.

Hsikiang drainage: 61—66 scales, M = 63.0;

Fukien (Minkiang drainage) specimens: 54—66 scales, M = 59.5.

The upper Yangtze and Hsikiang specimens may either be ascribed to the Paiho subspecies *Ps. sin machaerius* or considered intergrades between *Ps. sinensis sinensis* and *Ps. sin fukiensis*.

Genus **Rasborinus** Oshima, 1919.

The genus includes a single species with two subspecies.

Rasborinus lineatus lineatus (Pellegrin, 1907)

Holotype of *Rasborinus hainanensis* Nichols & Pope, 1927: A.M.N.H. 8382, Nodoa, Hainan Isl., 98.8 mm; A 2/16; L. lat. 39—38.

Rasborinus lineatus altior (Regan, 1913)

Holotype of *Rasborichtys altior* Regan: B.M.N.H. 1912.3.2.1, Singapore (surely introduced!); 52.2 mm; A 2/15; L. lat. 39.

Holotype of *Rasborinus takakii* Oshima, 1920: A.N.S.P. 49951, Citno, Taiwan, 62.3 mm; A 2/14; L. lat. 37—36.

Holotype of *Rasborinus fukiensis* Nichols, 1925: A.M.N.H. 8431, Yenping, Fukien, 68.1 mm; A 2/16; L. lat. 38; the two other available Fukien specimens have 14 branched anal rays.

Genus **Salmostoma** Swainson, 1839.

Salmostoma clupeoides (Bloch, 1782).

Holotype of *Cyprinus clupeoides*: Z.M.B. 3425, (Fig. 3) "Indian Ocean" (evidently not the Ocean, but some fresh water), 109.0 mm st. 1., A 3/12, L. lat. $78\frac{14}{3}83$, Sp. br. 20 strong symphyseal hook on the lower jaw; agrees with the already [5] recorded specimens of *S. clupeoides*, many of which were determined by F. Day himself.

Salmostoma phulo orissaensis Bănărescu, 1968

According to my recent revision of the genus [5], the species *S. phulo* appeared restricted to northern India (*S. ph. phulo*) and to Mahannadi drainage in Orissa, Northeast India (*S. ph. orissaensis*); the available specimens from Madras, Godavari and Kistna drainage, labelled *phulo* from different collections proved to belong to *S. novacula*. Yet one Madras specimen, F.M.N.H. 2350, 66.0 mm actually is a *S. ph. orissaensis*, having: A 2/18, L. lat. ± 89 (as against 99—112 in *S. ph. phulo*), Sp. br. 13 (as against 76—94 in *S. novacula*!). The very similar but perhaps not too closely related *S. novacula* and *S. ph. orissaensis* occur thus sympatrically in Madras.

Salmostoma acinaces (Valenciennes, 1842)

Syntypes of *Chela argentea* Day 1872: B.M.N.H. 1867, 7.24. 44—47, Bhavani (Bowany) R., 4 spec., 89.0—97.0 mm; A 2/15—17; L. lat. 43—44; Sp. br. 15—18. The largest specimen, 97.0 mm (B.M.N.H. 67.24.44), is designed as lectotype.

Genus **Chela** Hamilton-Buchanan, 1822

Chela laubuca (Hamilton-Buchanan, 1822)

Holotype of *Laubuca siamensis* Fowler, 1939: A.N.S.P. 68496, Trang, Thailand, 44.6 mm; A 2/20; L. lat. 35—36; depth 29.8%; head 24.6%. In spite of its elongate shape, upwards directed mouth and strong denivelation at nape ([6], Fig. 3), this fish is really a *Chela laubuca*, as accepted also by Smith [15].

Chela coeruleostigmata (Smith, 1931)

Holotype of *Laubuca coeruleostigmata*: U.S.N.M. 90287, Nakon Sawan, Thailand, 41.8 mm; D 3/11; A 2/20; L. lat. 35. Paratypes of the same: U.S.N.M. 90288, 5 spec., 44.0—48.0 mm; D 3/10—11; A 2/21—22; L. lat. $31\frac{7-8}{3-5}35$.

Holotype of *Chela mouhoti* Smith, 1945: U.S.N.M. 107959, Pasak R. at Pechabun, Central Thailand, 45.0 mm; D 3/10; A 2/21; L. lat $32\frac{7}{5}31$.

The comparison of the types of both nominal species confirmed my former assumption [6] that these are synonyms. There is a full intergradation in number of scales [see also the values of the specimens recorded formerly, 6]; as mentioned by Smith [15], the type of *mouhoti* has no vertical stripes above pectoral, but these may have vanished; some of these stripes are vanished also in a few paratypes of *coeruleostigmata*.

Chela (Malayochela) maassi (Weber & De Beaufort, 1912)

Holotype of *Eustira maassi*: Z.M.A. 110111, Kampar R., Sumatra, 29.2 mm; D 3/6; A 2/11; L. lat. $28\frac{6}{1}29$; Phar. teeth 5.2 — 2.5. The character on which the subgenus *Malayochela* is based, pharyngeal teeth on two rows, is thus confirmed in the holotype.

Genus **Longiculter Fowler**, 1937

Longiculter siahi Fowler, 1937

Holotype of *L. siahi*: A.N.S.P. 68014, (Fig. 4) Me Poon, 163.6 mm; paratype of the same: A.N.S.P. 68015, Me Poon, 162.0 mm. D 3/7; A 2/30—31; L. lat. 73—82; Sp. br. ± 107—128; D. phar. 4.2—2.4. Agree with Fowler's description.

[Genus **Oxygaster** Van Hasselt, 1823

Oxygaster oxygastroides oxygastroides (Bleeker, 1852)

Lectotype of *Leuciscus oxygastroides* (designed by Alfred [2]): B.M.N.H. 1866. 5.2.216, no locality; 103. o mm; A 2/28; L. lat. 40—41; Sp. br. 17; depth 27.3% of st. 1.

Holotype of *Chela siamensis* Günther, 1868; B.M.N.H. 1861, 10.8 17, Pachebon, Thailand, 83.0 mm; A 2/26; L. lat. $40\ \frac{8\frac{1}{2}}{3}$ Sp. br. 12; depth 31.6%. A normal *O. oxygastroides* with somewhat deeper body.

"Holotype" of *Oxygaster brachysoma* Fowler (unpublished name !) : A.N.S.P. 61300, Bangkok, 61.9 mm ; A 2/27 ; L. lat. ± 36 ; Sp. br. 12 ; depth 37.0 %, origin of dorsal slightly behind that of anal. This is the specimen recorded and illustrated by Fowler ([11], Fig. 4) as *O. siamensis* ; in the illustration, the position of the dorsal insertion is inadequate. Evidently, Fowler intended to describe this specimen as new species and identified it later with Günther's *Chela siamensis*.

Oxygaster oxygastroides ingerkongi Bănărescu, 1969

In the description of this subspecies, [8] a print mistake occurred, concerning the Catalogue number of the holotype : U.S.N.M. 135 ; actually it is U.S.N.M. 135 946. The 13 paratypes from the same series became U.S.N.M. 202860.

Oxygaster maculicauda (Smith, 1934)

Holotype of *Chela maculicauda* : U.S.N.M. 103373, Klong Ranoad, tributary to Tale Sap lake, 45.0 mm ; A 2/25 ; L. lat. $39\frac{8}{4}40$. Paratype of the same, 44.2 mm ; A 2/24 ; L. lat. $\pm 40\frac{8}{4}$. In general shape, rays and scales counts, this nominal species fully agrees with the sympatric *O.o. oxygastroides*, but has two small, well marked blackish spots on each caudal lobe. Such spots never occur in normal *oxygastroides*. *O. maculicauda* may be either a synonym of *oxygastroides*, based on two aberrant specimens, or a distinct, reproductively isolated species with quite restricted range and special ecological requirements.

Oxygaster williaminae (Fowler, 1934)

Holotype of *Parachela williaminae* : A.N.S.P. 57457, Chiengsen, 88.0 mm ; A 2/35 ; L. lat. 41—40 ; Sp. br. 22 ; depth 29.8 %. Fowler ascribed this species to *Parachela* because of the absence of pelvic fins. But one can easily recognize that this absence is accidental, the former insertion of the fins can easely be recognized. The species is an *Oxygaster*, closer to *oxygastroides* (the scales reach to above anterior margin of eye), but with very long pectoral fin (36.6 % of st. 1.) and with more anal rays than *O.o. oxygastroides* from Thailand and West Borneo (approaching in this character the East Borneo *O.o.ingerkongi*).

Oxygaster anomalurus pointoni (Fowler, 1934)

Holotype of *Chela pointoni* : A.N.S.P. 57456, Chieng Mai, 58.6 mm ; A 2/28 ; L. lat. $44\frac{7}{3}43$ [not 33, as given by Fowler [10])

I already [8] pointed the similarity between *pointoni* and *anomalurus*. Smith [14] records both forms from Thailand, mentioning for *anomalurus* 50—60 scales (probably he took these values from Weber & De Beaufort). In the nine available *anomalurus* specimens from Thailand (including the type of *pointoni*) I found 43—47 scales, while those from Indonesia and Malaya have 49—56, rarely 47, 48 or 57—60. I therefore consider *pointoni* as the Siamese subspecies of *anomalurus*.

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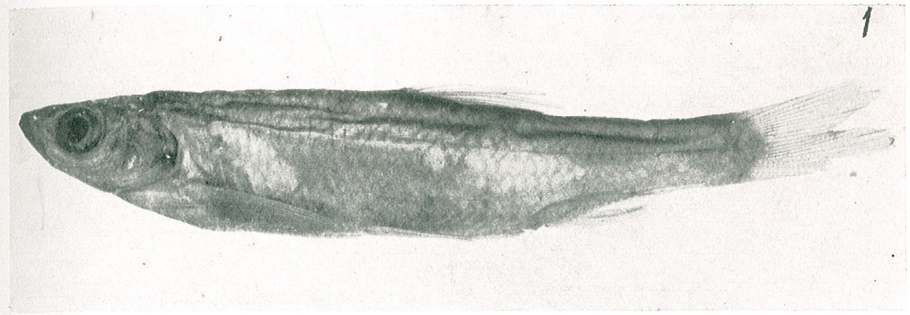

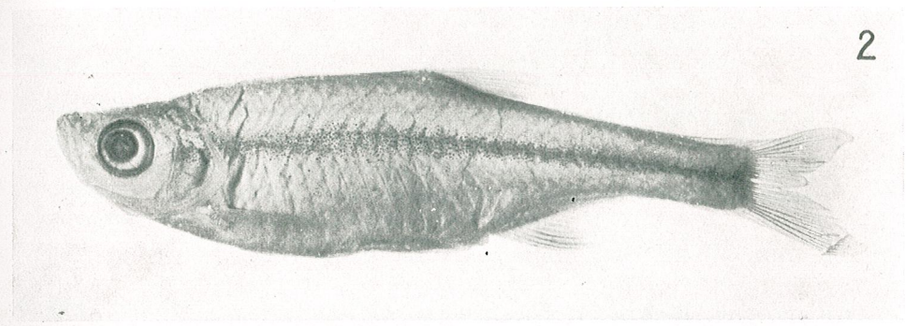

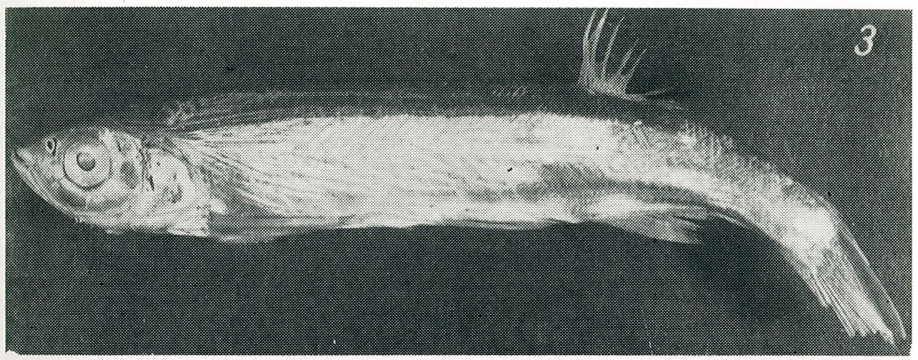

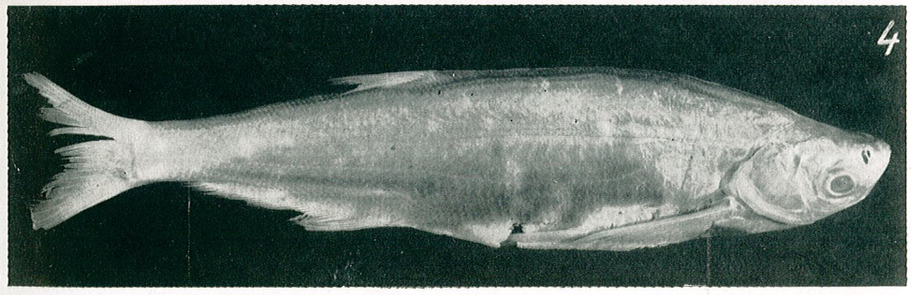

Fig. 1. *Hemiculter leucisculus* (Basilewski). Holotype of *Cultriculus akoensis* Oshima. A.N.S.P. 49953; Ako, Taivan.

Fig. 2.— *Ischikauia macrolepis macrolepis* (Regan). Holotype of *Rasborinus Formosae* Oshima. A.N.S.P. 49952. Mantsa, Taiwan. Courtesy of Dr. J. Böhlke.

Fig. 3. *Salomostoma clupeoides* (Bloch). Holotype, Z.M.B. 3425. India.

Fig. 4.— *Longiculter siahi* Fowler. Holotype, A.N.S.P. 68014. Me Poon. Courtesy of Dr. J. Böhlke.

LE SYSTÈME GÉNITAL MÂLE DE *SEGESTRIA SENOCULATA* (ARANEAE)

PAR

ELENA TRACIUC

The male reproductive organ is described in a primitive spider species (*Segestria senoculata* (Linnaeus, 1758), Dysderidae).
The male reproduction organ was examined in the adult and in the first nymphal instars, when the different parts of the reproductive organ are formed. The names of several parts are precised in relation to their origin.

Les recherches antérieures concernant l'appareil génital mâle des Araignées se réduisent au travail de Bertkau [1]. L'auteur décrit l'appareil génital interne de l'espèce *Phyloica domestica*, comme étant représenté par un type d'appareil ayant deux testicules. Ultérieurement, les traités de spécialité [3], [4] mentionnent ce type chez toutes les Araignées, bien que l'auteur [1] fasse une remarque dans son travail sur l'appareil génital de *Segestria bavarica*, chez lequel le testicule a l'aspect d'une masse en forme de U. Mais l'auteur ajoute que n'ayant travaillé que sur un seul exemplaire, il n'y a joint aucun dessin venant à l'appui de son affirmation. Il ne donne que l'image du palpe de type primitif de ce genre.

Dans le présent travail on décrit le type d'appareil génital de *Segestria senoculata* qui se présente sous la forme d'une masse testiculaire en fer-à-cheval.

MATÉRIEL ET TECHNIQUE

80 mâles de *S. senoculata* ont été capturés sous l'écorce d'arbres du massif de Gîrbova-Sinaia (Prahova). L'étude a porté sur des individus adultes et des nymphes aux stades I, II, III, IV. Les adultes ont été capturés avant et après la mue d'accouplement.

Pour les nymphes on a utilisé les fixatifs Susa et Zenker, et pour les adultes le Hollande*.

*) Nous avons constaté que c'est le plus approprié des fixatifs pour les Araignées adultes. Arch. anat. micr., 1911, vol. 13

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Le matériel a été inclus dans de la paraffine et sectionné à 5—7 μ. Les coupes sériées ont été coloriées à l'hémalun-éosyne. On a effectué des dessins à la chambre claire et des photographies. Pour les palpes on s'est servi des méthodes histologiques, des clarifications au KOH à 20 % et de dissections.

L'Adulte. — L'appareil génital du mâle adulte est constitué par plusieurs formations de différentes origines. Le testicule, les canaux

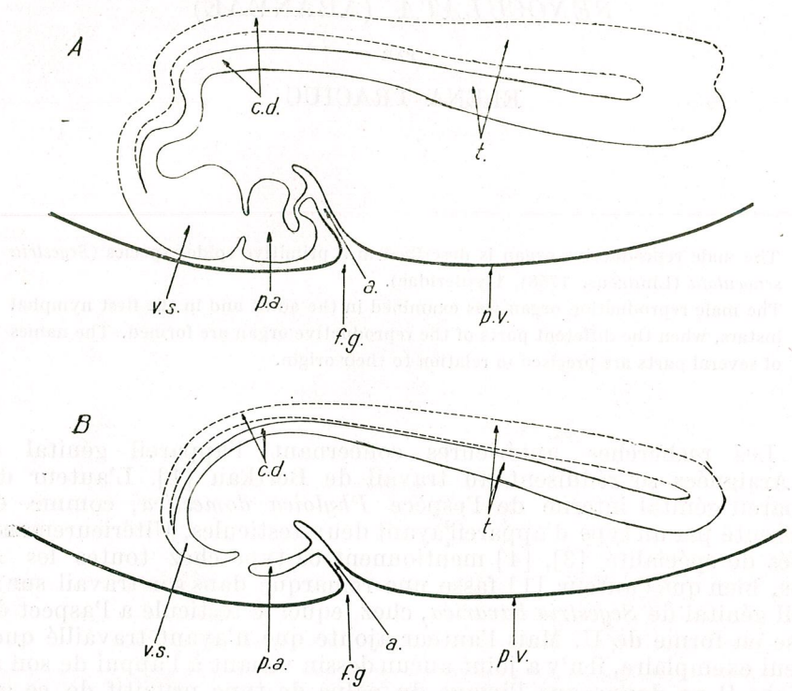


Fig. 1. — Vue latérale semi-schématique de l'appareil génital mâle de *Segestria senoculata*.
A. — Adulte.
B. — Nymphe, *phase III*.

déférents et la vésicule séminale proviennent de l'ébauche des glandes sexuelles. La poche atriale et l'atrium se forment par l'invagination de la paroi ventrale de l'abdomen.

Le testicule de *Segestria senoculata* a la forme d'un fer-à-cheval et est situé dans l'abdomen, sous l'intestin (fig. 1A). La courbure postérieure de celui-ci se trouve sous le cloaque et ses extrémités s'étendent jusqu'au tiers antérieur de l'abdomen où elles se prolongent par deux canaux déférents qui se recourbent antéro-ventralement en s'unissant pour former une poche qui s'étend sous la paroi ventrale de l'abdomen, devant la fente génitale (fig. 1A). Cette poche, qui peut être considérée comme étant une vésicule séminale, s'ouvre dans une autre poche que nous avons dénommée atriale, en tenant compte de son origine (fig. 2D). La poche atriale est

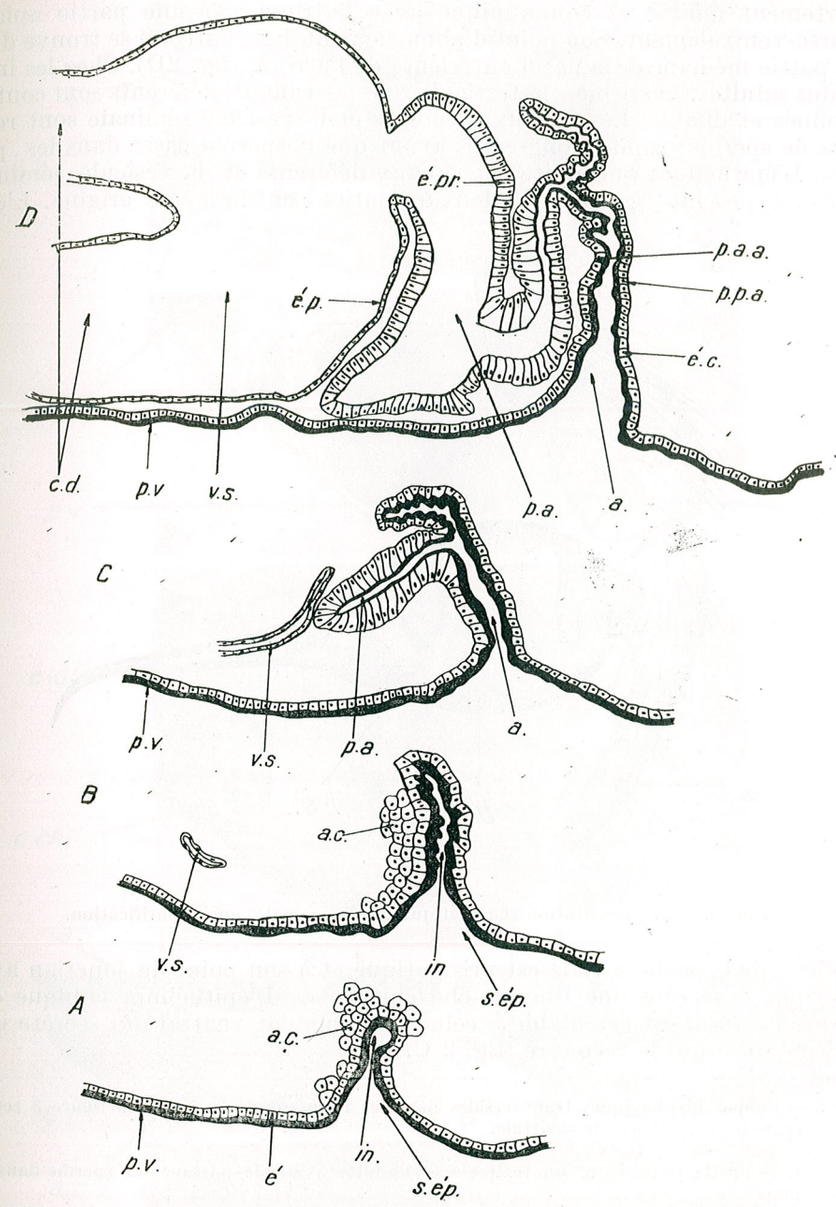


Fig. 2. — Les formations de l'atrium chez l'adulte ainsi que chez différents stades nymphaux (dessins à la chambre claire).
A. — *Ière phase du développement de l'appareil génital.*
B. — *IIe phase du développement de l'appareil génital.*
C. — *IIIe phase du développement de l'appareil génital.*
D. — Adulte.

fortement dilatée et communique avec l'atrium par une partie aplatie dorso-ventralement. Son point d'abouchement dans l'atrium se trouve dans la partie médiane de la paroi antérieure de l'atrium (fig. 2D). Chez les individus adultes, aussi bien le testicule que les canaux déférents sont contorsionnés et dilatés. Les canaux déférents et la vésicule séminale sont remplis de spermatozoïdes longtemps avant que le sperme passe dans les palpes. L'épithélium qui limite les canaux déférents et la vésicule séminale est de type plat (fig. 2D). Les deux formations ont la même origine. L'épithélium de la poche atriale est prismatique et à son point de jonction avec l'atrium il sécrète une fine couche chitineuse. L'épithélium cubique qui forme l'atrium est semblable à celui du tégument ventral qui sécrète une chitine dure qui le recouvre (fig. 2 C, D).

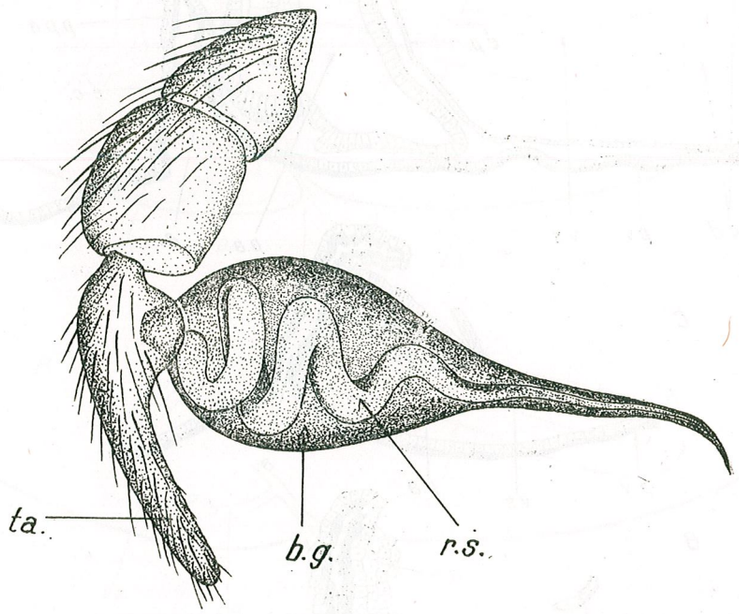
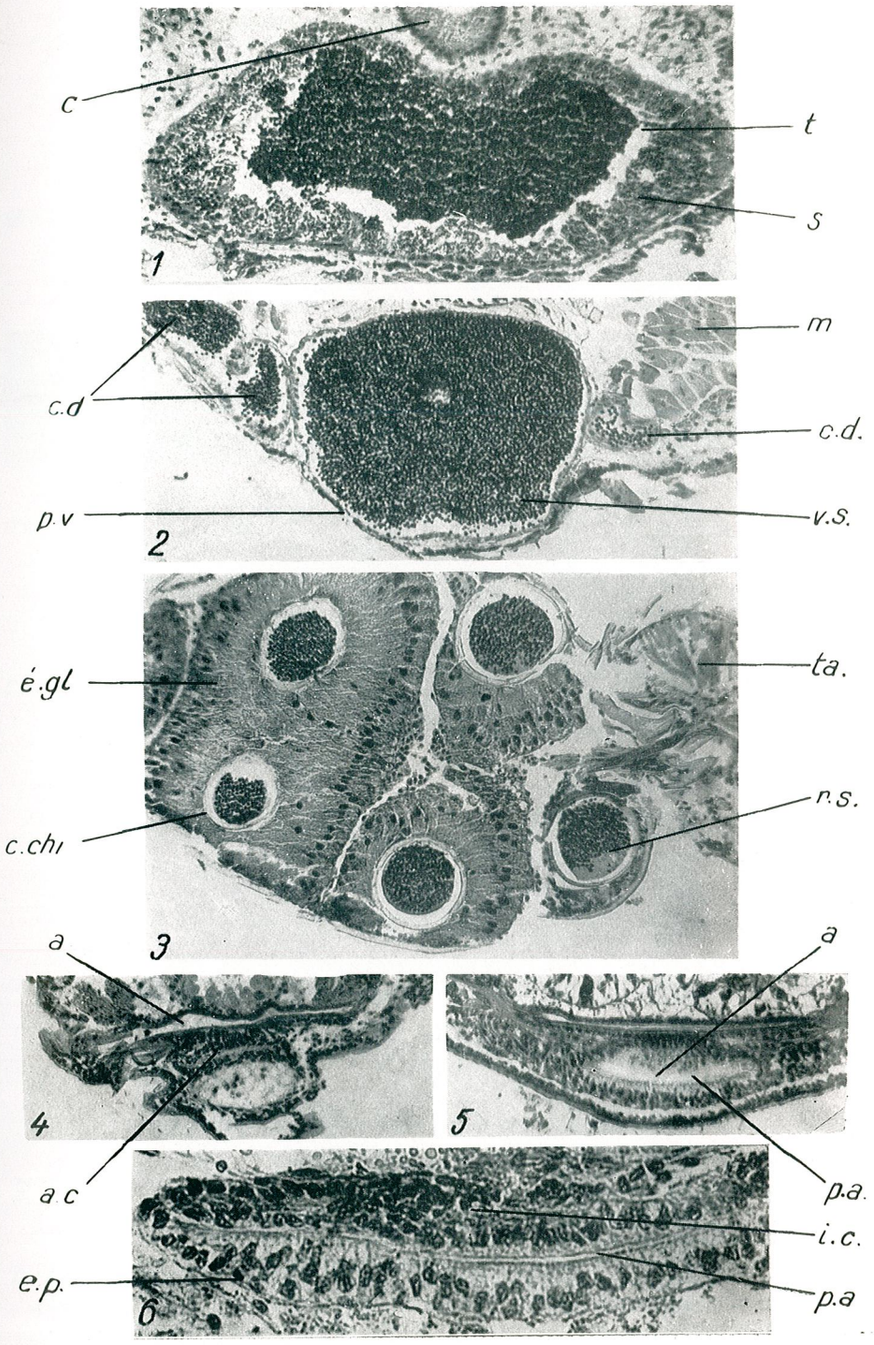


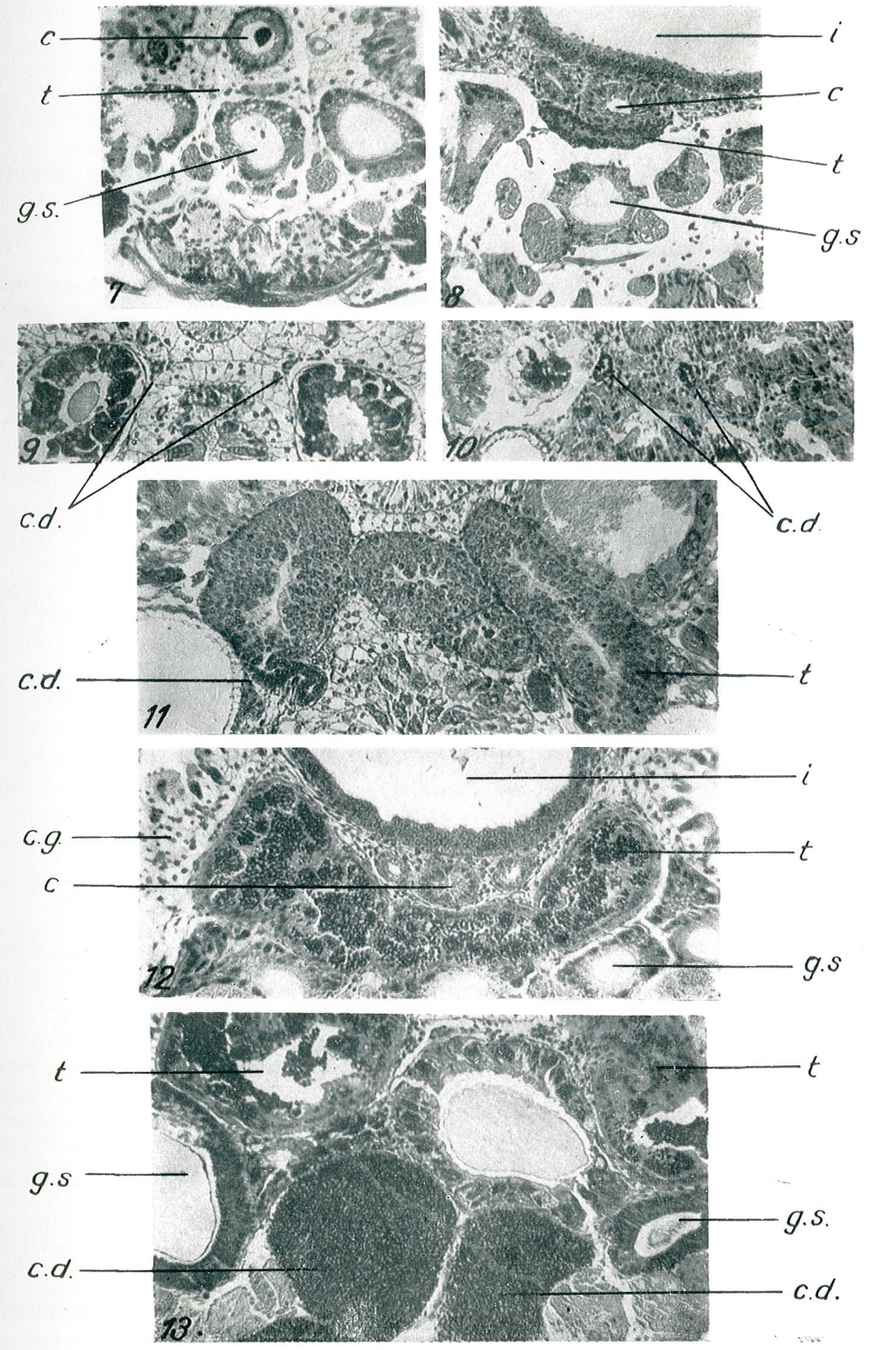
Fig. 3. — Représentation schématique du palpe mâle, après clarification.

Pl. I. — Coupes histologiques transversales dans les formations génitales. La figure 3 représente le palpe en coupe sagittale.

1. — Partie postérieure du testicule de l'adulte avant le passage du sperme dans les palpes.
2. — Dépôt de spermatozoïdes dans la vésicule séminale et les canaux déférents chez l'adulte.
3. — Section du palpe au niveau de la fonction avec le tarse.
4. — L'atrium chez les nymphes de la *IIe phase*.
5. — L'atrium et la poche atriale chez les nymphes de la *IIIe phase*.
6. — Infiltration des cellules de la vésicule séminale dans la paroi dorsale de la poche atriale (*phase III*).

PLANCHE I





Pl. II. — Coupes transversales dans le testicule et les canaux déférents.

7. — Le testicule chez la nymphe (*Ière phase*).
8. — Le testicule chez la nymphe (*IIe phase*).
9. — Les canaux déférents chez la nymphe (*Ière phase*).
10. — Les canaux déférents chez la nymphe (*IIe phase*).
11. — Le testicule et les canaux déférents chez la nymphe (*IIIe phase*).
12. — Spermatogenèse intensive dans le testicule chez la nymphe (*phase IV*).
13. — Dépôts de sperme dans les canaux déférents chez la nymphe (*phase IV*).

Le palpe est considéré comme un organe copulateur, étant modifié dans ce but. Un bulbe piriforme, partant du segment du tarse, s'achève par une partie aciculaire. Dans sa longueur, ce bulbe est traversé par un canal en spirale (trois tours de spirale) qui est plus large à sa base et se rétrécit vers son bout (fig. 3). La base de ce canal est en forme de cul-de-sac et son bout a un orifice minuscule. Sur tout son trajet ce canal est entouré par un épithélium glandulaire (Pl. I, 3) qui sécrète une couche de chitine qui le tapisse. Cette couche de chitine est traversée par des pores fins.

Les nymphes ne possèdent pas d'appareil génital complet.

Au premier stade de nymphe libre on observe seulement l'ébauche du testicule en forme de fer-à-cheval et les canaux déférents. Les cellules qui le constituent ne sont pas différenciées (quelques-unes se divisent). L'ébauche ne possèede pas de lumen (Pl. II, 7,9). La courbure postérieure du testicule a une position ventrale par rapport à la ligne médiane de l'abdomen, les canaux déférents se dirigeant vers cette ligne (fig. 1 B). C'est à ce stade que l'on remarque aussi la courbure antéro-ventrale de ces canaux.

Devant la future fente génitale apparaît une légère invagination du tégument (fig. 2A).

Donc, ce premier stade correspond à la première phase de développement de l'appareil génital. Les autres stades nymphaux ne se superposent plus aux phases de développement de l'appareil génital.

Chez les nymphes à la *II^e^ phase*, l'ébauche du testicule augmente et les canaux déférents s'élargissent (Pl II, 8, 10). L'invagination du tégument devant la fente génitale est plus profonde (Pl. I, 4a) et dans la partie antérieure de l'invagination on observe une agglomération de cellules provenant, probablement, de la multiplication de l'épithélium invaginé (fig. 2B).

A la *III^e^ phase*, le testicule présente déjà un lumen et les cellules qui se développent en direction de la ligne séminale ont le contour bien précisé (Pl. II, 11).

Les canaux déférents ont une paroi épithéliale qui délimite un lumen, ils sont recourbés antérieurement vers la paroi ventrale de l'abdomen en s'unissant pour former une poche qui, à ce stade, est fermée (fig. 2 C), c'est la future vésicule séminale.

Un fait important dans cette phase est que l'invagination de la fente génitale forme un atrium semblable à celui qui existe chez l'adulte (Pl. I, 5). A sa partie antérieure, la paroi de l'atrium, à son tour, subit une invagination médiane (fig. 1 B), qui ne prend pas la forme d'un tube, mais celle d'une poche aplatie dorso-ventrale (fig. 2c). L'épithélium qui limite l'atrium est cubique, celui de la poche atriale prismatique. Les cellules épithéliales de ces deux formations sécrètent une couche chitineuse qui les tapissent (fig. 2C). La partie antérieure, fermée, de la poche atriale, se trouve à proximité de la vésicule séminale formée par la jonction des deux canaux déférents, mais ne communique pas avec celle-ci.

La jonction de ces deux formations a lieu pendant la *IV^e^ phase de développement de l'appareil génital*, lorsque le trajet continu par lequel les éléments sexuels communiquent avec l'extérieur s'accomplit. Le bord posté-

rieur de la vésicule séminale avance vers la poche atriale, les cellules de la vésicule s'infiltrent parmi les cellules prismatiques de la poche atriale (Pl. I, 6). A ce stade, le testicule présente une intense spermatogenèse, le lumen étant rempli de spermatozoïdes. Ce qu'il faut relever au cours de cette phase c'est le rôle de dépositaire de spermatozoïdes qu'ont les canaux déférents. La différence de leur structure par rapport aux phases précédentes est remarquable (Pl. II, 12, 13). Nous mentionnons qu'à partir de la IV[e] phase jusqu'à l'état adulte (Pl. I, 1, 2) les canaux déférents gardent aussi bien leur structure que cette fonction de dépositaire.

DISCUSSION

A la suite de cette description, nous pouvons conclure que l'appareil génital interne du mâle de *Segestria senoculata* est constitué par un *testicule* en forme de fer-à-cheval, des *canaux déférents*, *une vésicule séminale*, *une poche atriale* et *l'atrium*. Ces formations sont d'origines différentes.

Afin de pouvoir dénommer avec précision et de manière adéquate les diverses formations il a été nécessaire de suivre leur développement et leurs fonctions. Depuis des exemplaires de la IV[e] phase jusqu'à l'état adulte, les canaux déférents et la vésicule séminale sont fortement dilatés et chargés de spermatozoïdes et, étant donné que cette constatation a été faite sur un grand nombre d'individus, nous pouvons tirer la conclusion que les formations mentionnées ont, pendant cette période, le rôle de dépositaire des spermatozoïdes. Il est probable que cette stagnation soit nécessaire en vue du fait que le dépôt du sperme (sur la toile), dans le but d'être absorbé par les palpes, se fait sous la forme d'une goutte qui s'élimine d'un coup.

La poche atriale est plissée chez l'adulte, ayant probablement le rôle d'un conduit qui peut se dilater et se remplir au moment de l'élimination du sperme.

Etant donné que le testicule de l'adulte se présente sous l'aspect d'une masse en U, il se pose le problème de sa formation.

Afin d'avoir de plus amples données à ce sujet, il a fallu étudier l'appareil génital aux premiers stades de développement postembryonnaire, lors de son apparition.

Il ressort de nos recherches que déjà du premier stade de développement, bien que le testicule soit sous forme d'ébauche, il est semblable à celui de l'adulte, c'est-à-dire l'ébauche se présente toujours sous la forme d'une masse en U.

D'ailleurs ce type d'appareil génital est assez fréquent chez les représentants d'autres ordres de la classe Arachnida, et les auteurs utilisent aussi bien l'expression « deux testicules soudés » que « un testicule en forme de U ». Nous avons utilisé le second terme car nous n'avons pas surpris au cours du développement postembryonnaire le moment de soudure. Il se pourrait que la soudure des deux testicules ait lieu au cours du développement ontogénétique de l'espèce respective, ou bien au cours de la phylogenèse à un certain moment. Nous pourrions tout aussi bien supposer qu'il

existe aussi la possibilité que l'espèce primitive étudiée ne possède qu'un seul testicule ayant la tendance à le séparer en deux testicules, sachant que les types évolués d'Araignées en possèdent toujours deux.

De ce point de vue, la discussion n'est pas close jusqu'à ce que nous tirions au clair certaines questions d'anatomie et d'organogenèse chez quelques familles d'Araignées que nous sommes en cours d'étudier.

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ABRÉVIATIONS

a, atrium ; a.c, agglomération de cellules ; b.g, bulbe génital ; c, cloaque ; c.chi, couche chitineuse ; c.d., canaux déférents ; c.g, cavité générale ; é, épithélium ; é.c, épithélium cubique ; é.gl, épithélium glandulaire ; é.p, épithélium plat ; é. pr, épithelium prismatique ; f.g, fente génitale ; g.s, glande séricigène ; i, intestin ; i.c, infiltration de cellules ; in, invagination du tégument ; m, muscle ; p. a, poche atriale ; p.a.a, paroi antérieure de l'atrium ; p.p.a, paroi postérieure de l'atrium ; p.v, paroi ventrale de l'abdomen ; r.s, réceptacle séminal ; s, spermatozoïdes ; s. ép, sillon épigastrique ; t, testicule ; ta, tarse ; v.s., vésicule séminale.

LE CYCLE ANNUEL DES CELLULES GLANDULAIRES DE LA VÉSICULE SÉMINALE CHEZ *ARMADILLIDIUM VULGARE* LATR. (ISOPODE TERRESTRE) DANS LES CONDITIONS CLIMATIQUES DE ROUMANIE

PAR

V. GH. RADU et C. CRĂCIUN

The authors started from the hypothesis that the secretory processes in the vas deferent of the Isopodes may have a cycle which phases are correlated with the spermatogenetic cycle. This is based on the functional connection between the final product of the spermatogenesis and the final product of the activity of the secretory cells of the vas deferent.

The results obtained in this work, based on morphological observations and biometrical dates, confirm our hypothesis. On the other hand, these reults open the perspective of some experimental reserches and a real interpretation for the Golgi apparatus morphophysiologic which in these cells, at the electronomicroscopical level, presents the morphological aspects in a variability not found till now.

Nous avons travaillé à plusieurs reprises [1], [2], [3] sur la structure cytologique des cellules glandulaires du canal déférent chez les isopodes terrestres, en insistant surtout sur l'appareil de Golgi, sur les aspects morphologiques de la sécrétion et sur les relations entre les deux sortes de cellules qu'on y voit, les « cellules prismatiques » ou « petites cellules » et les « grandes cellules », nommées aussi « cellules gigantesques ». Toutes les observations sur la structure fine de ces cellules ont été faites, naturellement, au microscope optique.

Récemment, nous avons repris l'étude cytologique de ces cellules au microscope électronique, étant donné que c'est un matériel de choix pour l'étude de l'appareil de Golgi et dès processus intimes de la sécrétion. Mais nous en avons obtenu des images tellement variées qu'il nous a été impossible, en tout cas extrêmement difficile, d'établir avec toute sûreté leur succession morphophysiologique réelle.

REV. ROUM. BIOL. – ZOOLOGIE, TOME, 16, N° 1, p. 29–37, BUCAREST, 1971

Pour mieux réaliser cette tâche, nous avons pensé que ce ne serait pas inutile d'établir tout d'abord le cycle, éventuellement les cycles sécrétoires de ces cellules et leurs relations avec le cycle spermatogénétique qui à son tour est conditionné par certains facteurs, entre lesquels, dans nos conditions climatiques, les saisons.

Sur le cycle annuel de la spermatogenèse nous avons déjà publié certains résultats [3], [4]. Nous y avons montré que chez les isopodes terrestres, dans les conditions du climat tempéré, bien que les processus de la spermatogenèse aient une durée assez longue, ils ne se répètent pas pendant le cycle annuel. Chacun des trois follicules de chaque testicule parcourt une seule fois par an le cycle entier de la spermatogenèse, mais entre les trois follicules il y a toujours un décalage chronologique de phase, on ne les trouve jamais dans le même stade spermatogénétique, ce qui prolonge l'activité spermatogénétique de l'individu pendant toute la saison chaude de l'année.

Il était intéressant de savoir s'il y a quelque corrélation fonctionnelle entre l'activité spermatogénétique et l'activité sécrétoire des cellules glandulaires du canal déférent, si cette activité comporte un seul cycle annuel ou si elle se répète plusieurs fois pendant une année.

Dans ce qui suit, nous présentons les résultats de nos premiers essais dans le sens indiqué plus haut dans la même espèce, *Armadillidium vulgare* Latr., dont nous avons étudié le cycle spermatogénétique.

MÉTHODE ET TECHNIQUE

Nous avons utilisé les mêmes préparations histologiques sur lesquelles nous avons étudié le cycle spermatogénétique [4]. Nous avons utilisé une méthode statistique sur le procédé suivant ;

Le canal déférent a été coupé toujours dans le sens longitudinal. Pour l'étude nous avons choisi la coupe sagittale, c'est-à-dire celle du milieu de l'organe, dans le but d'avoir sous le microscope des cellules dont la position soit perpendiculaire sur la membrane basale.

Sur les coupes ainsi choisies, nous avons délimité une portion d'épithélium longue de 2000 μ, située toujours approximativement dans le même endroit de la vésicule séminale.

Dans tous les cas nous avons compté les deux sortes de cellules et nous avons pris les deux dimensions, largeur et hauteur, que nous avons notées séparément dans un tableau pour chaque canal déférent.

Nous avons fait ces opérations sur des individus pris dans la nature à des intervalles de deux mois. Chaque fois nous avons pris plusieurs individus (de 2 à 7) et nous avons analysé un seul testicule par individu. Voici la liste de ces prélèvements :

février 2 individus	août 3 individus
avril 7 individus	octobre 4 individus
juin 5 individus	décembre 5 individus

Le tableau 1 reflète d'une manière assez claire le procédé que nous avons utilisé pour faire ressortir le plus nettement possible les variations numériques et dimensionnelles que subissent les deux sortes de cellules pendant une année. Nous attirons tout spécialement l'attention sur le fait que dans ce tableau nous n'avons pas introduit séparément tous les chiffres notés pour chaque canal déférent. Nous avons recouru à des chiffres moyens. Par exemple, pour les sept testicules que nous avons étudiés dans le mois d'avril, nous avons pris la moyenne des nombres des cellules prismatiques, la moyenne de leur hauteur maximum et minimum et de leur largeur, etc. La même chose pour les cellules géantes, la même chose pour les autres mois.

La fig. 1 représente l'ensemble d'une coupe longitudinale sagittale faite dans la région réniforme (vésicule séminale) du canal déférent et chacune des six autres, 2—7, représente un petit fragment de l'épithélium glandulaire étudié, microphotographiés au même grossissement.

RÉSULTATS

La variation du nombre des cellules. Une courte considération des chiffres de la ligne horizontale 3 du tableau 1 nous montre que la moyenne annuelle du nombre des cellules glandulaires qui apparaît dans une portion de 2 000 μ d'une coupe longitudinale est de 46, dont 21 cellules géantes et 25 cellules prismatiques ou, en pourcentage 45,7% géantes et 54,3% prismatiques.

Mais, durant une année, il y a des variations importantes. Nous examinerons ces variations en commençant par le mois de février, moment où, dans le climat de notre pays, l'activité physiologique des isopodes terrestres doit être la plus ralentie. Jusqu'au mois d'avril (fig. 2), le nombre total des cellules diminue de 11%[1], après quoi il augmente rapidement de 35% jusqu'aux mois de juin-juillet. Dès le mois d'août jusqu'au mois d'octobre le nombre des cellules diminue de nouveau fortement jusqu'à 30% pour remonter enfin, faiblement, de 7% jusqu'au niveau duquel nous sommes partis au mois de février.

Il est important de remarquer que les deux catégories de cellules coexistent toujours, que l'amplitude de leurs variations numériques, prise à part, est approximativement la même (fig. 3) et surtout que le rapport numérique entre les deux sortes de cellules C_g/Cp (cellules géantes : cellules prismatiques) est à peu près constant, variant entre 0,9 (juin) et 0,7 (octobre) et toujours avec la domination numérique des cellules prismatiques.

VARIATION DES DIMENSIONS DES CELLULES

La largeur moyenne annuelle des *cellules géantes* est de 54,4 μ et l'ampleur de la variation de 37%. La dimension maximum se trouve en octobre, elle diminue lentement pendant l'hiver, puis rapidement pendant le printemps jusqu'au minimum qui est atteint au mois de juin. Dans la période août-octobre elle remonte à son maximum. Les *cellules prismatiques* ont la largeur moyenne annuelle seulement de 35,3 μ, évidemment plus petite que celle des cellules géantes, et l'ampleur de la variation de cette dimension est de 40%, donc légèrement plus haute que celle des cellules géantes. Mais, chronologiquement, la variation en largeur des cellules prismatiques est à peu près inverse par rapport à celle des cellules géantes (voir le tableau et la fig. 3,5 b).

[1]) Pour que la représentation des variations soit vraiment fidèle à la réalité, nous avons rapporté l'ampleur de la variation au même chiffre, 46, qui est le nombre des cellules au mois de février. Il est intéressant que ce chiffre correspond exactement à la moyenne annuelle du nombre des cellules.

Tableau 1

Variation numérique des différentes dimensions des cellules glandulaires, sur une portion de 2000 μ d'une section longitudinale de la vésicule séminale chez *Armadillidium vulgare* Latr., pendant une année.

	Moyenne des données numériques \ MOIS		FÉVRIER	AVRIL	JUIN	AOÛT	OCTOBRE	DÉCEMBRE
1	Moyenne du nombre des Cg.		20,5	19	27	21,6	17,7	20
2	Moyenne du nombre des Cp.		25,5	22	30	25	25,5	24
3	TOTAL Cg. + Cp.		46	41	57	46,6	43,2	44
4	Valeur en %	a) Cg.	44 %	47 %	48 %	47 %	41 %	45 %
		b) Cp.	56 %	53 %	52 %	53 %	59 %	55 %
5	Largeur moyenne des cellules (en μ)	a) Cg.	57,5	53,7	41,6	53,3	61,6	58,9
		b) Cp.	33,4	42,9	28,9	34,8	36,2	35,5
6	Moyenne des hauteurs maxima (en μ)	a) Cg.	40,9	48,6	94,5	86,1	67,7	57,9
		b) Cp.	28,8	32,4	60,4	46,2	34,6	27,7
7	Moyenne des hauteurs minima (en μ)	a) Cg.	15,7	23,6	50,4	35,7	30,2	22,6
		b) Cp.	12,6	14,4	21,4	21,0	12,6	12,6
8	Hauteur moyenne des cellules (en μ)	a) Cg.	25,7	35,1	74,0	60,5	43,4	38,1
		b) Cp.	16,8	20,5	37,3	32,7	24,4	16,5
9	Moyenne de la surface totale des Cg. (en μ^2)		30.111	36.360	81.447	68.996	46.557	41.567
10	Moyenne de la surface totale des Cp. (en μ^2)		13.988	19.744	33.330	27.923	22.524	13.701
11	Somme des surfaces moyennes totales des Cg. + Cp. (en μ^2)		44.099	56.104	114.777	96.919	69.081	55.268

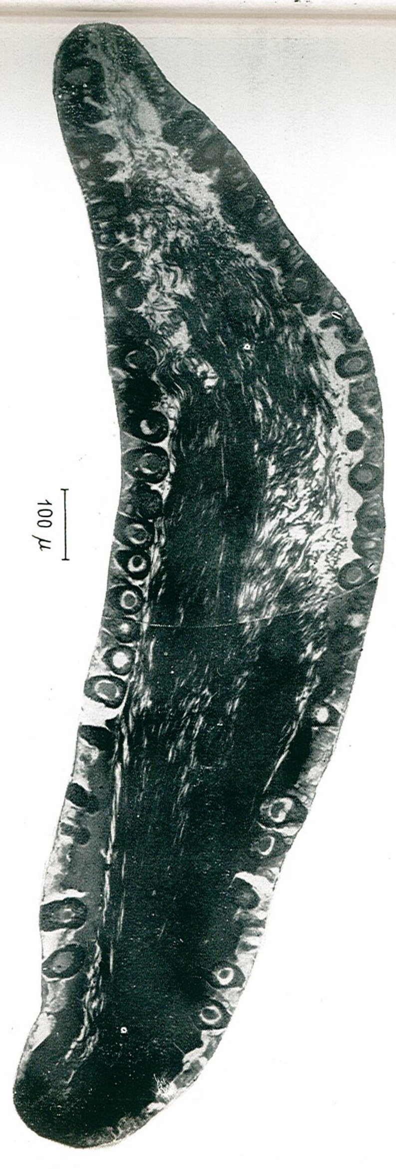

Fig. 1. — *Armadillidium vulgare* Latr. Section longitudinale de la région réniforme (vésicule séminale) du canal déférent (mois de juillet). Méthode : Kolatschev-Nassonov.

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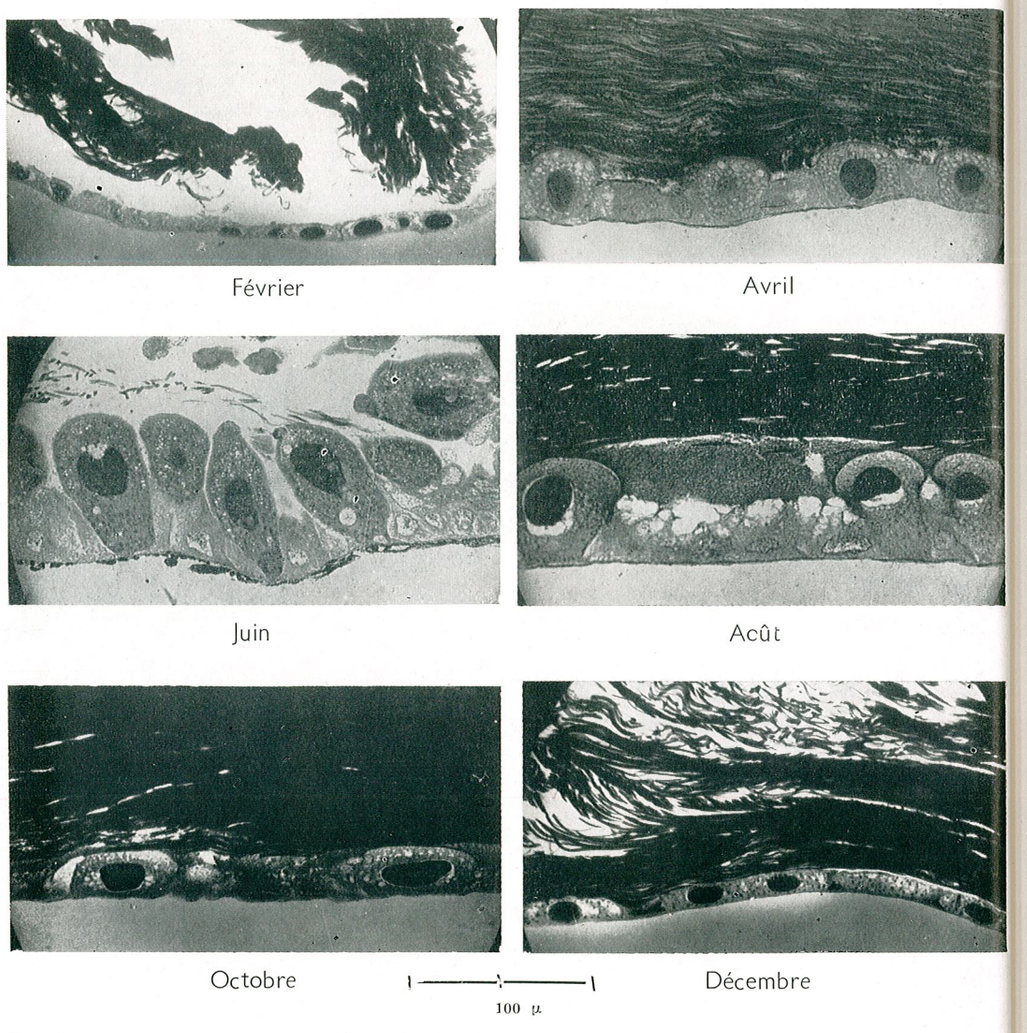

Fig. 2. — *Armadillidium vulgare* Latr. Section longitudinales de la région réniforme (vésicule séminale) du canal déférent. Différents aspects de la structure de l'épithélium glandulaire pendant un cycle annuel.

La hauteur moyenne annuelle des *cellules géantes* est de 40 µ donc sensiblement plus petite que la largeur. Pratiquement, on peut placer ces cellules dans la catégorie des cellules cubiques, mais la variation de leur hauteur est grande, atteignant une valeur de 121%, donc trois fois plus forte que celle de la largeur. La hauteur maximum, prise toujours comme valeur moyenne, se trouve au mois de juin, après quoi elle baisse lentement jusqu-au mois de février, où elle atteint le minimum. En traversant les mois d'avril et de mai, elle fait un véritable bond jusqu'à la hauteur maximum que nous avons trouvée en juin (voir le tableau et la fig. 3, 8a). Les *cellules prismatiques* ont la hauteur moyenne annuelle de 24,7 µ, donc presque la moitié de la hauteur des cellules géantes et en même temps bien plus inférieure que leur propre largeur. On pourrait donc les placer dans la catégorie des cellules plates, mais ici encore, comme dans le cas des cellules géantes, la hauteur varie de 126% (voir le tableau et la fig. 3, 8b).

On peut donc conclure, à ce chapitre, que les deux dimensions, hauteur et largeur, des deux sortes de cellules, ont une variation annuelle très prégnante et, bien qu'à des valeurs différentes, cette variation est évidemment parallèle, donc dans le même sens.

La *surface totale* des cellules, prise séparément pour les deux catégories de cellules, confirme très bien la variation des deux dimensions linéaires. Dans le cycle annuel, cette variation est de 170% pour les cellules géantes et de 143% pour les cellules prismatiques. Le sens de la variation est le même que celui de la hauteur, presque superposable, avec les mêmes périodes de maximum et de minimum, ce qui signifie que la variation de la surface et, en fin de compte, du volume des deux sortes de cellules glandulaires est déterminée par la variation de leur hauteur (fig. 3). D'ailleurs, il n'est pas difficile de s'imaginer que les choses ne peuvent pas se passer autrement. Dans leur développement dimensionnel, les cellules ne trouvent d'espace libre que vers la lumière du canal déférent, donc dans le sens de leur hauteur.

DISCUSSIONS

Les images et les données biométriques, transposées aussi en graphiques, que nous avons présentées dans ce travail, attestent, avec une claire évidence, l'existence d'un cycle dans l'activité sécrétoire des cellules glandulaires du canal déférent chez *Armadillidium vulgare*.

La variation numérique, d'une ampleur significative (39%), peut être interprétée uniquement dans le sens que, dans les processus de sécrétion, un certain nombre de cellules se détruisent et d'autres cellules prennent leur place.

Nous ne pourrions pas nous prononcer catégoriquement, dans ce travail, sur le mode de régénération de l'épithélium glandulaire, bien que nous possédions déjà certaines images assez significatives. Il paraît qu'il existe une période de multiplication en masse des cellules glandulaires aux mois de printemps (avril-juin).

Jusqu'ici, nos considérations se sont rapportées à deux catégories cellulaires que nous avons traitées séparément bien que, d'après l'opi-

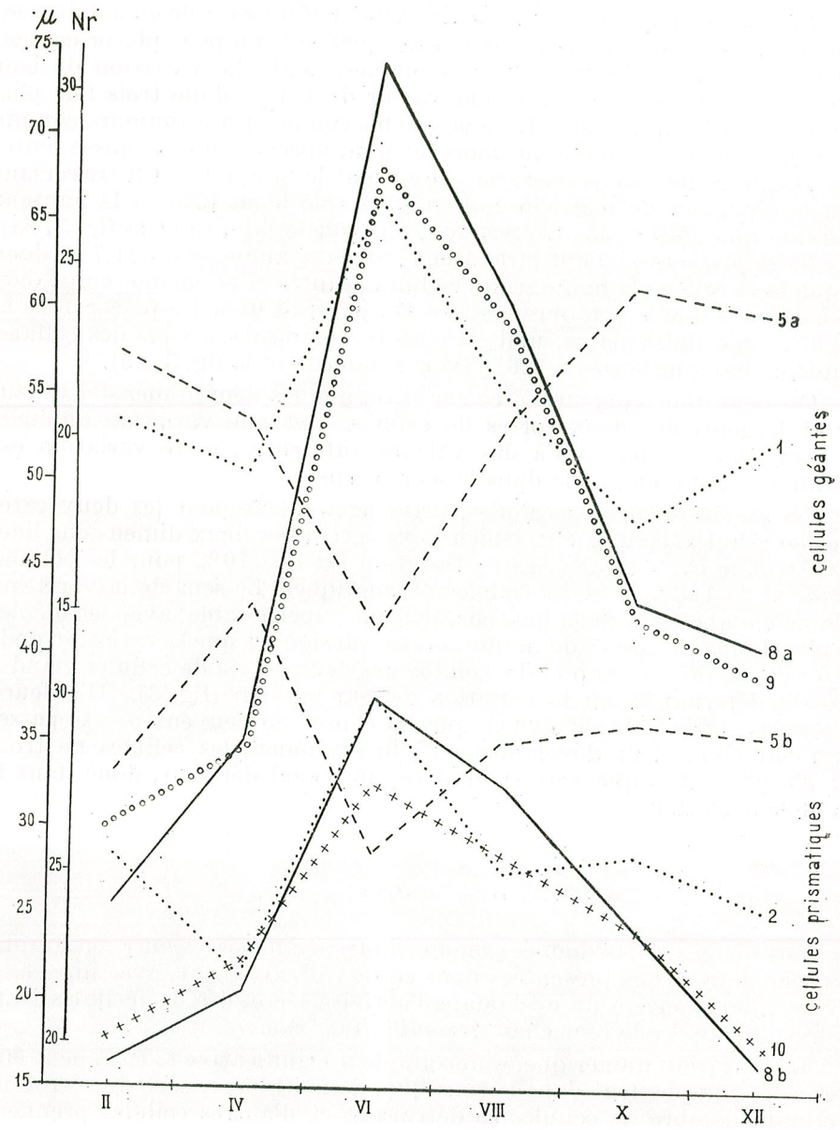

Fig. 3. — *Armadillidium vulgare* Latr. Graphiques représentant la variation saisonnière des différentes dimensions : nombre, hauteur, largeur et surface totale des deux sortes de cellules : cellules prismatiques et cellules géantes, de la région réniforme (vésicule séminale) du canal déférent.

..... = nombre des cellules.
– – – = largeur „ – „ –
——— = hauteur „ – „ –
ooooo = surface totale des cellules géantes
+++ = –„– –„– –„– prismatiques

Les numéros, accompagnés ou non d'une lettre, correspondent aux chiffres et aux lettres du tableau. Pour les graphiques 1 et 2 l'abscisse intérieure.

nion que nous avons exprimée dans nos travaux antérieurs, il n'y ait là qu'une seule sorte de cellules, mais qui, dans leur cycle sécrétoire, apparaissent sous deux aspects principaux : *petites, de type prismatique* au début de la phase préparatoire de la sécrétion, elles s'accroissent rapidement, surtout en hauteur et prennent une taille vraiment gigantesque (fig. 3).

Le passage d'un stade à l'autre se fait remarquer surtout par le fort développement de la bordure en brosse (microvili) et par la transformation du noyau qui, de forme irrégulière ou très irrégulière et pauvre en chromatine, prend, au fur et à mesure qu'il grandit, une forme régulière, sphérique ou ovoïde, très riche en chromatine. Quelque fois il est bien difficile de décider sur certains stades de transition si, dans le classement statistique, ils doivent être répartis du côté des cellules prismatiques ou celui des cellules géantes, ce qui pourrait introduire un peu de doute sur la valeur de la méthode statistique dans notre cas. De plus, dans la catégorie des cellules prismatiques ou petites cellules, ont été comptées aussi les cellules qui sont dans le stade d'élimination massive du produit de sécrétion, pendant lequel elles diminuent en hauteur, perdent leur bordure en brosse et leur noyau devient irrégulier et pauvre en chromatine. D'ailleurs, les graphiques de la fig. 3 expriment assez clairement ce fait. On voit ici que, dans la période juin-octobre le nombre des cellules géantes diminue de 45%, pendant que celui des cellules prismatiques ne diminue que de 17%, quoique pendant ce temps, la majorité des cellules prismatiques présécrétoire aient dû se transformer en cellules géantes. Nous pensons que le nombre relativement grand des cellules prismatiques dans cette période de vive activité sécrétoire est dû justement au fait que les cellules géantes, de par leur modalité d'élimination mérocrine du produit de sécrétion, reprennent l'aspect de cellules prismatiques en augmentant ainsi la proportion de celles-ci.

On peut donc exprimer le cycle sécrétoire des cellules glandulaires du canal déférent chez *Armadillidium vulgare*, de la manière suivante :

Cellules prismatiques jeunes
(petites, dans le stade présécrétoire)
↓
Cellules géantes
(stade d'élaboration)
↓
Cellules prismatiques vieilles (stade d'élimination massive, mérocrine, du produit de sécrétion)

Si, théoriquement, nous aurions eu la possibilité de poursuivre les mêmes cellules dans tous leurs stades de développement, nous aurions obtenu le graphique de la fig. 4 qui, après ce que nous avons dit plus haut, ne nécessite pas d'autres commentaires. Toutefois nous attirons l'attention sur la courbe qui représente la surface des cellules dont la partie finale, dans la phase d'élimination, se termine en pointillé vers le 0. Par cette

représentation en pointillé nous ne savons pas si à la fin de leur cycle sécrétoire les cellules reprennent un nouveau cycle ou disparaissent. C'est un problème que nous nous proposons d'élucider. Mais, le fait que pendant l'été le nombre total des cellules augmente sensiblement (voir le tableau et la fig. 3) doit signifier, d'après nous, que le contraire de cette

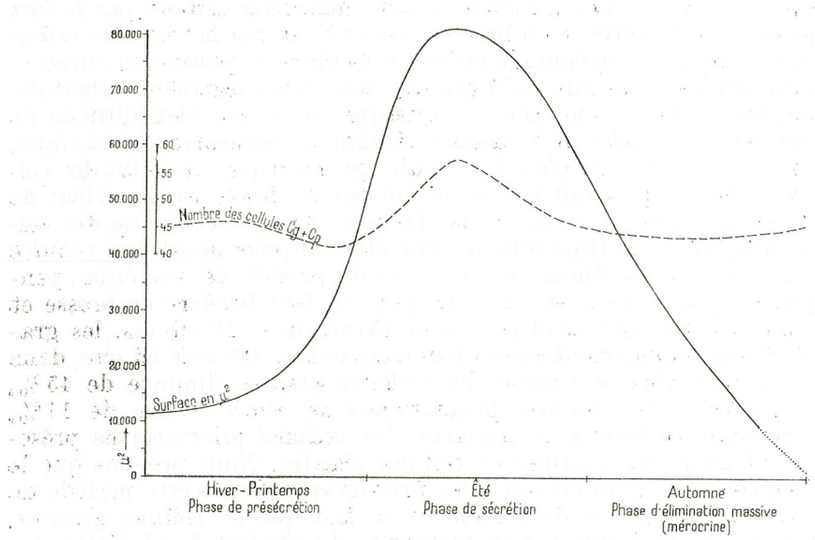

Fig. 4. — *Armadillidium vulgare* Latr. Graphiques syntéthiques concernant la variation du nombre et de la surface totale des cellules glandulaires, sur une portion de 2 000 μ de l'épithélium sécrétoire, pendant un cycle annuel. Cg. + Cp. =cellules géantes + cellules prismatiques.

multiplication ne peut être que la disparition, sinon de la totalité, au moins d'une partie du nombre des cellules qui ont fini leur cycle sécrétoire.

Un dernier fait sur lequel nous insisterons, c'est l'aspect le plus frappant qui caractérise morphologiquement l'épithélium glandulaire de la vésicule séminale chez *Armadillidium vulgare* : à aucun moment de son cycle sécrétoire cet épithélium n'a une constitution homogène ; il est formé constamment de « deux sortes » de cellules, géantes et prismatiques, toujours mélangées entre elles. Il est donc à conclure que, bien qu'il existe manifestement un cycle sécrétoire saisonnier, les cellules glandulaires n'ont pas une évolution synchrone dans les processus de la sécrétion.

De ce fait on peut tirer, sans doute, deux conclusions : 1) ces cellules glandulaires ont une période d'activité, un cycle propre plus court que le cycle général annuel ; 2) elles ne se développent et n'entrent pas en activité simultanément, à un moment donné du cycle annuel, mais par des poussées qui se succèdent plusieurs fois pendant le cycle général.

En liaison avec ce fait, nous rappelons que chez *Armadillidium vulgare* dans les trois paires de follicules testiculaires, les processus de

la spermatogenèse ne sont pas synchroniques, chaque paire de follicules se trouvant à un moment donné dans un autre stade spermatogénétique que les autres. Nous posons ici la question s'il n'y aurait pas une certaine relation de causalité entre ces deux processus rythmiques qui mènent à des finalités strictement contingentes (constitution des spermatozoïdes et la réalisation des conditions optimales pour leur vie et leur fonction biologique) et nous nous proposons d'en aborder l'étude.

Implicitement il sera nécessaire d'établir la modalité et le moment (ou les moments) de la multiplication des cellules glandulaires.

CONCLUSIONS

1. L'activité sécrétoire de l'épithélium glandulaire de la vésicule séminale chez *Armadillidium vulgare* se développe par des cycles annuels, comportant des variations saisonnières.

2. Le cycle d'activité glandulaire de cet épithélium concorde chronologiquement avec le cycle spermatogénétique, ce qui laisse à supposer l'existence d'une corrélation déterminante entre ces deux processus étroitement subordonnés l'un à l'autre.

3. La durée du cycle sécrétoire d'une seule cellule est plus courte que la durée du cycle annuel, ce qui implique l'apparition et l'entrée en fonction de nouvelles cellules pendant les phases actives du cycle annuel. D'ici résulte la constitution hétérogène (cellules petites-prismatiques et cellules géantes) de l'épithélium.

4. Certains indices donnent à penser que la multiplication et l'entrée en activité des nouvelles cellules se passent par poussées qui se répètent plusieurs fois, probablement trois fois, pendant le cycle annuel.

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Centre de Recherches Biologiques, Cluj
Section de Zoologie

SOME ELECTRON MICROSCOPIC OBSERVATIONS ON SPERMATOGENESIS IN *GRAPHOSOMA ITALICUM* MÜLL. (HEMIPTERA – PENTATOMIDAE)

BY

VIORICA TRANDABURU and T. TRANDABURU

Some successive aspects of the transformations occurring in *Graphosoma italicum* Müll. are presented, beginning with the secondary spermatocyte stage up to the spermatozoon. Thus, the mitochondria diffusely distributed in spermatocytes, merge and form the nebenkern in the spermatid. The latter, in its turn, divides into two mitochondrial bodies which in the mature sperm show a particular structure of the cristae. The single axial filament is characteristic but, occasionally, the presence of two axial filaments is recorded in the spermatozoon. The changes occurring in the constitution ot the nuclei are also studied. Observations are discussed in connection with the results of researches in other groups of insects.

Lately, our knowledge about spermatogenesis in insects has considerably increased, as a result of electron microscope studies. In this respect, the investigations of André [1], Baccetti and Bairati [2], Baccetti [3], Bairati [4], Bawa [5], David [6], Furieri [7], [8], Gatenby and Tahmisian [10], Sakai and Shigenaga [20] [21], Werner [23] [24], Yasuzumi and Oura [25] etc. should be mentioned, which contributed to the elucidation of certain problems regarding the morphological changes of various cell structures and of their space relationships during the phases of the spermatogenesis process.

With regard to the Hemiptera, important contributions were made by the studies of Furieri [7], Payne [17], Pratt [18], Tandler and Moriber [22], Werner [24].

In this context, the present paper deals with some aspects of the evolution of the male sexual cells in *Graphosoma italicum* studied at the electron microscope from the secondary spermatocyte up to the mature sperm stage.

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MATERIAL AND METHOD

Adult specimens of *Graphosoma italicum* Müll. were dissected in fixation solution unde the binocular and the testes were removed. Fragments < 1 mm³ were fixed 2 hours in a 2.5 % glutaraldehyde solution in 0.15 M phosphate buffer at pH 7.4. The material was washed thre times for 2 hours in phosphate buffer and then post-fixed in the same buffer to which 1 % OsO_4 was added (MILLONIG, 1962). The preparations were dehydrated in acetone and embedde in Vestopal W. The ultra thin sections obtained with an ultratome LKB were stained in an aqueou solution of uranyl acetate and contrasted with lead citrate (REYNOLDS, 1963). The grids wer examined in a JEM-7 electron microscope (80Kv).

RESULTS

Although most of our observations were centered on some aspects related to the spermiogenesis process we considered, however, advisable to include also some data on the changes occurring in the spermatocyte.

Most secondary spermatocytes in a seminal cyst are characterized by the presence of a large nucleus rich in chromatin. The nucleus contains a single nucleole and in its vicinity a mass of heterochromatin with a characteristic structure is observed (Fig. 1, **1**). Numerous mitochondria with a few cristae are spread in the cytoplasm. Ribosomes are abundant, aggregated in clusters or arranged as polysosomes. The smooth endoplasmic reticulum is poorly developed, the tubules having a very narrow lumen.

At the end of telophase II, in spermatid, mitochondria are grouped at one pole of the nucleus and, subsequently, they increase in volume and become branched. Their agglomeration and fusion gives rise to the classical nebenkern. In this stage (Fig. 1, **2**), similar to that of "peloton" described by ANDRÉ [1], mitochondria are very intricately twisted and inside them tubules are distinguished with granules on their surface. The other cytoplasmic constituents of the early spermatid are rather scarce and poorly developed. However, the presence of ribosomes, of some smooth endoplasmic reticulum with its narrow tubules, of dyctiosomes and of autophagosome-like formation is noticed. The nucleus is spherical, with the condensed chromatin, under the form of a thin layer at the periphery or agglomerated in a few clusters.

In the more advanced spermatid the nucleus becomes elongated (about 4 times the cross diameter), whereas the heterochromatin having a peripheral arrangement, forms a continuous osmiophilic layer (Fig. 2, **3**).

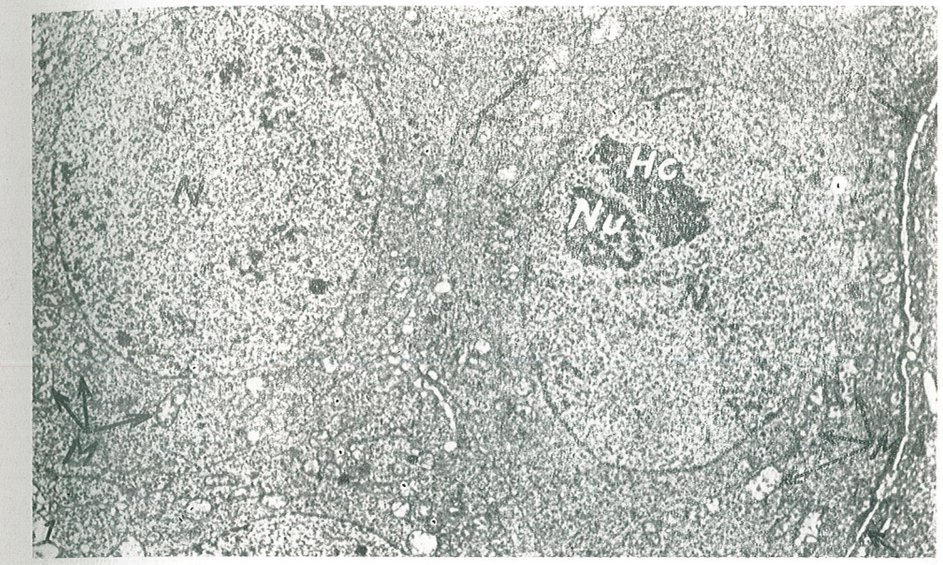
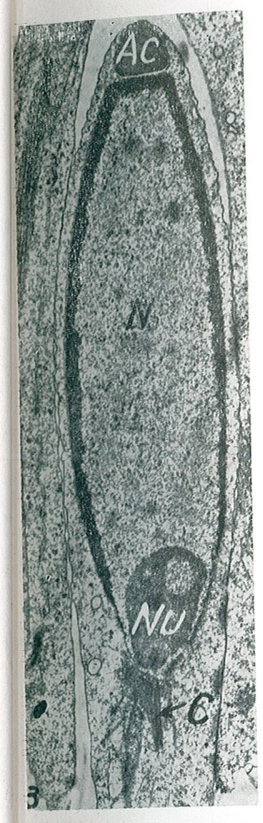
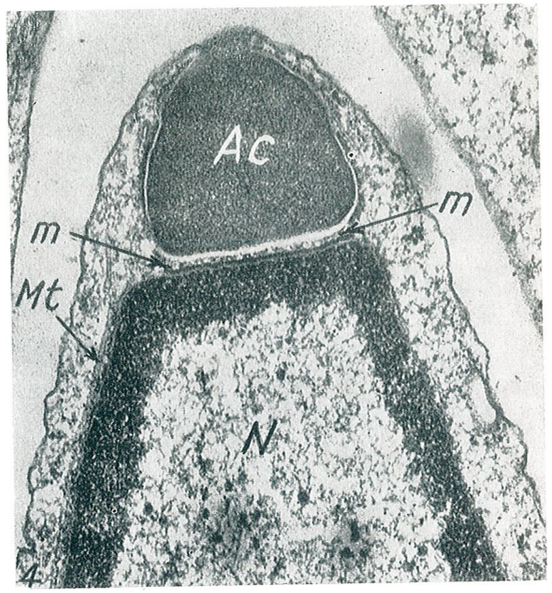


Fig. 1. — **1**: Secondary spermatocytes. Their nucleus (*N*) contains one nucleole (*Nu*) and a mass of heterochromatin (*Hc*). The cytoplasm contains numerous mitochondria (*M*) and ribosomes (*R*). Prolongations of the nutritive cells (*P*) can be observed. × 5.560. **2**: Young spermatid with Nebenkern constituted (N_k). In the cytoplasm, the tubules of the agranular endoplasmic reticulum (*Ar*) may be distinguished, as well as ribosome aggregates and an autophagosome (*F*). × 18.700.

Fig. 1. —





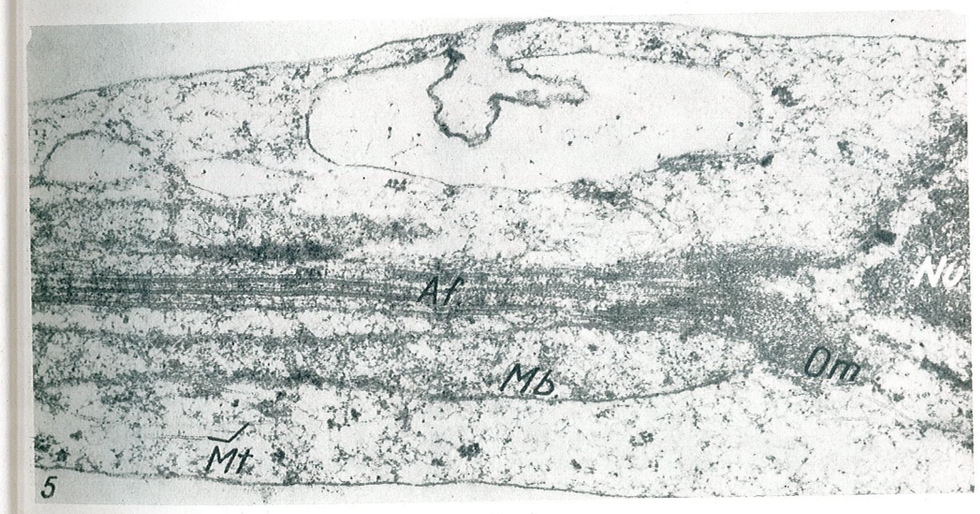
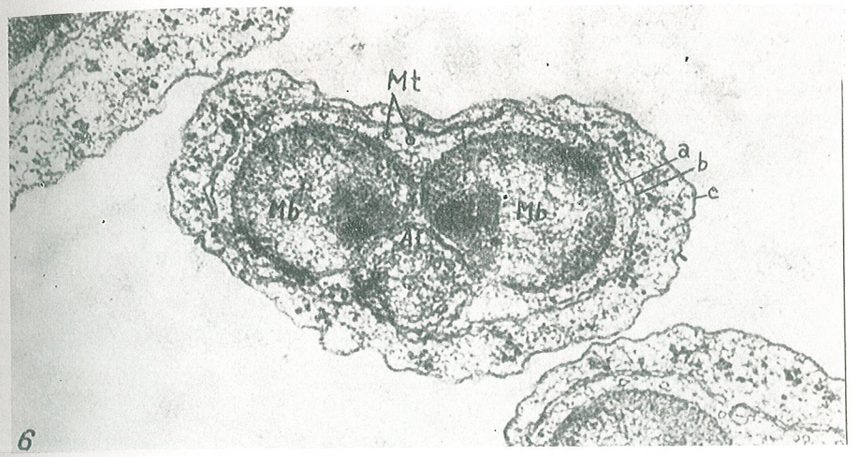
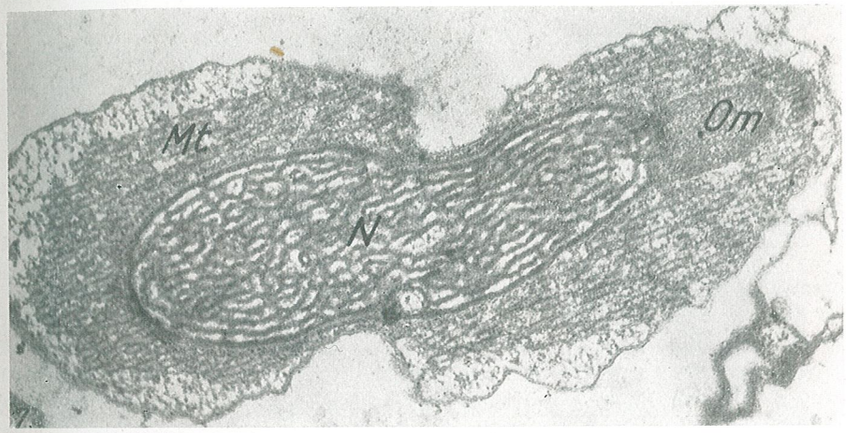


Fig. 2. —

Fig. 2. — **3** : General view of the head of a more advanced spermatid, sectioned longitudinally. The nucleus is elongated ; the acrosome is seen in its anterior part (*Ac*), while the nucleole and the centriole (*C*) in the posterior part. × 12.000. **4** : Details of the anterior part of the same spermatid. Between the acrosome and the nucleus a membrane (*m*) can be distinguished. The microtubules (*Mt*) are near to the surface of the nucleus membrane. ×43.500. **5** : — Longitudinal section through the tail of a spermatid. The transversal osmiophilic stripes of the axial filament (*Af*) can be observed, as well as the presence of the osmiophilic mass (*Om*) of the two mitochondrial bodies (*Mb*) and of the microtubules. × 32.200.





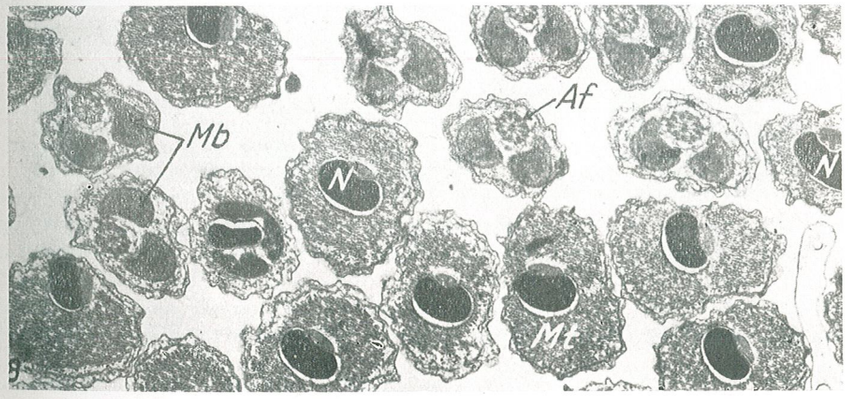


Fig. 3. —

Fig. 3. — **6** : Cross section through the tail of a spermatid. The arrangement of the mitochondrial bodies and of the microtubules in relation to the axial filament (*Af*) can be seen. These structures are, in turn, surrounded by three plasmalemmas (*a*, *b*, *c*). × 95.500. **7** : Head of an advanced spermatid. The nucleus contains thick fibers oriented longitudinally ; numerous microtubules exist in the cytoplasm. × 44.600. **8** : Almost mature spermatozoa cross sectioned at various levels. The compact and osmiophilic content of the nuclei should be noted. × 17.400.

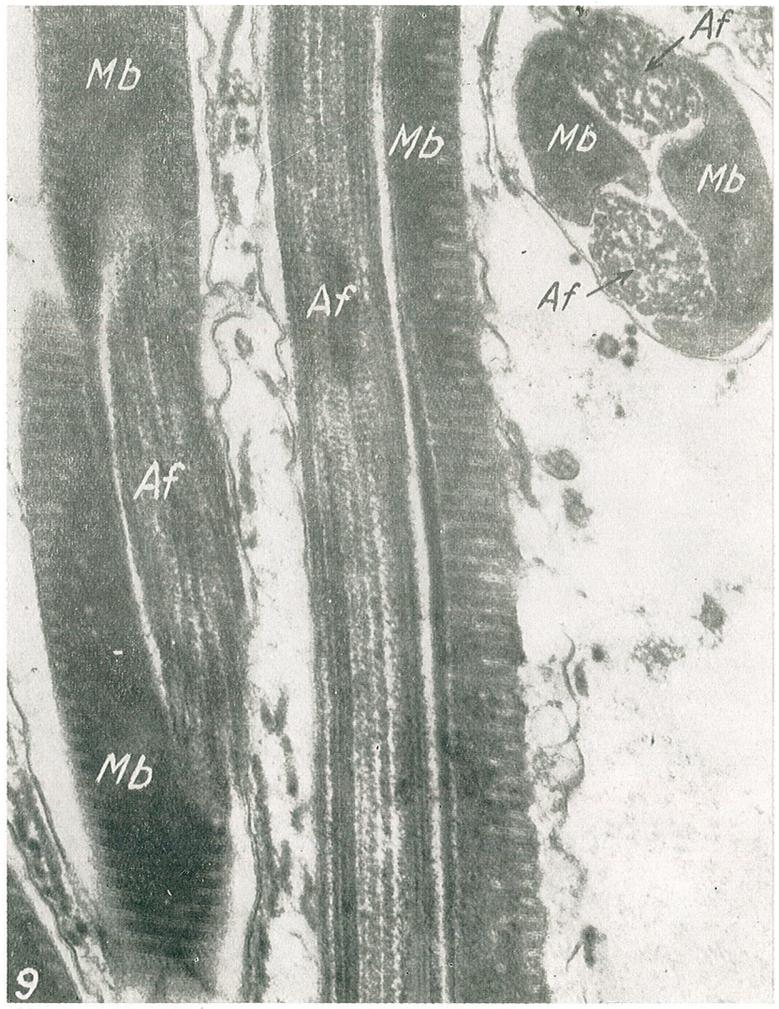


Fig. 4. — **9**: Spermatozoon tails, showing the structure of the cristae of the mitochondrial bodies. Above, at the right, a tail with two axial filaments. × 61.250. Same legend as figs 1—3.

The condensation is less pronounced towards the posterior pole of the nucleus where the nucleole is located. The acrosomal vesicle is situated at the anterior part of the nucleus. The acrosome surface oriented towards the nucleus is level and in the space between it and the nucleus a membrane is observed (Fig. 2, **4**), resembling the interstitial membrane described by KAYE [13] in the house cricket. The acrosome gradually extends on one side of the nucleus. The centriole may be seen at the posterior portion of the nucleus (Fig. 2, **3**). Beside it there is a finely granular osmiophilic zone, resembling that described by WERNER [24], in *Nepa rubra*. The axial filament presenting osmiophilic cross stripes proceeds from the centriole (Fig. 2, **5**). The two mitochondrial bodies with no special structure, resulting from the division of the nebenkern, extend along the axial filament. In a cross section microtubules orderly disposed in a single row are observed around mitochondrial bodies (Fig. 3, **6**). These structures are surrounded by two plasmalemmas with varying spaces between them. On their outer side there is a cytoplasm layer, sometimes rather thick, in its turn delimited by a third plasmalemma. In the cytoplasm layer, between plasmalemmas *b* and *c* (Fig. 3, **6**), autophagosome-like formations as well as elements of the smooth endoplasmic reticulum and ribosomes are occasionally visible. This layer might represent either lamellar prolongations of the nutritive cells, or the cytoplasmic remnant of the spermatid which is to be eliminated.

In a still more advanced stage, structural changes of the chromatin occur in the spermatid nucleus. Thus, at first thick filaments (Fig. 3, **7**) are observed, which subsequently fuse into lamellae, so that finally, due to their maximum condensation, the nuclei appear homogenous and dense (Fig. 3, **8**).

In the mature sperm we can see that both mitochondrial bodies acquire a characteristic structure. In each body a dense, osmiophilic matrix, structureless or sometimes with a fibrillar structure can be detected. Lamellar expansions, in the form of continuous circular cristae penetrating superficially in the matrix, derive from the membrane of the mitochondrial body (Fig. 4, **9**). The cristae and the matrix between them are 250Å thick. Two osmiophilic stripes, each of a thickness of 60 Å, are distinguished in the crests.

DISCUSSIONS

The nuclear changes occurring in the spermatid were previously described by André [1], Baccetti and Bairati [2], Gall and Bjork [9], Grassé [11], Kessel [15], Werner [23], [24] and others. These changes have also been recorded in the species studied by us. Thus, in the early spermatid with a spherical nucleus, the chromatin is finely dispersed. As the nucleus elongates, its contents becomes fibrous, then the fibers become associated in the form of lamellae oriented longitudinally to the longer axis of the nucleus and, finally due to the utmost condensation of the chromatin, the nucleus appears perfectly homogenous.

The phases of the nebenkern formation have been described by André [1] in Macroglossidae and Pieridae and by Pratt [18] in *Murgantia*

histrionica. We however, emphasize the fact that in the case of *Graphosoma italicum*, after the division of the nebenkern into two mitochondrial bodies, these latter show in the spermatozoon a characteristic infrastructure. Two osmiophilic stripes, of 60 Å thick, are found within the mitochondrial cristae. Other authors, such as André [1] in Pieridae, and Macroglossidae, Furieri [7] in *Pyrrhocoris apterus*, Herold and Münz [12] in *Peregrinus maidis* spermatozoa and Werner [23] in *Cicindella campestris* described a specific structure of the mitochondrial bodies which, however, is not the same as that described by us.

The abundant microtubules occupying almost the whole cytoplasm of the head region of the spermatid play a role in modifying the form of the nucleus during spermiogenesis, as suggested by other authors too: David [6], Kessel [14], [15], Werner [24], etc. Tandler and Moriber [22] have described them in *Gerris remigis*, also around the acrosome and within it, these maintaining its rigidity but having also the role of transporting the synthesized proteins and the polysaccharides. We were unable to detect microtubules within the acrosome, but only some microtubules extending from the surface of the nucleus towards the acrosome.

Kessel [14] [15] mentions that as the spermiogenesis process is advancing, the microtubules in the head region disappear and agglomerate in the midpiece and in the tail, in the mature sperm having a role of support and motility. As regards the microtubules observed by us around the mitochondrial bodies, we consider that they could also play a role in their elongation along the axial filament.

CONCLUSIONS

1. The secondary spermatocytes have a large nucleus rich in chromatin and numerous diffusely spread mitochondria in the cytoplasm.

2. Within the spermatid, the mitochondria are agglomerating at one pole of the nucleus, coalescing and forming the nebenkern.

During the evolution of the spermatid, the nucleus undergoes several changes both as regards the form and structure of the chromatin. Thus, a transition is noted from the spherical form having the chromatin arranged peripherally in a thin layer, to the elongated form with condensed chromatin, the nucleus becoming homogenous and dense.

3. In the spermatozoon, characteristic is the structure of the cristae of the mitochondrial bodies. The axial filament is single but occasionally the presence of two axial filaments is recorded.

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ON THE STRUCTURE OF THE ADRENAL IN SOME INDIAN SNAKES

BY

J. H. SABNIS

There has been increasing recognition recently of the importance of the adrenal function in non-mammalian vertebrates. The monograph "The adrenal cortex" by Chester Jones, which appeared in 1957, has been particularly important in this somewhat belated recognition.

The anatomy of the adrenal glands of snakes has received little attention except for the brief descriptions of Janequiera (1944), Benzedeczky et al [1]. Sabnis [9] has described the structure of the adrenal in *Naja naja* and *Natrix piscator*. This study was made in order to contribute further information on the structure of the adrenal in the primitive and specialized snakes.

MATERIAL AND METHOD

This study is based on the examination of specimens of each of the following species

Ophidia	Family :	Typhlopidae — *Typhlops*
	,,	Boidae — *Python molurus*
	,,	Viperidae — *Echis carinatus*

The specimens were collected in the vicinity of Nagpur. In all the cases, the tissues were fixed in neutral formalin, dehydrated in graded alcohols, embedded in paraffin wax and sectioned at 5 to 10 microns. Sections were stained either with Ehrlich's haematoxylin and counterstained with eosin, or with Masson's trichrome method.

OBSERVATIONS

Of the existing families the Typhlopidae are of a particular interest. Typhlops and its allies are small worm-like creatures. They are specialized

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for burrowing life. In spite of their high degree of specialization the Typhlopidae have been regarded as primitive snakes. The Boidae, though also retain vestiges of the pelvis, possess many of the characters of the more advanced and typical snakes. The Viperidae are in many ways the most specialized of all poisonous snakes. It is hoped that a study of the

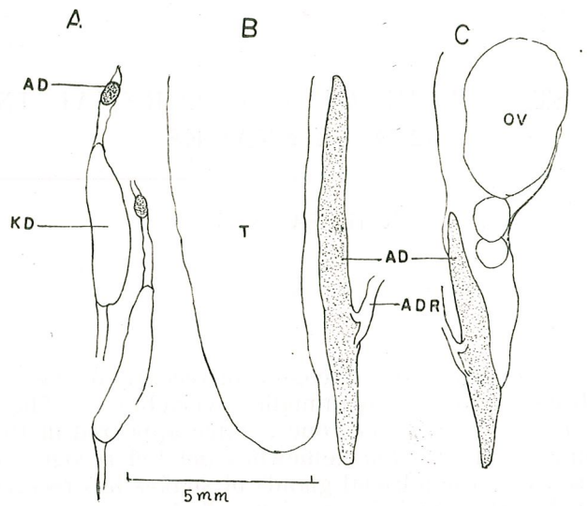

Fig. 1. — Diagram of the adrenal of *Typhlops* (*A*), *Python molurus* (*B*) and *Echis carinatus* (*C*). *AD*, adrenal; *ADR*, adrenal artery; *KD*, kidney, *OV*, ovary; *T*, testis.

adrenal structure of some oriental snakes will add to our limited knowledge of them.

Considering the shape, the adrenals of snakes may be roughly divided into two kinds: the typical elongated and round. The adrenal of *Python molurus* and *Echis carinatus* are elongate bodies with tapering ends and are situated near the caudal end of the gonad. They are enveloped in mesovaria or mesorchia (Fig. 1). A similar arrangement of the adrenal has been observed in *Naja naja* and *Natrix piscator* [9]. In *Typhlops* the adrenal is a round body and not associated with the gonad but is situated near the cranial end of the kidney (Fig. 1).

HISTOLOGY

In *Typhlops* and *Echis carinatus* the adrenal is composed of a ventral interrenal and a dorsal chromaffin encapsulated component (Fig. 2 *A* and 2 *C*). The chromaffin region is approximately one third as thick as the interrenal tissue. It is usually thickest midcentrally, but is thin at each pole. The chromaffin tissue is compact and not arranged in cords as in the case of the interrenal substance. In *Python molurus* the dorsal chromaffin

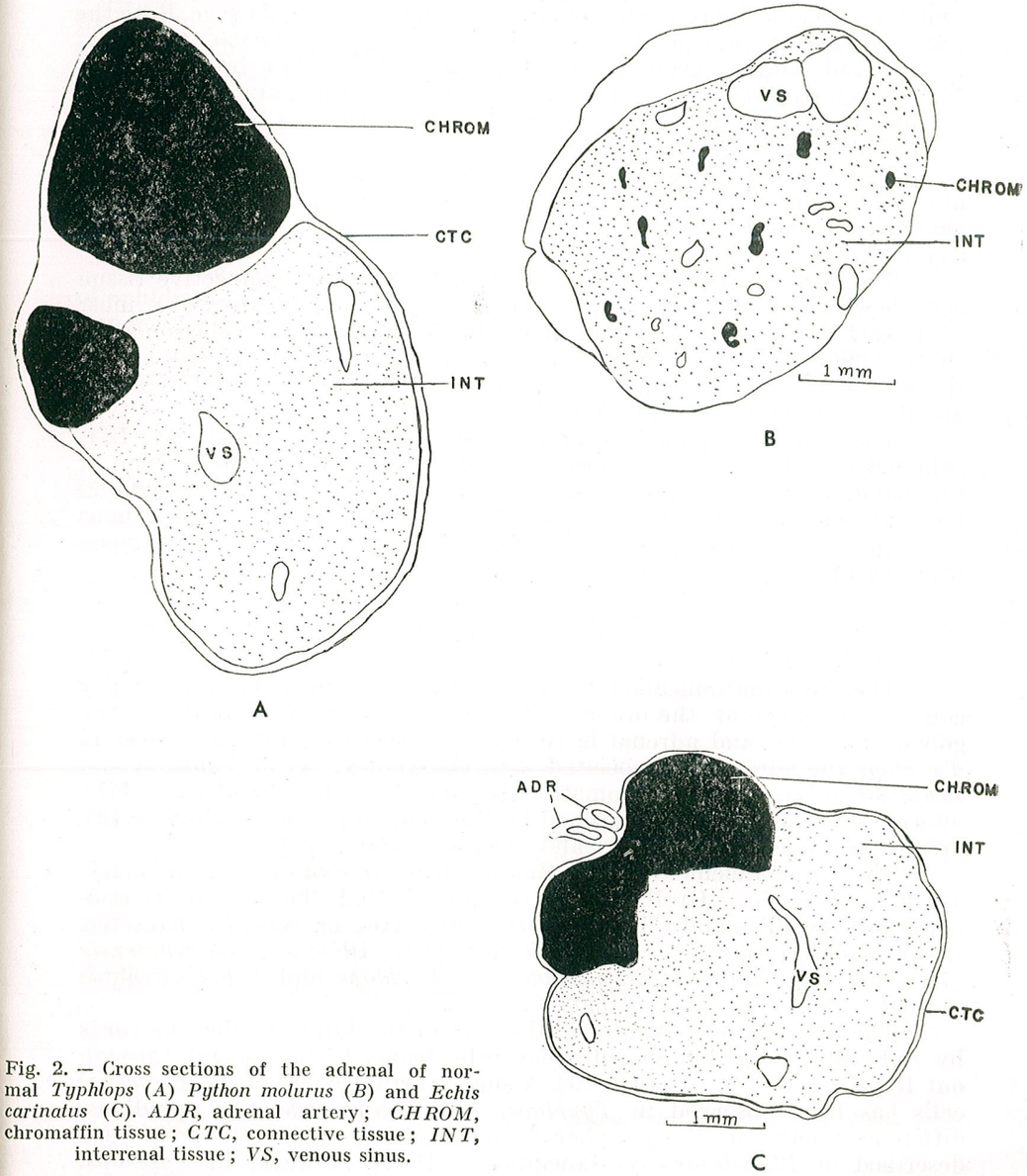

Fig. 2. — Cross sections of the adrenal of normal *Typhlops* (*A*) *Python molurus* (*B*) and *Echis carinatus* (*C*). *ADR*, adrenal artery; *CHROM*, chromaffin tissue; *CTC*, connective tissue; *INT*, interrenal tissue; *VS*, venous sinus.

encapsulating portion is absent. Single cells or small groups of chromaffin tissue are frequently seen between the interrenal cords. The adrenal elements in *Python* are more mixed and hence avian in character (Fig. 2*B*).

The cromaffin tissue is composed of two types of cells, one peripheral and the other one more central or adjacent to the interrenal tissue. Both the cell types are low polygonal (9 μ in *Typhlops*, 12 μ in *Python* and *Echis*) in size and contain granules with characteristic yellow brown colour. The nucleus of all types is 4 to 5 μ in diameter. It is interesting and perhaps significant that the acidophilic chromaffin type cells are mixed with the interrenal cells.

The interrenal tissue is composed of irregularly anastomosing cords of radially columnar cells separated in *Typhlops*, *Python* and *Echis* by venous channels. The cord is about 36μ in diameter in *Typhlops*, 54μ in *Python* and 66μ in *Echis*.

The adrenal gland is enclosed completely by thin connective tissue capsule which is in intimate contact with a supporting mesentery over most of its surface. Collagenous reticular elastic fibers stained by Masson's trichrome method, are clearly visible in the capsule, in the adrenal and in the wall of the vascular sinuses and blood vessels. Collagenous fine fibrils are also seen among the cells.

The adrenal is pink in colour because of its high vascularity. It is supplied by relatively large adrenal artery entering the dorsal portion of the gland. Its arterial supply consists of an extensive subcapsular arterial plexus from which the blood passes into the vascular sinuses. The venous drainage is accomplished by way of a series of coalescing vascular sinuses draining into the large vein.

DISCUSSION

The gross anatomical relationship of the adrenal in ophidia seems fairly constant throughout the order, though the supporting mesenteries of the gonad, gonoduct and adrenal have not yet been described. However in *Typhlops* the adrenal is associated with the kidney. As in *Typhlops* and *Echis carinatus*, a dorsal chromaffin cap overlying the ventral mass of the interrenal tissue has been described in *Naja naja* and *Natrix Piscator* [9], *Gerrhonotus multicarinatus* [8] and *Xantusia vigilis* [5].

The adrenal elements in *Python molurus* are more mixed and hence avian in character. Grollman [3] considered that the adrenal components in Crocodiles and Chelonia were more mixed or avian in character. However descriptions of *Emys orbicularis* [10], *Alligator mississippiensis* [7], *Naja naja* and *Natrix piscator* [9], *Typhlops* and *Echis carinatus* present a picture similar to that of lacertilia.

The arrangement of interrenal tissue in the form of tube like cords by radially arranged high columnar cells seems to be general through out Reptilia [5], [6], [7], [9], [10]. A similar arrangement of the interrenal cells has been observed in *Typhlops*, *Python* and *Echis*. In ophidians, differences between the peripheral and centrally lying cells have been described in *Philodryas* by Janequiera, (1944). However in *Typhlops*, *Python*, *Echis* and most of the other ophidians so far studied, only one morphological type of interrenal cell has been described.

The arrangement of chromaffin cells in irregular compact masses is similar in Reptiles. Cromaffin cellular differentiation has been reported in several species of reptiles. Two medullary cell types are usually described, both chromaffin in reaction. In *Typhlops* and *Echis* eosinophilic cells are limited to a distinct zone adjacent to the interrenal elements. This arrangement has been described in *Alligator* [7], in *Xantusia vigilis* [5], in *Naja naja* and *Natrix piscator* [9]. Histochemical and physiological studies are now in progress to investigate the structure and arrangement of these cells among ophidia.

CONCLUSIONS

1) The adrenals of *Typhlops* and *Echis carinatus* are similar to those of ophidians. They compose dorsal medullary and ventral interrenal components.

2) The adrenal of *Python molurus* is more mixed and hence avian in character.

3) The interrenal tissue is composed of high columnar cells radially arranged in cords.

4) The stroma of the snake's adrenal consists of the collagenous reticular fibers.

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A FURTHER STUDY OF THE ACTIVITY OF *MONODONTA LINEATA* (DA COSTA) BY MEANS OF AN AKTOGRAPH

BY

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A new aktograph for studying the activity of *Monodonta lineata* in and out of water is described. A direct relationship between activity and temperature was found to exist. Light does not seem to be an essential factor as activity persists even in the dark. Experimental results related the ability of this animal to respire oxygen better in air than in water between 13°—14° and 24°C and vice-versa below and above this temperature range and with its subaerial mode of existence on the shore.

Previous tests carried out on *Monodonta lineata* by means of an aktograph [2] revealed a direct relationship between the activity of this snail in relation to temperature between 8° and 16°C. Elsewhere [1] it was also noticed that its aerial rates of oxygen uptake exceeded the aquatic ones between 13°—14°C and 24°C and that some activity took place in the dark.

The purpose of the present investigation was to extend the previous study of the activity of *Monodonta* in relation to temperature. Tests were carried out at temperatures between 3.5°C and 24°C. It was also set out to investigate (a) whether any relationship might exist between the type of activity here studied and the rates of aerial and aquatic respiration at different temperatures and (b) the activity of this snail in the dark.

MATERIALS AND METHODS

To study these problems, an aktograph was devised to reproduce on a smoked drum all activities around the waterline, registering the exact time when these took place, as well as

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recording the time spent in air and in water. The model shown in figure 1 was made entirely of Perspex except where mentioned. It consisted of a tubular animal compartment 23 cm long by 3.2 cm internal diameter, connected with another tube 14 cm long and 1.3 cm

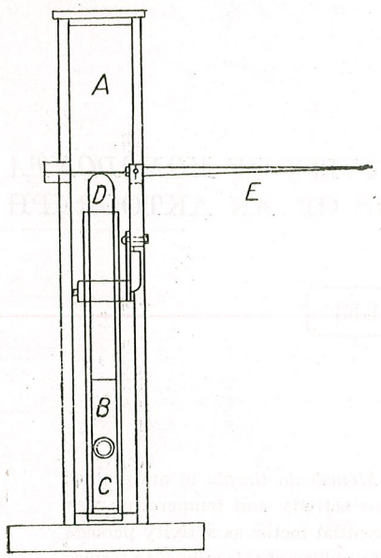

Fig. 1. — The aktograph. *A*, animal chamber ; *B*, tube connected with *A* ; *C*, horizontal connecting tube ; *D*, float ; *E*, lever.

internal diameter, by means of a horizontal, 2.5 cm long tube of the same diameter as the latter, about 2.5 cm above the circular platform supporting the animal chamber.

The narrow side-tube carried a movable collar split at one end which could be slid up and down and clamped at the desired height along the tube by a tightening screw threaded through both sides of the collar gap. Screwed to the collar was a U-shaped brass piece carrying a lever made from 1/5 mm steel mounted on a horizontal shaft which rotated on bearings. The side-to-side flexibility coupled with rigidity in the vertical plane of this lever provided negligible frictional resistance and high sensitivity to this scriber on a smoked drum. The other end of the lever was twisted horizontally to sit on the smooth dome-shaped top of a very light, test-tube like, air-filled, glass float in the side-tube. A light counterpoise cemented at this end of the lever, together with a loop of thin wire riding over the lever and cemented at two diametrically opposite sides of the glass float, maintained the lever permanently resting on the float.

To set the apparatus ready for an experiment, it was first filled to a fixed level with sea water, a snail introduced the right way up on the bottom of the animal chamber, and finally the top was sealed by a piece of bolting silk secured by means of a rubber band to prevent the animal from crawling out. This maintained free communication with the atmosphere.

The principle on which it works is simple. Water in the two intercommunicating tubes is permanently at the same level, and any change in the water level in the animal chamber produces simultaneously an identical change in the water level in the side tube in which the float operating the up and down oscillations of the lever supported. Therefore the float bobs up and down to accompany every rise or fall of the water level in both tubes registering instantly each event on a smoked drum. Thus, if a submerged snail rises to the surface, immediately it starts breaking through the surface the level of the water in both tubes goes down, the float sinks down dragging the lever behind it so that the writing end goes up registering a vertical line on the

drum the height of which depends on the degree of emersion. The reverse occurs when an emersed snail returns back to the waterline and below the surface. This way, even the very slightest movements of the snail around the air/water interface is instantly recorded during the whole test period.

When setting an experiment, the drum is rotated through a few millimeters to mark the level of the lever when the snail is immersed, then the lever is pushed lightly to mark the starting point. The time of start is noted on the drum. Knowing the height of a full emersion, the exact position of the snail in relation to the surface at any moment throughout the whole experimental period can be assessed. A 24 hs drum which gave a 28 cm long trace made possible the identification of the exact time at which each movement recorded occurred. The time an animal spent above water could also be calculated by measuring the width of all oscillations above the initial baseline.

A (Casella) thermohygrograph placed alongside the aktograph registered the temperature and relative humidity throughout each experiment. Notes were kept on conditions of light and other observations made during inspections at each experiment. Adult snails were used only once except on certain occasions when the same individual was used on a succession of days to record its activity over a few days. Except for a few tests carried out at 10°C or below, and a few others inside a dark cupboard, the majority of these experiments were performed under natural shaded daylight conditions at room temperature in the laboratory.

RESULTS AND DISCUSSION

In these experiments activity was measured on the basis of the number of emersions and immersions which were registered as ripples on a 24 hs drum. The results obtained from over 80 experiments each lasting 24 hs are summarised in figure 2.

Notwithstanding the individual variation within the species, certain points emerge fairly clearly.

Here, as in the previous experiments [2], temperatures below 8°C seem to inactivate *Monodonta* and confine it to complete submersion. The aversion to cross the waterline ceases around 8°C. Between 8° and 10°C, although the activity is low, most snails climbed to the surface and beyond, apparently indifferent whether to settle at or above the surface partly or fully emersed. This figure also shows clearly that there was a progressive increase in activity as the temperature rose from 9°C to reach a peak around 19°C, after which there was a rapid decrease as the temperature rose to 24°C. In general, activity and emersion seem to be preferred over a good part of the 9°—24°C. Above this temperature range inactivity and submersion appear to set in.

Not infrequently the same level of activity was reached at a given temperature when relative humidities differed by 10% to 15%. This would indicate that the main factor operating inside the apparatus was air or water temperature.

In the laboratory, the relative humidity normally dropped with rising temperature and vice-versa. Furthermore, emersed snails lose water by drainage from the mantle cavity under gravity, particularly during ventilation, a process described elsewhere [1]. The mobility and kinetic energy of water also increases with temperature. Altogether, these three events provoke a higher rate of water loss at the higher temperatures in emersed

snails, and these are driven to moisten their "lungs" more often to promote gaseous exchange. In other words, the higher the temperature, the stronger become the temperature-induced desiccation effects, and the more frequent the returns of emersed snails back to water.

Below 8°C *Monodonta* is inactive and submerged. Obviously it can easily satisfy its oxygen requirements from sea water. Between 8° and

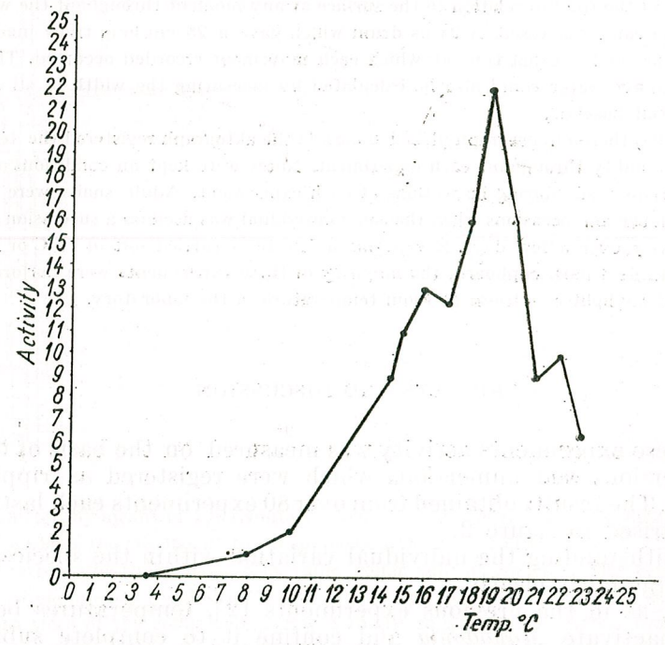

Fig. 2. — The relation between activity and temperature of *M. lineata*.

12°C *Monodonta*'s activity in and out of water increases and it starts preferring emersion to immersion.

With rising temperature the metabolic rate of the active snail increases while the solubility of oxygen in sea water diminishes. Apparently, like *Monodonta turbinata* (Micallef and Bannister, 1967), *M. lineata* is better adapted to respire oxygen in air than in water as its amphibious midlittoral mode of existence would demand. This animal was found to have a higher rate of oxygen consumption in air than in water between 14° and 24°C [1]. If it is permissible to surmise that this animal seems unable to extract as much oxygen from water as from air this then appears to be one of the causes compelling it to increase its visits to the surface and settle there partly or fully emersed, utilizing atmospheric oxygen. When the activity levels registered between 14° and 24°C were compared with the aerial and aquatic rates of oxygen uptake at the same range [1], it was found that the highest number of emersions was scored at 18°—20°C, which more or less coincides with that at which the widest gap between the rates of aerial and aquatic respiration occurs.

Besides, there seems to be increase and decrease in the activity in and out of water closely parallelling the widening and narrowing gap between the higher rate of aerial oxygen consumption over the aquatic between 14°—24°C. This leads to the reasonable conclusion that the present type of activity seems largely provoked by the decrease in oxygen concentration in sea water coupled with the respiratory make-up of *Monodonta*, which seems to be better adapted to breathe in air than in water within this temperature range.

Therefore, while air temperature and the associated desiccation effects persuade this animal to make periodical dives under water to flush its mantle cavity, the increasing inability to obtain oxygen from water drives it back to the surface and beyond. Ecologically this can be interpreted to mean that in the warm season when *Monodonta* is more active and consuming more oxygen, this animal probably settles nearer the waterline when emersed at low water, whence it can make quick returns to water and vice-versa. Presumably, at the lower temperatures, when metabolic rates drop to a lower level, this animal still visits the surface partly to exchange its oxygen-depleted air bubbles trapped inside the mantle cavity, and partly through their innate predominant negative geotaxis [1].

Exposures for a few days at 22°—24°C inactivate *Monodonta*, and eventually persuade it to settle below water. Such temperatures cause the animal to fall to the bottom of the animal chamber and eventually die. Figure 3 shows that the animal was very active on the first day when exposed to this temperature range. The same animal on the second day

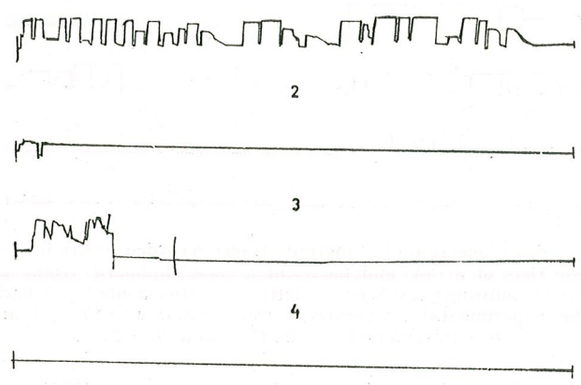

Fig. 3. — Four aktograph recordings of the activity of *M. lineata* kept for four successive days at 22°C to 24°C. *1*, very active on the first day; *2*, little activity and about 24 hours emersion; *3*, high activity for about 2 hours and about 21 hours immersion; *4*, no activity and total immersion throughout the whole period. The snail died at the end of the fourth day.

showed very little initial activity followed by complete emersion and inactivity for about 24 hs. On the third day, activity lasted about 2 hours, then it was immersed for over 21 hs. Finally, on the fourth day there was no activity at all and the animal immersed for the who le 24 hs period after

which it died. Here, high metabolic rate, low oxygen concentration and the ill-effects of prolonged action of high temperature on the tissues, probably eventually produced a state of continous dyspnoea and death from hypoxia and tissue damage within a few days. Elsewhere [1] it was shown that if *Monodonta* is kept for a succession of days at a temperature only a few degrees above the "optimal", activity diminished from day to day, and within less than a week the animal usually died. This animal seems to be adapted to temperatures not exceeding 20°—22°C since it succumbs to sustained temperatures above this limit within a few days.

The effect of light on this form of activity under these test conditions appears more difficult to explain. Thus, while light in general seems to promote activity, darkness in a closed cupboard or during the night often proved ineffective to curtail activity, which was found to continue for a few minutes to several hours in several instances as shown in figure 4.

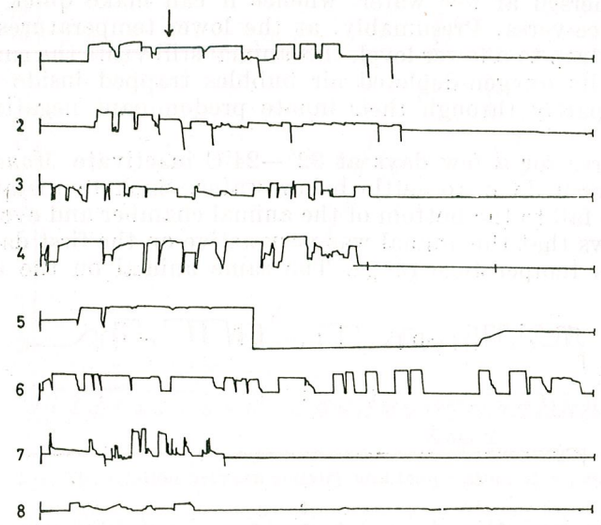

Fig. 4. — Selection of aktograph traces recorded partly in daylight (left of arrow) and partly in a dark cupboard (right on arrow), showing persistence of activity of *Monodonta* in the dark. The experimental temperatures were : 1 and 2 = 17°C ; 3 and 4 = 19°C ; 5 and 6 = 20°C ; 7 and 8 = 21°C.

Therefore, although in the majority of cases, more activity was registered in the light than in the dark, it would appear that light, at least in these experiments, was playing a less important role than temperature in controlling activity, other factors not excluded, and that it is not absolutely essential for this type of activity.

Finally it must be stated that this form of activity appears to be inseparably connected with air and water temperatures and with the effects of temperature on metabolism, other factors not being excluded. Furthermore, the fact that elsewhere [1] it was shown that like the present type of activity, the temperature of selection and maximum speed

of crawl were registered at around 18°—20°C leaves hardly any doubt that temperature seems to be the major factor controlling the activity of this snail in many if not all its various forms.

CONCLUSIONS

1. A new aktograph for studying the activity of *Monodonta lineata* is described.
2. Spontaneous activity in and out of water was recorded in over eighty experiments each lasting 24 hs in *Monodonta*.
3. Activity seems to be governed directly by temperature.
4. Light does not appear to be an essential factor as activity persist even in the dark.
5. Experimental results relate to the ability of *Monodonta* to respire oxygen better in air than in water and with its subaerial mode of existence on the shore.

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DER EINFLUSS DER GEHIRNERREGUNG AUF DIE GLYKÄMIE BEI *CYPRINUS CARPIO* L.

VON

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The influence of electrical stimulation of 15 points from various parts of the brain on glycemia in *Cyprinus carpio* L. was investigated. In the most cases more or less hyperglycemia was produced. There are clear differences concerning the time of its appearance and duration. As a general rule glycemia increases in the first 3—5 hours, begins to decrease to the initial level at 24 hours and reaches this one after 48 hours. The lowest glycemic values were found after stimulation of the telencephalon. After the stimulation of the caudal part of the telencephalon the return of normal glycemia occured more rapidly than in the control. The most pronounced hyperglycemies were produced after stimulation of the metencephalon and myelencephalon. The intervention of the encephalon in the regulation of glycemia in the carp is very possible and even the existence of some glycoregulatory centers localized in some of its parts should not be excluded.

Die Untersuchungen, die an einigen Arten von Cyclostomata und Fischen vorgenommen wurden, haben erwiesen, daß die Schädigung, integrale Zerstörung oder Extirpation von Teilen des zentralen Nervensystems meistens Hyperglykämie von großen Ausmaßen und langer Dauer bewirken [1], [5], [10], [11], [13]. Bei den Lurchen gibt es ebenfalls einige Arbeiten, die sich mit dieser Frage des Zuckerhaushaltes befassen [2] — [4]. Bei diesen bewirkt die elektrische Reizung des Telenzephalons, Mesenzephalons und Mielenzephalons Hyperglykämie und Glykurie und umgekehrt, Hypoglykämie und rascheren Schwund der Hyperglykämie und der bewirkten Hypergalaktosämie, wenn das Diencephalon gereizt wird [4]. Eine infolge der Reizung des Telenzephalons, Dienzephalons, Myelenzephalons oder Mesenzephalons eingetretene Hyperglykämie wurde auch bei den Kriechtieren verzeichnet [12].

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Der Einfluß, den bei Fischen die Reizung des zentralen Nervensystems auf die Glykämie ausübt, wurde bisher noch nicht untersucht. Diese Tatsache veranlaßte uns dazu, Untersuchungen vorzunehmen, deren Ergebnisse den Gegenstand vorliegenden Beitrags bilden.

MATERIAL UND METHODE

Die Versuche wurden auf zwei Sommer alten Zuchtkarpfen vorgenommen, deren Körpergewicht zwischen 400 und 600 g betrug.

Die gefangenen Karpfen wurden in großen Aquarien mit stetigem Leitungswasserdurchfluß gehalten. Die Wassertemperatur schwankte zwischen 16 und 25°C. Die Versuche wurden bei 18—24°C etwa 2 Wochen nach dem Einfangen der Karpfen in Laborbedingungen durchgeführt. Während der ganzen Versuchsdauer wurden die Tiere nicht gefüttert. Es wurde mit Versuchsgruppen von 4—6 Tieren gearbeitet. Nur die Kontrollgruppe bestand aus 12 Tieren.

Zum Zwecke der Operation wurden die Fische mit einem für Fische speziell angepaßten Gerät fixiert [14].

In derjenigen Gegend des Gehirns, die gereizt werden sollte, entfernte man zuerst die Haut, dann bohrte man mit einem Trepan die Schädeldecke mit einer Öffnung von etwa 6 mm Durchmesser an, und legte den betreffenden Teil durch die Beseitigung der überliegenden Fettschicht frei.

Um einen oder den anderen Gehirnteil an verschiedenen vorherbestimmten Punkten zu reizen, verwendete man mit Viniflex isolierte NiCr-Elektrodenpaare mit einem Durchmesser von 5μ; die Entfernung zwischen den Elektroden beträgt 50—100μ; etwa 0,5 mm der Spitze ist nicht isoliert.

Das Aufsetzen der Elektroden auf die Reizpunkte wird anhand eines Mikromanipulators Zeiss bewerkstelligt.

Das Außenende der Elektroden wurde mittels Leitern an die Bornen eines EMA-Generators von rechteckigen Impulsen angeschlossen. Die Dauer eines Reizes betrug 15 s, bei einer Spannung von 3V, einer Intensität von 2 mA und einer Frequenz von 100c/s.

Nachdem der betreffende Teil erregt wurde, bedeckte man ihn mit einer sterilen Agarschicht (1—2%) und setzte die Fische wieder ins Aquarium. Die Blutproben wurden durch Herzpunktion vor der Erregung und 3, 5, 24 und 48 Stunden danach entnommen. Der Zuckerspiegel wurde nach Hagedorn-Jensen bestimmt.

Es wurden insgesamt 15 Punkte erregt, die wie folgt aufgeteilt waren: 1, 2 im Telenzephalon, 3—5 im Mesenzephalon, 6—10 im Metenzephalon und 11—15 im Myelenzephalon (Abb.1A)

Aufgrund der glykämischen Werte, die für jede einzelne, einem Reizpunkt entsprechende Versuchsgruppe bestimmt wurden, ermittelten wir den Mittelwert. Diese Werte sind in Prozenten des glykämischen Ausgangswertes in Abb. 1 B angegeben.

Während der Versuche verhielten sich die Tiere normal; bei der Kontrolle, nach Abschluß der Versuche, wurde keine Infektion der gereizten Gehirnteile festgestellt.

ERGEBNISSE

In Abb. 1 B sind die glykämischen Werte aufgestellt, die in verschiedenen Zeitabständen bestimmt wurden, nachdem dem Enzephalon in den in Abb. 1A angegebenen Reizpunkten ein Reiz angelegt wurde.

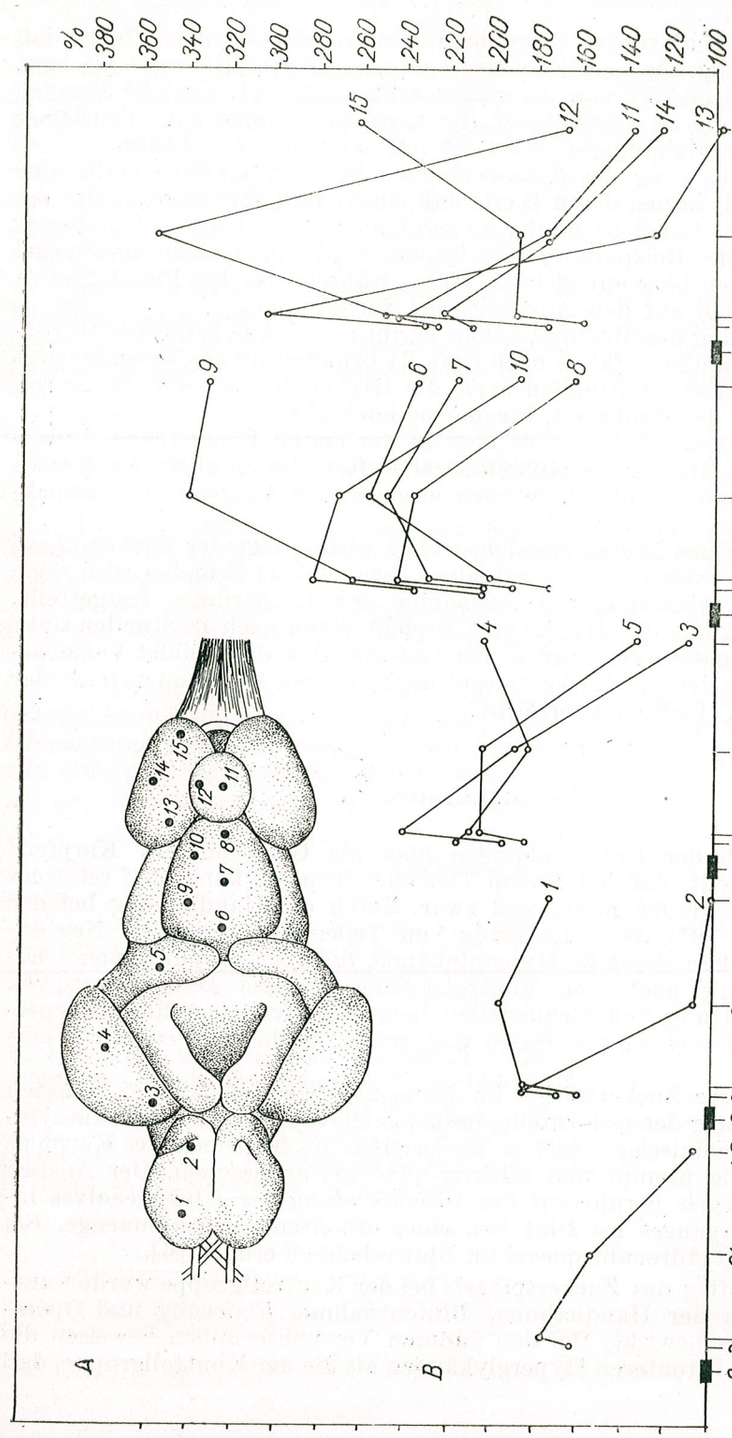

Abb. 1. — A) Lokalisierung der Reizpunkte in den verschiedenen Gehirnteilen. B) Die den Reizpunkten entsprechenden Hyperglykämiekurven. Jedem Reizpunkt entspricht der bei einer Versuchsgruppe erzielte Mittelwert. Die mittleren Glykämiewerte, die in verschiedenen Zeitabständen nach dem Reiz (0 Uhr) bestimmt wurden, sind in Prozenten vom Ausgangswert (100) ausgedrückt.

Aus der Analyse der angeführten Daten folgt in erster Reihe, daß bei der Kontrollgruppe der Zuckerspiegel nach 3 Stunden um 63% bzw. nach 5 Stunden um 77% vom Ausgangswert gestiegen ist. Nach 24 Stunden ist eine Abnahme zu verzeichnen, die nach 48 Stunden noch deutlicher wird, denn der glykämische Wert ist nur noch um 7% höher.

Bei der Reizung des Telenzephalons bemerkt man ebenfalls eine leichte Hyperglykämie, deren Wert bloß ein wenig höher liegt als der der Kontrollgruppe. Der Verlauf der hyperglykämischen Kurven ist aber je nach der Lage des Reizpunktes verschieden. Bei Punkt 1 bleibt der Spiegel etwa 48 Stunden lang auf gleicher Höhe, während er bei Punkt 2 etwa nach 24 Stunden auf den Ausgangswert zurücksinkt.

Die Reizung des Mesenzephalons ergibt eine etwas betontere Hyperglykämie, die in allen Fällen nach etwa 24 Stunden auf den Ausgangswert zurücksinkt. Nach 48 Stunden sank die Glykämie etwa auf die Norm, mit Ausnahme des Punktes 4, für den sie hoch blieb.

Beim Metenzephalon stellt man in der ersten Phase (nach 3 bis 5 Stunden) einen Anstieg des Glykämiewertes fest, der im allgemeinen etwa 24 Stunden bestehen bleibt, wonach er gegen den Ausgangswert zurücksinkt.

Nachdem das Mielenzephalon gereizt wird, steigt der Zuckerspiegel im Blut in den ersten 3 bis 5 Stunden, aber nach 24 Stunden wird seine offensichtliche Abnahme, mit Ausnahme der 12. Gruppe, festgestellt, bei welcher er kaum den Höchstwert erreicht. Etwa nach 48 Stunden sinkt er auf den Ausgangswert zurück. Eine andere Ausnahme bildet Versuchsgruppe 15, bei der der Zuckerspiegel im Blut erst 48 Stunden nach der Reizung seinen Höchstwert erreicht.

DISKUSSION

Anläßlich der Untersuchungen über die Glykämie des Karpfens wurde festgestellt, daß bei diesem Tier eine Hyperglykämie auf reflexem Wege bewirkt werden kann, und zwar, durch die Handhabung bei der Blutentnahme [9]; die Schädigung von Teilen des zentralen Nervensystems bewirkte ebenfalls Hyperglykämie, die aber sowohl unvergleichlich stärker als auch von unvergleichlich längerer Dauer war [10]. Die durch Reizung von Gehirnteilen beim Karpfen eingeführte Hyperglykämie wird meistenteils durch das sympathische Nervensystem hervorgerufen.

Der höhere Zuckerspiegel im Blut in den ersten 3 bis 5 Stunden nach der Reizung der Gehirnteile, bestätigt die Feststellung, daß die sympathisch-adrenalinischen Zucker fördernden Mechanismen des Karpfens verhältnismäßig prompt und effektiv [7]—[9] ansprachen. Der Anstieg des Zuckerspiegels beruht auf der Intensivierung der Glykogenolyse infolge des Überganges ins Blut von einer erhöhten Adrenalinmenge. Bei Insulten ist der Adrenalinspiegel im Blut erheblich erhöht [6].

Der Anstieg des Zuckerspiegels bei der Kontrollgruppe wurde wahrscheinlich von der Handhabung, Blutentnahme, Fixierung und Operation des Tieres bewirkt. Bei den anderen Versuchsgruppen beweisen die erzielten, viel betonteren Hyperglykämien als die der Kontrollgruppe, daß

sich sämtliche Faktoren der Versuche, denen das Tier unterzogen wurde und die zuckerspiegelsteigernden Effekt haben, summierten. Der Verlauf der erhaltenen hyperglykämischen Kurven unterscheidet sich sowohl nach der Stellung des Reizpunktes des betreffenden Teiles, als auch nach dem Gehirnteil selbst. Das Ausmaß der erhaltenen Hyperglykämien nimmt vom Telenzephalon zum Myelenzephalon zu. Dadurch könnte man voraussetzen, daß das Metenzephalon und das Myelenzephalon bei der Erhöhung des Zuckerspiegels im Blut eine größere Rolle spielt, denn hier registrierte man die höchsten Werte.

Es ist schwer zu behaupten, daß es Zentren gibt, die den Zuckerhaushalt regeln und in einem der untersuchten Teile lokalisiert sind, denn deren Reizung in den verschiedensten Teilen, bewirkte für den betreffenden Teil eine Zuckerspiegelerhöhung der gleichen Ordnung.

SCHLUSSFOLGERUNGEN

1. Die elektrische Reizung des Enzephalons bei *Cyprinus carpio* L. bewirkt für etwa 48 Stunden eine offensichtliche Steigerung des Zuckerspiegels im Blute.
2. Die niedrigsten Hyperglykämiewerte wurden beim Telenzephalon registriert und die höchsten beim Metenzephalon und beim Myelenzephalon.
3. Die Lokalisierung von zuckerhaushaltregelnden Zentren in den untersuchten Gehirnteilen ist schwierig, denn ihre Erregung in den verschiedensten Gegenden bewirkt einen Anstieg des Zuckerspiegels im Blut von etwa gleicher Intensität und Dauer.

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EFFICIENCY OF THE UTILIZATION OF FOOD WITH THE MAIN PROTEINIC SOURCE FROM GREEN ALGAE ADMINISTERED TO GROWING HEN CHICKENS

BY

GH. BURLACU, M. PARASCHIV, N. SĂLĂGEANU, MARGARETA BALTAC and DUMITRA IONILĂ

The nutritional value of a food diet from which proteins of animal origin were replaced mostly by proteins from green algae was studied, on growing chickens of the Leghorn breed and was recorded at the age of 3—4 weeks a smaller nutritional value, expressed in metabolizable energy (ME) and in net energy (NE) than that of the diet with main protein source of animal origin (2,880 Kcal ME end 1,954 Kcal NE, as against 3258 Kcal ME and 2,127 Kcal NE/1kg dry matter). At 11—12 weeks the discordance between these values diminishes however, due to chickens increased capacity of digesting algae at that age (3,208 Kcal ME and 2,419 Kcal NE, as against 3,373 Kcal ME and 2,633 Kcal NE/1kg dry matter).

In some experiments previously effected on growing or adult white rats, we found that green algae administered in the food, as main or sole source of protein, have an appreciable biological value (73—80%) [2] but are digested in a relatively percentage (57.72%) [1]. These results confirmed the previous researches carried out by Powel [quot ed by ([4]), Shugara and Fink [quot ed by [5]), Zglobică et al [16] a.o., on rats and growing chickens or on piglings. Some research workers recorded, however, a slight turning to account of green algae in rats and particulary in broiler chickens. We thus quote the works of Witt et al (quot ed by [15]), Vernî et al [15], Leweille and coll. [quot ed by [9]), Koreleski [9], Garbovska [6] a.o.

Considering the still existing controversy concerning the nutritional value of green algae, we proposed to study on growing hen chickens th nutritional value of some diets from which the proteins of ani

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origin were mostly replaced by proteins from green algae (species *Chlamydomonas reinhardi*). Below, we are presenting results obtained.

MATERIAL AND METHOD

Sixteen 3 weeks'old, pullets of the Laghorn breed were put into study, of which 2 homogenous lots of 8 heads each were formed. The food diets shown in table 1 were administered to the lots.

From the table it ensues that the two diets differ in the first place by the substitution of meat and fish meal with green algae meal, namely : to the 10 % meat and fish meal we substituted 20.2 % green algae meal, substitution being made on the basis of crude protein content of these fodders, assessed by us, and on the basis of the digestibility of these proteins. The digestibility values of these proteins were taken from literature, in the case of meat and fish meal, and were assessed by us in rats in the case of alga meal. Alga meal was obtained within the Section of plant physiology of the "Traian Săvulescu" Institute of Biology.

Table 1

Food dietes investigated

Diet composition	Control diet %	Experimental diet %
Maize	65	57.67
Sunflower	7	6.20
Soia groats	8	7.08
Green algae meal	—	20.20
Meat meal	5	—
Fish meal	5	—
Fodder lees	2.5	2.21
Skim-milk powder	2	1.77
Bone meal	1,5	1.33
Calk	2.5	2.22
Premix *	1.5	1.33
Total	100.0	100.00
Gross energy Kcal/kg D.M. (dry matter)	4469	4492
Crude protein %	20.4 %	21.1 %

* Mixture of vitamins and mineral salts for hen chickens, manufactured by "Zoofort," Bucharest.

Experiments consistent in the investigation of the energy and nitrogen balance in 3—4 and 11—12 weeks'old chickens fed on the two above mentioned diets. Chickens were fed on these diets at two nutritional levels, namely;

1 — ad libitum

2 — with a ration equivalent to 2/3 of the level of ad libitum diet.

Balance experiments lasted time 18 days each, divided into two periods, of which the first period is of 11 days, during which the balance of rations administered ad libitum was investigated, and the second period of 7 days, during which the balance of the ration reduced to 2/3 was investigated. In the last 6 days of the first period and in the last 4 days of the second period, the quantity of ingested food, the quantity of faecals + urine excreted were recorded and the energy metabolism was determined for 24 hours twice each time The caloricity and the dry matter and crude protein contents of ingesta and excreta were analysed by using the current techniques indicated in previous works [1] [2]. Respiratory exchanges were assessed

by using an open circuit installation with the possibility of a concomitant metabolism investigation in 4 animals (or groups of animals). On the basis of nitrogen balance values, deposited protein caloricity was computed (N × 6.25 × 5.66 cal.) (13), and by the difference as against the total net energy, the caloricity of deposited fat.

At the age of 3—4 weeks, the evidence of ingestion and excretion as well as the assessement of energy metabolism were made on the entire lot, and the age of 11—12 weeks, individually. Experiments were carried out at a 25°C temperature of the ambient surroundings.

RESULTS OBTAINED

Is found that the metabolizable energy of control and experimental diets differ substantially in favour of the control ration, in the first month of the chicken's life (72.9% as against 62.8%). It thus ensues that the substitution of meals of animal origin (of meat and fish) with green alga meal entails a weaker digestibility of the ration. Alga meal diminishes, apparently, also the digestibility of the other components of the ration as the diminution by about 10% of digestibility of test ration is greater than the combined effect of the totalization of the separate digestibility of green alga meal with that of ration. We find, however, that the discordance between the values of metabolizable energy of the two rations at the age of 11—12 weeks is much smaller (75.5% to 71.4%), which means that at this age chickens can digest green algae better than at the age of 3—4 weeks. It is likewise noteworthy that both food rations were better digested by chickens at the age of 11—12 weeks than at that of 3—4 weeks.

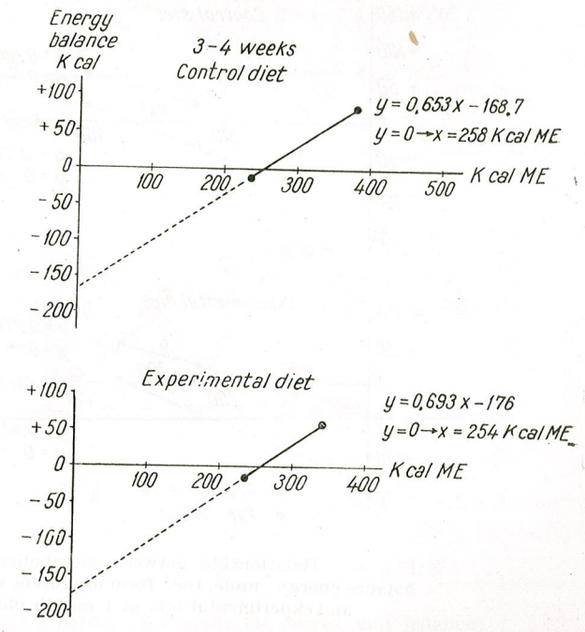

Fig. 1. — Relationship between metabolizable energy and balance energy in control and experimental lots of 1 month of chickens.

By comparing the values of metabolizable energy of food rations with those of net balance) energy values and with deposited or desassimilated energy such as protein or as fat, we recorded that these are to be found in a linear relationship, the regression straight lines and the equations of linear functions being shown in figures 1—4. We as certain, at first, that at the age of 3—4 weeks, chickens assimilate the metabolizable

energy of control diet, as net energy, in a proportion of 65.3% and of experimental diet in a proportion of 69.3%, thus somewhat greater (Fig. 1). Likewise from figure 1 we see that the metabolizable energy necessity for maintenance (y = 0) is very similar in the two lots fed on the rations investigated (258 Kcal, respectively 254 Kcal/kg 0.75/24 hours), the value of the basal energy metabolism (x = 0) being likewise similar (168.7 Kcal respectively 176 Kcal/kg 0.75/24 hours).

In figure 2 we see that the depositing of protein and fats is similar in both control and test lots, and that these vary with the metabolizable energy of diets according to linear functions whose regression gradiens are smaller in protein depositing (28.4% respectively 41.7%). We also note the fat that in both rations the increase as total energy, i.e. also of protein and fat, is deposited after the rations ensure a metabolizable energy of

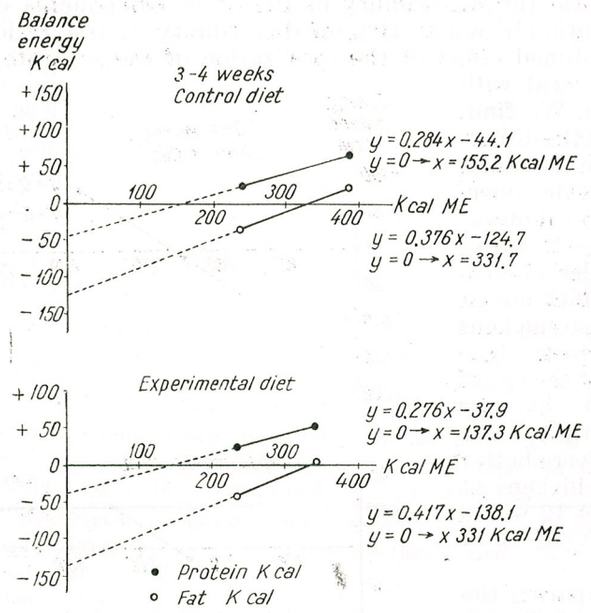

Fig. 2. — Relationship between metabolizable energy and balance energy, unde the form of protein and fat in control and experimental lots of 1 month old chickens.

minimum 331.1 cal/kg 0.75/24 hours. We remark, however, that the protein increase is realized also at lower levels of the ration in metabolizable enesgy, namely at 155.2 Kcal in control diet and at 137.3 Kcal in experimental diet, which means that the necessity of crude protein for the maintenance of functions is, in the lot fed on control diet, of 9.72 g/kg 0.75/24

hours.

$$\left(\frac{155.2 \text{ Kcal ME/kg } 0.75/24 \text{ hours} \times 0.284\% \text{ protein/g D.S.}}{3.258 \text{ Kcal ME/1 gD.M.}}\right)$$ and in

the lot fed on experimental diet of 10.239 g/kg 0.75/24 hours

$$\left(\frac{137.3 \text{ Kcal/ME/kg } 0.75/24 \text{ hours} \times 0.211\% \text{ protein/g D.S.}}{2.820 \text{ Kcal ME/1 g D.M.}}\right).$$

At age of 11—12 weeks (Fig. 3) the metabolizable energy of both food diets are better assimilated as net energy than at the age of 3—4 weeks, namely in proportion of 78.1% in control diet and of 75.4% in

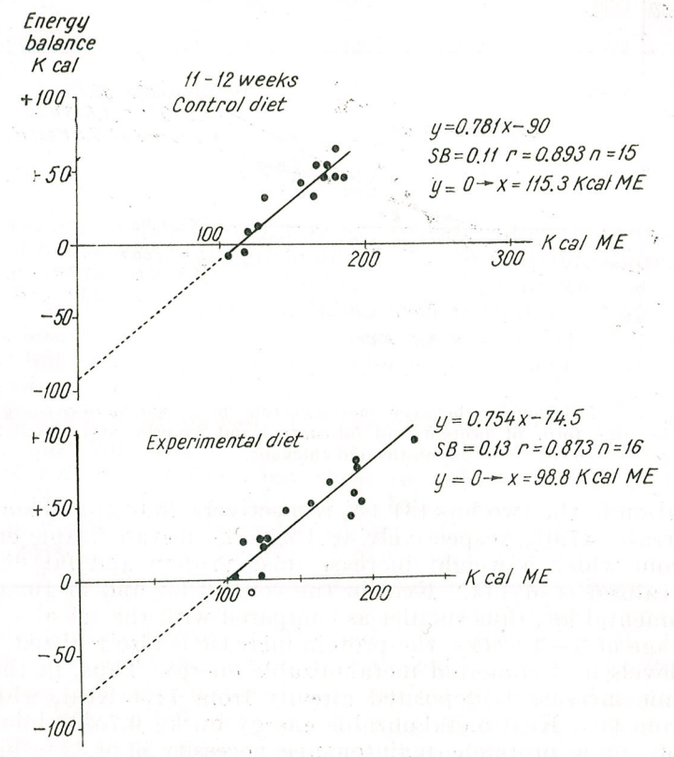

Fig. 3. — Relationship between metabolizable energy and balance energy in control and experimental lots of 3 months' old chickens.

experimental diet. At this age the necessity of metabolizable energy for maintenance on kg 0.75 /24 hours is certainly smaller than at the age of 3—4 weeks, being of 115.3 Kcal in the control lot and of 98.8 in the experimental lot, the pullets of the control lot having a basal metabolism of 90 Kcal/kg 0.75/24 hours, while those of the experimental lot of 74.7 Kcal/kg

0.75/24 hours. Figure 4 shows the increase evolution expressed in protein and in fat. As at the age of 3—4 weeks we find that proteinic increase related to metabolizable energy evolves on a regression gradient with a simi-

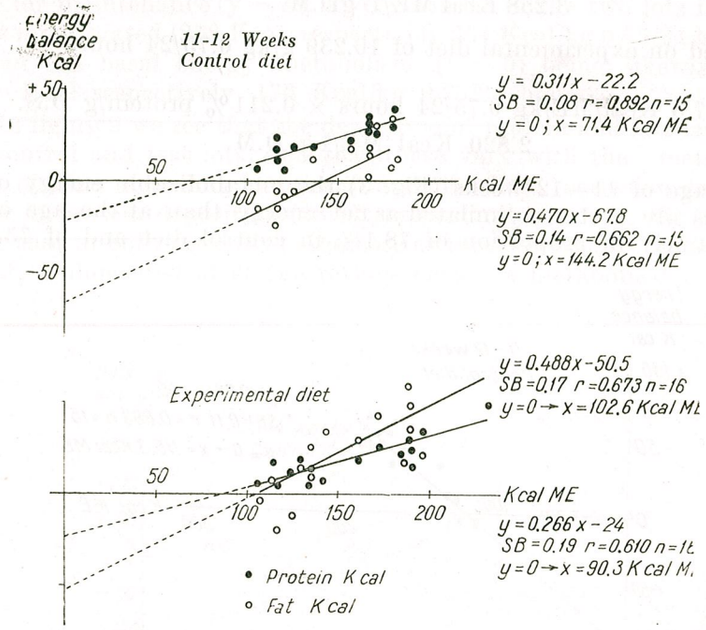

Fig. 4. — Relationship between metabolizable energy and balance energy under the form of protein and fat control and experimental lots in 3 months' old chickens.

lar inclination in the two lots (31.1% respectively 26.6%) and smaller that of fat increase (47.0% respectively 48.4%). The metabolizable energy minimum from which a weight increase (also protein and fat deposits) is actually realized is of 144.2 Kcal in the control lot and of 102.6 Kcal in the experimental lot, thus smaller as compared with the age of 3—4 weeks. As at the age of 3—4 weeks, the protein increase is also realized, however, at lower levels of assimilated metabolizable energy. Thus, in the control lot proteinic increase is deposited already from 71.4 Kcal, while in the test lot from 90.3 Kcal metabolizable energy on kg 0.75/25 hours, which corresponds to a proteinic maintenance necessity of 4.32 g/kg 0.75/24 hours in the lot fed the control diet and of 5.92 g/kg 0.75/24 hours in that fed with experimental diet.

From all tehese experimental data we can reach the conclusion that the test ration is assimilated similary with the control diet, the protein of green algae thus replacing efficiently the protein of animal origin of the control ration. We note, however, particularly in the first weeks of growth, that chickens cannot digest green algae too well, which brings about a lower gross efficiency of ration of green algae.

In table 2 we show the nutritional value of the two investigated rations expressed in Kcal in 1 kg dry matter diet.

Table 2

Nutritional value of investigated food rations

Diet	Age	Nutritional value (Kcal/kg D.M.)		
		Gross energy	Metabolizable energy	Net energy
Control	1 month	4469	3258	2127
	3 months	4469	3373	2633
Experimental	1 month	3392	2820	1954
	3 months	4492	3208	2419

DISCUSSION OF RESULTS

The results obtained in our research concerning the gross and net efficiency of food ration with green algae as main proteinic source, confirm those obtained by the authors quoted in the introduction of this work (Powel, Shugara and Fink, Tamia, Malek, Fodor, Heitman, Zglobică, a.o.), as well as those determined by us in previous researches [1] [2]. It thus results that but with a high biological value, comparable to that of proteins of animal origin.

As regards the comparison of the results obtained by us in both food diets, with those obtained by various authors, also in chickens, on different fodders and rations, we find that these resamble those given by Fraps [quoted by [10]), Sibbald et al [14]. McIntosh et al [11] and Hill et al [7]. Thus as regards metabolizable energy (ME), McIntosh et al, find a ME in wheat which varies between 3,080 and 3,290 Kcal and in maize between 3,420 and 3,500 Kcal/kg D.M., depending on the degree of crumbling of fodder, while Hill et al, assesses a metabolizable energy in equilibrated food rations, between 3,340 and 3,390 Kcal/kg D.M. As to the variation of metabolizable energy value depending on age, ascertained in our researches, this comparable to that assesed by Sibbald and coll. who determines in maize a ME of 3,550 Kcal/kg D.M. in 2 weeks' old chickens, and of 3,510—3,750 Kcal in 16 weeks' old chichens. We note, however, that Renner and Hill [16] did not record any significant variation of metabolizable energy with age except only in certain fodders (e.g. suet, administered as lipidic source).

Net energy values of rations investigated by us, are likewise comparable with those investigated by Fraps [quot ed by [10]) and with those obtained by Hill et al [7]. Thus, Fraps records in maize a net energy of 2,528 Kcal/kg D.M., while Hill et al a value of 2,490 Kcal in the above mentioned ration.

We must nevertheless point out that Hobls [8] recorded in 10—12 weeks' old chickens a net efficiency of only 50—55% and contrary the results obtained by us, he descertained a decrease with age of the efficiency of metabolizable energy as net energy.

CONCLUSIONS

Fodd diet from green algae, as main proteinic source administered to growing chickens of the Leghorn breed has at the age of 3—4 weeks smaller nutritional value, expressed in metabolizable energy (ME) and in net energy (NE), than that of the diet with main proteinic source of animal origin (2820 Kcal ME and 1954 Kcal NE/1 kg D.M., in experimental (diet, as against 3259 Kcal ME and 2127 Kcal. NE/1 kg D.M. in control diet).

At 11—12 weeks the discordance between these values diminishes however, due to chickens' increased capacity of digesting algae at that age (3208 Kcal NE/1 kg D.M. in the experimental diet as against 3375Kcal ME and 3633 Kcal NE/1 kg D.M. in the control diet.

We further note that at both ages the investigated food diets have a close net efficiency (net energy/metabolizable energy), which means that the digestible substance both rations, inclusive of proteins, are similary assimilated.

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FREQUENCY ANALYSES OF THE ELECTRICAL ACTIVITY OF THE LIMBIC SYSTEM STRUCTURES DURING DIFFERENT FORMS OF BEHAVIOUR

BY

N. O. TIMOFEEVA and B. I. KOTLYAR

On rabbits in the conditions of free moving the frequency changes were investigated with the spontaneous forms of behaviour (orientive reaction to the environment, hanger, defensive state).

The analyses of EEG hippocampus, mammillary bodies, the anterior thalamus nuclei and g. singuli has shown the correlation between the frequency and the character of the electrical activity and the behavioral reactions of an aimal.

The existence synchronous activity is related to the orientive component of the animal's behaviour, and the increase of the rhythmical oscillations frequency — with the summation of afferent stimulations in the limbic circle structures.

Electroencephalografic investigations of the different forms of behaviour have revealed a certain connection between the bioelectrical activity changes and behavioral reactions. In a number of works the correlation was revealed between the EEG changes and the unconditioned feeding and defensive [4] [13] [25] [48], orientive ([11] [36] [41] [46] and many others) and the conditioning [2] [10] [17] [27] [32]. As EEG phenomenon, accompaning the behaviour theta activity most of all expressed in hippocampus and some other central formations in response to the presentation of different stimuli and conditioning tasks were repeatedly described. Some investigators connect this appearance with the state of stress [7], [8], others connect it with orientive reactions to unknown situations. [22].

Taking into consideration the functional significance of the limbic system in the formation of emotional, mativational behaviour [9] [38] and in visceral regulation [5] and also a close morphological connection inside the limbic circle [33] [39] we made and attempt to trace the frequency changes in the electroencephalogram of these rabbit's brain struc-

tures in different forms of behaviour. The frequency of the wave activity was used as a reliable indicator of a functional state of the investigating structures [1] [3] [28].

METHODS

The work was carried out on 7 adult male rabbits in the conditions of free moving [26]. Bipolar electrodes made of nichrome wire, diameter 70—100 mk were implanted stereotaxically according to the Sawer's Everett and Green brain rabbit's atlas coordinates [42] to the dorsal hippocampus, mammillary bodies, the anterior thalamus nuclei and g. cinguli. Electroencephalogram was registered on a 4-channel electroencephalograph "Medicor". Sponteniously with the recording of the EEG frequency spectral analyses was made with help of an analyzer "Alvar" which gives as an opportunity to observe the frequency distribution from 2 to 28 Hz during 5 sec. Several forms of the spontaneous behaviour of the animal which was placed in sound-proof screen chamber were analysed. The observation of the animals was made through a special visir apparatus. The results of the analyses were summerised for 90 second (18 five-second intervals) for each type of the activity and then were averaged to all the seven animals. On the grounds of middle data the graphics were built reflecting frequency distribution in different forms of behaviour. At the end of the experiments the morphological control was made showing the localisation of electrodes in the pointed structures.

RESULTS

The animal was subjected to the experiment in 7 days after the operation. The first placements of the rabbits the chamber were accompanied by the explicitly revealed orientative-investigating reaction, consisting of the continuous motor activity, smelling , being on alert, standing up to the paws and a number of other behavioral reactions. Spontaneously with the given form of behaviour on EEG in all the limbic structures synchronic activity with the frequency 7 osc/sec (Fig. 1*A*) predominated. Intensivity of a given frequency 2,5—3 times increased the one observed on other spectre frequencies (fig. 2B). Sound stimulation presented on this ground did not cause any frequency changes in EEG. The character of the electrical activity changed when the animal got adopted to the environment. The analyses of the electrical activity of hippocampus, mammillary bodies, anterior thalamus nuclei and g. cinguli has shown that synchronic as well as asynchronic oscillations are registered on the animals in a calm state. As a rule differences in predominance, different oscillations of EEG were shown and depended of the anymals state.

For example, in a EEG sitting rabbit more regular type of the activity prevailed (Fig. 1 *B*, *C*). The spectre of frequencies is at that period is changed to the slow-wave area, that is to say, the intensity of frequencies from 4 to 7 osc/sec increases the intensity of other frequencies to 1,5 time. (Fig. 2 *A*). The same phenomenon in a rabbit's EEG is noticed by other authors [14] [22] [36] [40].

Deprivation of food for 2 or 3 days in animal again caused the appearance of the seeking and investigating reactions.

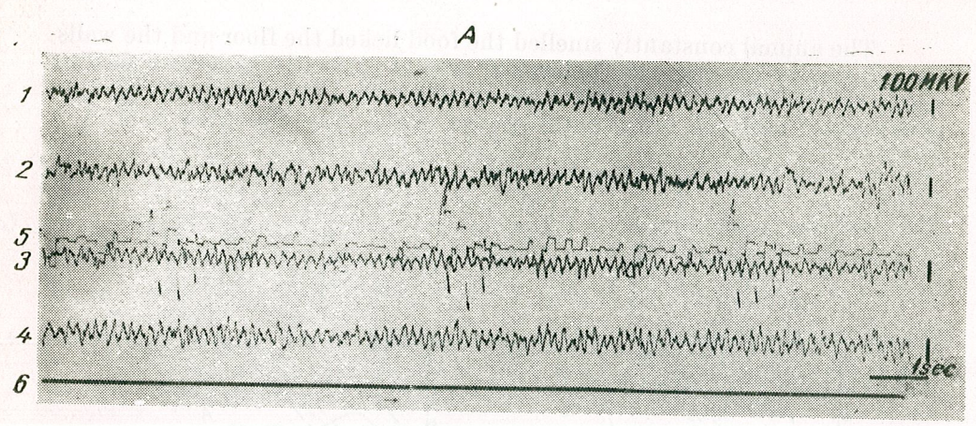


Fig. 1. — Electrical activity of the limbic system structures of the rabbit's brain during (*A*) the orientative reaction to the environment of an awake, sitting (*B*) and lying (*C*) rabbit, 1 — hippocampus, 2 — mammillary bodies, 3 — anterior thalamic nuclei, 4 — g. cinguli. 5 — frequency analysis of the hippocampus electrogram, 6 — sign of stimulation.

The animal constantly smelled the food licked the floor and the walls, its orientative-investigating behaviour was of expecitly expressed feeding character. In limbic structures EEG in this form of behaviour the synchronization with the frequency 8 osc/sec which predominated in electro-

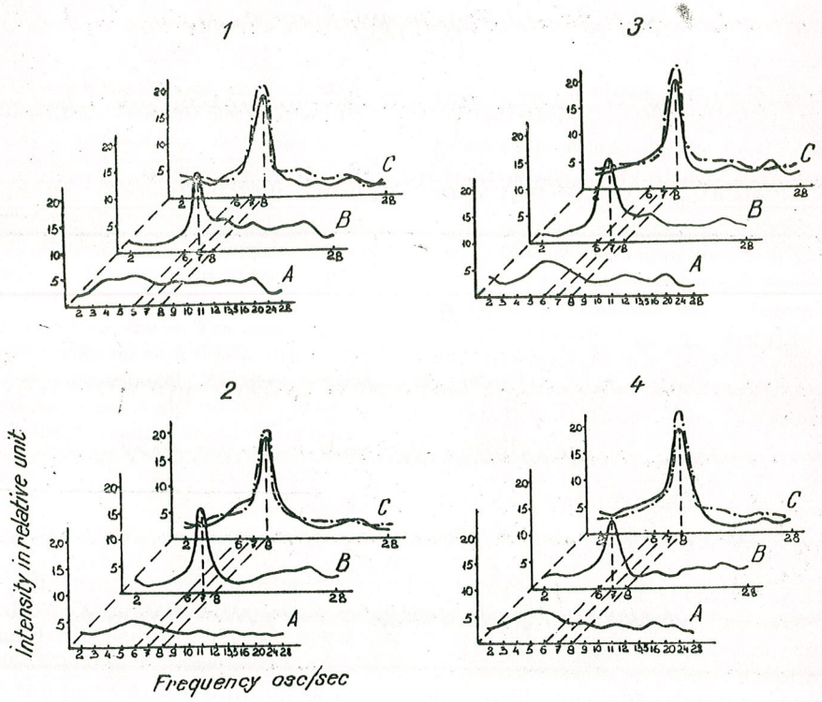
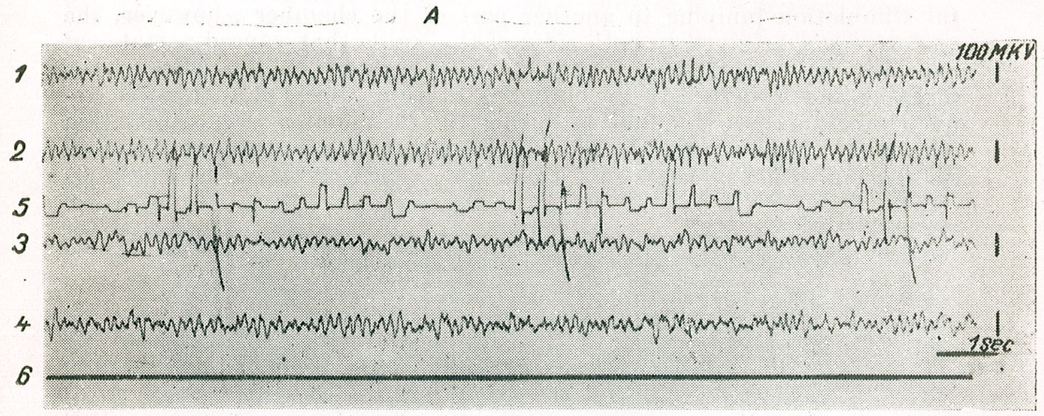
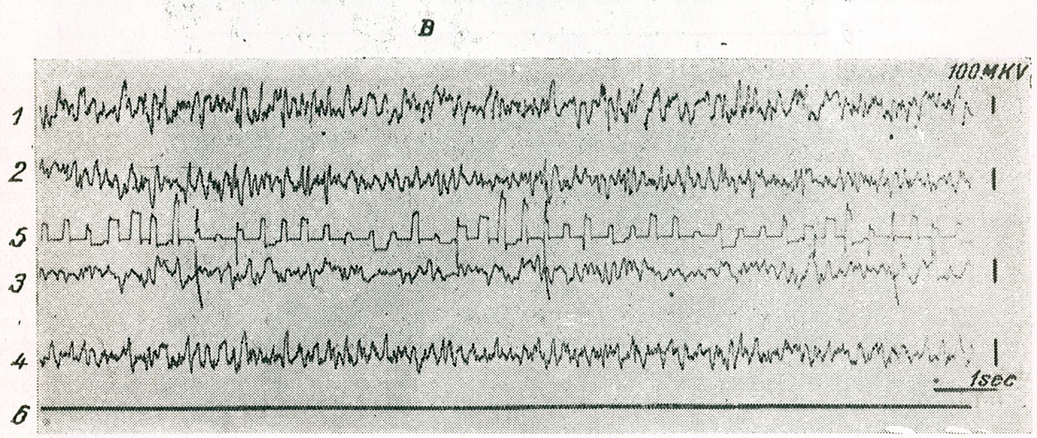


Fig. 2. — Distribution of frequencies in the EEG of the hippocampus (1), mammillary bodies (2), anterior thalamic nuclei (3), and g. cinguli (4) with the different forms of behaviour.
A = a calm awaking state; *B* — orientative reaction to the environment; *C* — dominant feeding and defensive (brokten line) state.

gram during 3 or 4 hours of continuous registration was observed (Fig. 3A). This intensity of the given frequency 3.4—5 times increased the intensity of other frequencies (Fig. 2 *C*). Sound stimulation given on the background of feeding domination did not cause any changes in EEG. The feeding of an animal in the chamber led to the gradual decrease of frequency of synchronous activity and to the appearance of asynchronous oscillations. Routtenberg [4] observed a similar phenomenon in the rats during deprivation of food and satiation. Masticating movements were accompanied by the asynchronous oscillations at a greater amplitude (Fig. 3 *B*), however if it while eating the animal dropped the food a burst of synchronous oscillation 7 osc/sec, appeared and the animal started seeking the food.

Defensive reaction were caused by the electric current blow in the paws (the threshold size is individual for each animal), the current was given through the metallic grille in the floor. The animal could avoid pain-





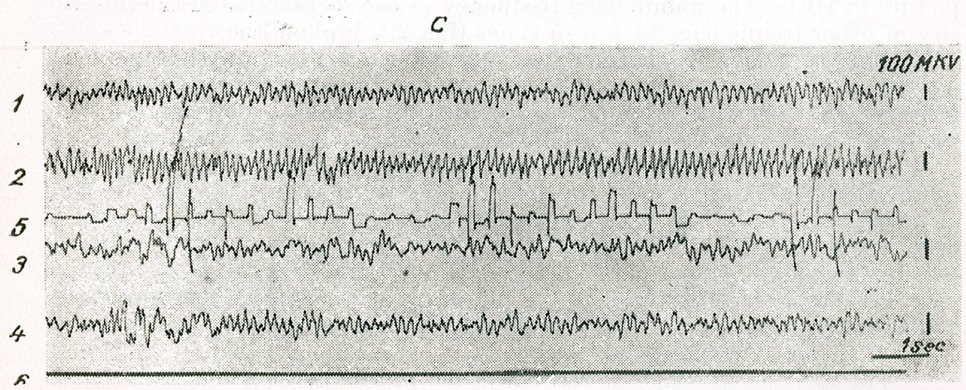


Fig. 3. — Character of the electrical activity in the limbic structures of rabbit deprived of food (*A*) during eating (*B*) and during the defensive dominant (*C*).

ful stimulation jumping to another part of the chamber , however, the current could be given to both sides of the grille that caused a state of alertness and frightfulness. After a current shock on the EEG of the investigating structures a synchronous rhythm appeared with the frequency of 8 osc/sec dominating during 15—20 minutes after stimulation (Fig. 3 *C*). Moreover, usually the animal was sitting immobile but strain. As the relaxetion of the stress state, the synchronization gave way to

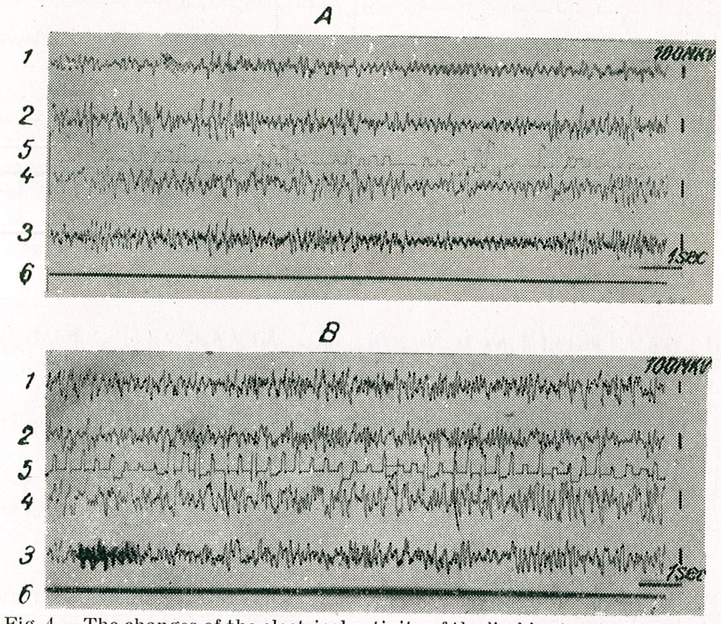


Fig. 4. — The changes of the electrical activity of the limbic structures during the dream. *A* — dream state. *B* — deep sleep. Same legend as in figure 1.

the asynchronous activity. The repeated stimulation caused the similar picture in EEG. The dominating frequency (8 osc/sec) increased the intensity of other frequencies to 4.5—5 times (fig. 2 C broken line).

It is necessary to mention that any drive activity was accompanied by the increase of synchronization frequency. For example, the forms of behaviour like chewing, kicking and scratching were not followed by long burst of synchronous activity, it rather precedes this activity.

Repeated long existence in the chamber caused the sleep which was reflected on the rabbit's EEG by the two essential pictures. The beginning of sleep dream state was characterized by high-amplitude nonregular oscillations lasting for 2 or 3 seconds which were changed from time to time by slow amplitude synchronous activity with the frequency of 5—6 osc/sec (Fig. 4 *A*) that, probably reflects the moments of awakening, because the sound stimulus, given on the background of high-amplitude oscillations causes the same changes as on EEG.

The next sleep stage is the one when in all the investigating structures high-amplitude slow waves appear, on which high-amplitude oscillations are modulated (Fig. 4 *B*). In this case the spectre of frequencies

presents more a distribution with a slight shift to 6—8 osc/sec, however, the oscillations themselves are asynchronous. During the prolonged registration of EEG during the sleep it became possible to observe the so-called paradoxical sleep phase when the high-amplitude wave activity suddenly gave way to the slow-amplitude synchronous rhythmics in all the structures with a frequency of 5—6 osc/sec. In some cases a short desynchronisation preceded synchronization. Outwardly the animal was as usual in sleep state. We did not make any detailed analyses of the behaviour in EEG during the sleep but the picture presented is similar to the one described before by Grastyan and Karmos [20], Brown, Stryne, Weiss et al [52].

It is necessary to notice that the changes of electrical activity described appeared in all the investigating structures with a great regularity and simultaneousness. Only 2 of 7 animals did not reveal explicitly expressed synchronous activity in g. cinguli that can probably be explained by the variation of the place of electrodes implantation. The electrodes of these animals were placed in the anterior part of the girus closely connected with the motor cortex [44] [45]. In other animals they were implanted to the cingular and retrosplenial areas which have direct connections with the stuctures of the hippocampus circle. Thus, we registered the electrical reactions in the structures of limbic system with the different forms of behaviour and noticed the increase of frequency of synchronous activity with the intensive orientative-investigating behaviour in a new environment and with the different functional and emotional states of the organism (Fig. 2).

DISCUSSIONS

Observing the data received first of all we consider it necessary to pay a special attention to the 3 essential moments, firstly, to the simultaneous appearance of synchronous activity in EEG of all the limbic structures with the different forms of behaviour. This very fact proves a close morphological [35] [39] and functional connection of the given formations. Probably, only such a synchronity in their activation can provide an adequate reaction of the organism to both the internal and external changes.

Other problem is the definition of informational significance limbic structures EEG reactions which accompanied the behaviour. There is a lot of material about the interrelation of behaviour and EEG changes. However their comparison presents a certain difficulty because they are received on different objects and on different stages of ontogenetic development. In this sense, the most interesting seem to be the investigations of Green, Arduini [22], Novicova, Farber [36], Banzekina [8], Petsche, Stumpf [40], Brown [11], Routtenberg [41] and many others, in which the appearance of the rhythmical tetha activity in a new situation to an afferent stimulation was described, and the given EEG picture was related to the behavioral reaction of attention "wakefulness" orientative reaction and extinction. The regular activity registered by us with a frequency of 5—8 osc/sec usually accompanied the awaking state of the animal; the

exception is the picture of the "paradoxical" sleep related by some authors to the eye movements and the possible dreams [30]. Moreover, the different frequencies corresponded to the different forms of behaviour. It seems to be valuable to notice that common for all the levels of awakening (calm state, reaction to environment, state of banget and defensive reaction) was the orientative-investigating behaviour, expressed in a smaller or greater degree. Asynchronous oscillations usually corresponded to the unactive calm state of an animal, to the act of food or dream, in other words, to the states in which the orientative reaction is inhibited. Similar data were received on cats by Lissak, Grastyan [31] in the conditioning situation with the presentation of the conditioned or differential signal. The appearance of the rhythmical activity was connected with the orientative reaction to the given stimuli, and desynchronisation with the extinction.

Thus, comparing the animal's behaviour and the electrogram of the limbic structures, it is possible to draw a conclusion of a close correlation of the synchronous activity and orientative-investigating behavioral reactions and also of the important role of the hippocampus circle structures in the formation of this reaction and consequently in the estimation of the significance of the signal in preparing the central nervous system in general to the presupposed activity.

The participation of the limbic structures to the formation of the orientative behaviour is proved by the results of other investigations. Thus in the investigations of Valdman and Koslovscaya [50] and Koridze [25] it is shown that the stimulation of the limbic formations leads to an increased orientative reaction. Komisaruk's and Olds [24] tests are also interesting; the latter investigator, observed the activation of separate limbic structures neurons during the behaviour connected with such phenomena as smelling and alertness that speaks about the specific character of the given formations in relation to the described investigating activity.

Finally, the last problem is the appraisal of the frequency increase of the sychronous activity, shown by us in the example of the intensive orientative-investigating reaction and the formation of feeding and defensive dominant expressed in the domination of 7—8 osc/sec frequencies. Observing many data of extirpation and stimulation of the limbic structures [23] [37] [49] we can see that apart the orientative-investigating activity the called formations exert a great influence over feeding and defensive behaviour. One can suppose that the increase of the synchronization frequency in EEG of the hippocampus and other structures is the result of the integration of the unconditioning states leading to the intensificaton of the activity of the investigating structures. In this con'ents the data of the increase of synchronization frequency during the conditioning development [17] [28] [32] [51] present a certain interest. According to Kotlyar and Kalyuzhny [28] this frequency increase is connected with the increase of the excitability level of the cortical and subcortical structures, that can be explained by the summation of the additional afferent information [7] [29]. Does the same way the additional afferent stimulation lead to the frequency increase of the rhythmical activity? Taking into consideration the dissimilarity of the different hippocampus zones [19] and its polysensority [15] [16] [19] [21] [43] it is possible

to suppose that not all the synaptic and neuronal units are actionated to the novelty signal supporting a certain level of excitation of the structure. The activation of new structural units takes place at a certain functional state (hunger, pain), in other words, at an additional afferent stimulation (olfactory, tactile, interoceptive) received from outward and inward systems of the analyzors. As one of such a mechanism can serve the stimulation of integratory synapsis hippocampus piramids, arousing during the frequency increase of afferent impulsing or converging to them the impulses of different modalites [6] as a result of which the excitability level structure increases that probably leads to the frequency increase of the rhythmical activity.

It is impossible to exclude the influence of the cortex and of the reticular formation upon the electrical activity of the limbic structures carried out through a whole system of morphological connections [33], [34], [39]. Stumpf [47], for example, showed that there is a certain dependance between the theta-activity frequency of the hippocampus and the degree of excitation of the reticular formation. It is also shown that the stimulation of the entorhinal cortex might cause the spike and switch activity and, in some cases, the theta-activity [18]. It is evident from these data that there exists a complex interaction of specific and nonspecific systems in the formation of the wave activity, of the structures of the limbic system.

CONCLUSIONS

1. The correlation is shown between the forms of an active state of an animal and the electrical activity frequency of the limbic system structures.

a) A calm awaking state is reflected on the EEG with frequencies of 4—7 osc/sec, presented equally. The oscillations can be of a synchronous and asynchronous character;

b) orientative-investigating behaviour in an unknown situation is accompanied by a synchronous rhythmics of 7 osc/sec, the intensity of which was 2,5—3 times higher than the intensity of other specter frequencies;

c) in an unconditioned feeding and defensive state correlates the domination of 8 osc/sec frequencies; the intensity of a given frequency is 3.5—5 times higher than the intensity of other frequencies.

2) Synchronous activity appears in the investigating structures simultaneously testifying a close functional interconnection of the limbic formations in the organization of the orientative-investigating behaviour.

3) It is supposed that the increase of synchronous oscillations frequency in the EEG of the limbic structures with the different functional states (hunger, pain) takes place on the account of the summation of the additional afferent stimulations.

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TH. BUŞNIŢĂ, GH. BREZEANU, M. OLTEAN, VIRGINIA POPESCU-MARINESCU, ELENA PRUNESCU-ARION *(Monografia zonei Porţile de Fier. Studiul hidrobiologic al Dunării şi afluenţilor săi*—Monograph of the Iron Gates Region. The Hidrobiological Study of the Danube and its Affluents). Ed. Acad. R.S. România, Bucharest, 1970, 270 p., 27 fig. , 44 tabl.

This volume is the first one published as part of the "Monograph on the Iron Gates Region". It is the result of a long and constant study, begun in 1957 and culminating during the 1966—1969 period and it is bringing new and valuable scientific data to the limnological study of the Danube.

Besides the foreword signed by Acad. Şt. M. Milcu and the preface signed by Prof. Th. Buşniţă, the work contains ten chapters, a synthesis of the hydrobiological characteristics of the Danube between the kilometres 943 and 1055, referring also to its main affluents, to its inundation zones as well as to some of the springs existing in this region before the establishing of the new basin of the lake to be formed at the Iron Gates.

The analysis of the problems has a well established chronology starting from the natural environment, presenting data concerning the origin of the Danube valley as well as geographical, geomorphological and climatic considerations. The analysis of these factors is made easier by the presence of a table containing a lot of important data, recorded at Turnu-Severin, regarding both the temperature and the average precipitations which occurred in a span of time of more than half a century (1896—1955).

The particular hydrological features of this region are studied under the aspects directly influencing the specific biotope and biocenoses of the Danube, the rivers, the phreatic waters and the springs, each treated separately. These data together with those already mentioned concerning the geomorphology, climatology, etc., are facilitating a deeper understanding of the chapter IV, by analyzing the physico-chemical characters of these waters.

The greatest part of the study deals with the composition and structure of the biocenoses (three chapters), whose importance results at the very beginning of chapter V entitled *Composition and structure of the Danube biocenoses* under chapter *Microphytic biocenoses* where the authors mentioned : "The algal microfloral study (...) is deprived of historical antecedents (...). Practically, a bibliography of the subject earlier than 1960, does not exist". The same thing is to be seen also in the next chapters and we are giving here but an example : "(...) only the Slătinicul Mare brook was explored as yet in its zone of confluence (...)". (p. 113) The authors give not only complete lists of the species they found there, but also a lot of quantitative and qualitative data concerning organisms and their variations according to numerous factors.

In the economy of the volume, the ichtyofauna is looked upon with a special concern. The composition and dynamics of the main species of fishes of the Danube and of its affluents, as well as the ecological aspects are carefully studied. Under a special heading , *The Parasito-*

faune of the Danube fishes, the parasites of 292 specimens of fishes belonging to 27 species are studied. A number of 77 parasites were found : protozoa (9), monogenea (20), cestoidea (8), trematoda (18), nematoda (12), acanthocephalia (4), hirudinea (1), crustacea(4).

The last but one chapter — *Zoogeographical considerations on the aquatic fauna of the Iron Gates region* — is based on the zoopaleontological aspects of the aquatic fauna (although it is difficult with the exception of some systematic groupings, to do an accurate analysis of the fauna from a paleontological point of view) as well as on the zoogeographical aspects, taking into account the present spreading of the various taxa and their origin.

The prospect treating of the modifications that will take place in the accumulation lake region is particularly important. These possible qualitative and quantitative modifications and their consequences are fully considered. The authors show that the introduction and the adaptation of new invertebrate species, as that of some mysids and cumaceans, should have a great importance for the life of the lake.

According to the new modifications, the ichtyofauna should acquire new features : a number of species as *Alburnus alburnus*, *Rutilus rutilus*, *Abramis brama*, etc., will largely develop while others like *Gobio gobio* and *Sabanejevia aurata* will probably disappear. The fish will be much bigger than the present one, the authors estimating it to 30—40 kg/ha.

Data drawn out from more than 160 papers mentioned in the bibliography were completed by a lot of original ones, increasing thus the value of the work. Yet its value shall be even greater when the profound transformations which are taking place — with both their aspects of creation and degradation — should determine the appearance of new biotopes, of new associations following new laws and new principles of development, thus allowing a better knowledge of the influence such an important hydrotechnical work had on the biological equilibrium of this zone.

This volume is useful to researchers of both hydrobiology, and hydrology.

Modest Guţu

AVIS AUX AUTEURS

La « **Revue Roumaine de Biologie — série de Zoologie** » publie des travaux originaux d'un haut niveau scientifique de tous les domaines de la biologie animale : morphologie, physiologie, génétique, écologie, taxonomie, etc. Les sommaires des revues sont complétés par d'autres rubriques comme : 1. La vie scientifique, qui traite des manifestations scientifiques du domaine de la biologie : symposiums, conférences, etc. 2. Comptes rendus des travaux de spécialité parus en Roumanie.

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