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REVUE ROUMAINE DE BIOLOGIE

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NEW CUMACEA FROM THE NORTH-WESTERN ATLANTIC: *CERATOCUMA PANAMENSIS* N. SP., *CIMMERIUS COSTLOWI* N. SP. AND SOME COMMENTS UPON *PETALOSARSIA DECLIVIS* (G. O. SARS)

BY

MIHAI BĂCESCU and ZARUI MURADIAN

The authors deal with Ceratocumidae and Pseudocumidae from the Western Atlantic Ocean, especially with those found in the R/V Vema stations. They describe and figure: *Ceratocuma panamensis* n. sp. from Eastern Panama waters, *Cimmerius costlowi* n. sp. from the Eastern shore of the U.S.A.; they also complete the description and spreading of *Petalosarsia declivis* from the material taken in the Eastern Labrador waters. A spreading map for the last species is also given.

Continuing our studies [1] on the Cumacea of the R/V Vema Expedition, sent for investigation purposes by the *Lamont Geological Observatory*, and the *American Museum of Natural History* as well as on those of stations made by the *Duke University Marine Laboratory* — Beaufort, in this paper our concern goes to the Ceratocumidae and Pseudocumidae families, namely:

1. **Ceratocuma panamensis** n. sp. (Fig. 1 and 2)

Description of preadult female. The whole tegument finely reticulated with strong scales, especially on the appendages, becoming prominently serrated ridges on uropoda, antennae and maxilliped III. Carapace wide ± oval (Fig. 1 *A*), nearly twice as wide as deep (Fig. 1 *C*), with 7 pairs of strong lateral spiniform tubercles. On the upper side only two truncate tubercules, hillock-like, situated in the posterior third of the carapace, and a bifid apophysis posteriorly directed, on the mediodorsal posterior portion. Carapace 2.2 mm in length, 1.32 mm in width. Frontal lobe large with a small median protuberance reminding the place of the completely missing eyes. Five pairs of peraeonites, free, equal in length, decreasing in width towards tail, last peraeonite with rather plaeonite aspect, as the peraeopod is also absent.

First peraeonite with only one epimeral projection; II, III and IV each with one pair of strong latero-dorsal spine-like projection, whilst the Vth last peraeonite bears such spiniform projections dorsally directed, as in plaeonites. Peraeonite II bears on its mediodorsal line a replica of the carapace, an anteriorly directed bifid apophysis. At the basis of Mxp. III and Prop. I—III, each with one pair of reduced oostegites

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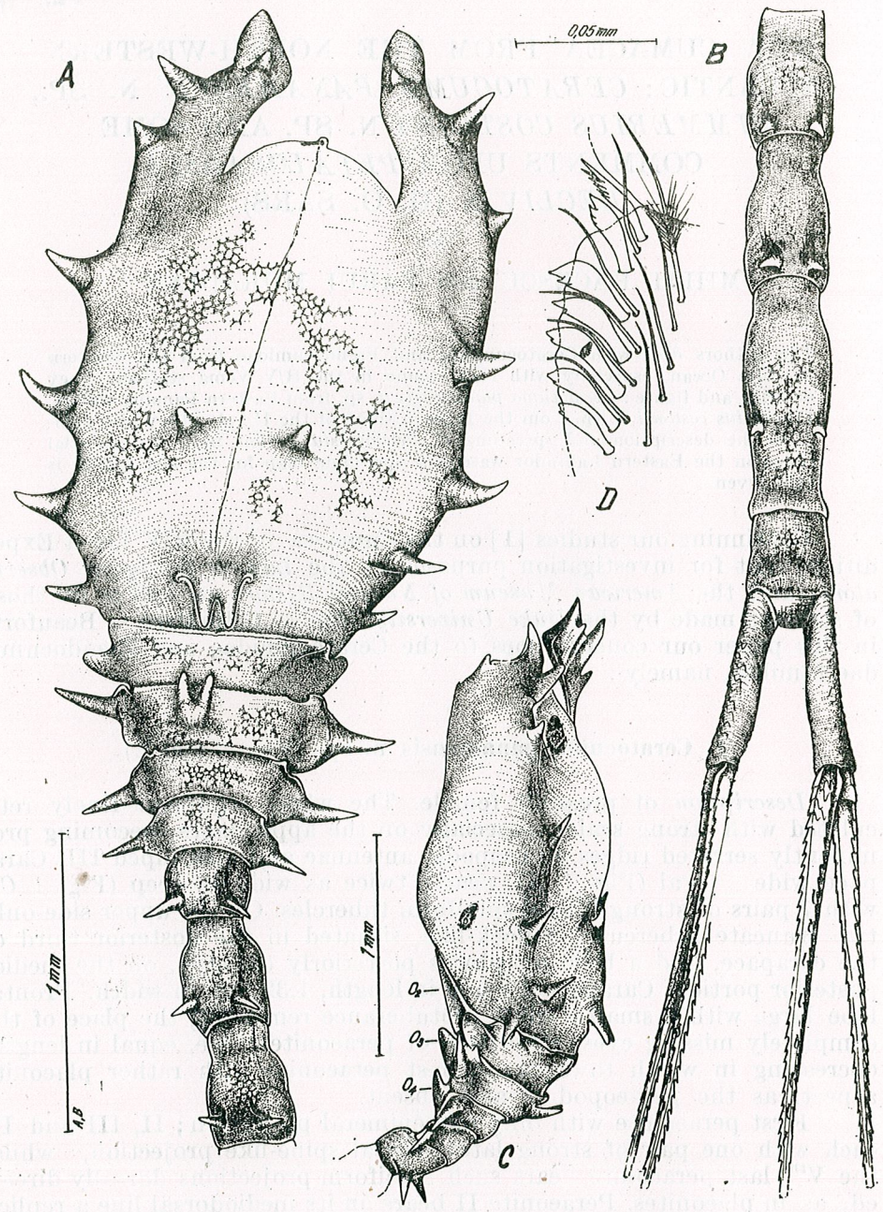

Fig. 1. — *Ceratocuma panamensis* n. sp. ♀ P. *A*, carapace and thoracomers, as seen from above; *B*, abdomen; *C*, anterior part, in lateral view; *D*, separate phanera of infero-interior edge of the carpus of maxilliped I (Orig.).

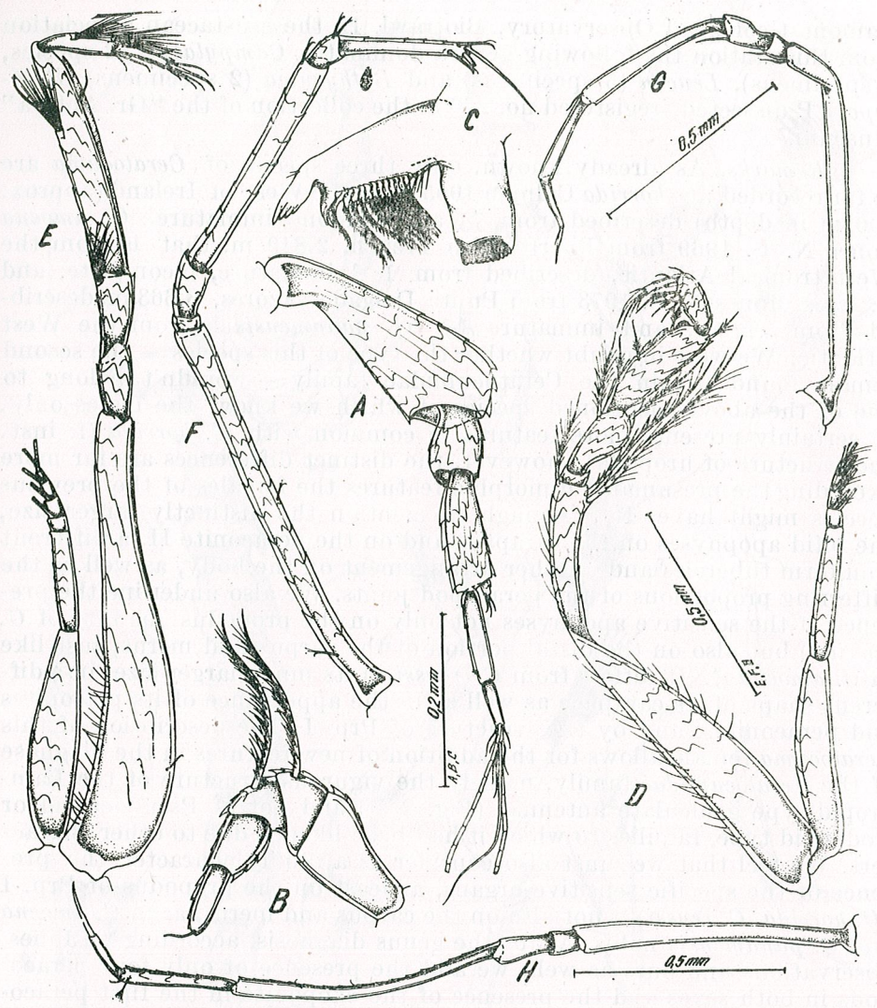

Fig. 2. — *Ceratocuma panamensis* n. sp. ♀ *P. A*, antennula; *B*, antenna; *C*, terminal part of left mandible; *D*, maxilliped III; *E*, peraeopod I; *F*, peraeopod II; *G*, peraeopod III; *H*, peraeopod IV (Orig.).

Fig. 1 *C*, *O* 2—4). Antennula (Fig. 2 *A*) of ceratocumid type. Antenna (Fig. 2 *B*) consisting of 5 joints, geniculated between joints 2 and 3. Oral pieces somewhat as in *Ceratocuma horrida*. Peraeopod I (Fig. 2 *E*) with tubercles bearing sensitive setae, not only on the propodus but also on the carpus and merus. Prp. II (Fig. 2 *F*) without exopodite. Plaeopods absent. Uropods somewhat resembling *C. horrida*. *Length* ♀ P, 8.1 mm (carapace, 2.2 mm; thoracomers, 1 mm; plaeomers, 2.9 mm; uropods, 2.06 mm). *Material*: one single female specimen with small marsupial lamellae, in St. 101 Lamont, N—E Panama, 10°11′ N and 78°30′ W, 1,616—1,534 m,

Lamont Geological Observatory, Biotrawl. In the crustacean association from this station the following genera dominate : *Campylaspis* (2 species, 6 specimens), *Leucon* (5 specimens) and *Bathycuma* (2 specimens). *Holotype* ♀ P dissected, registered no. 239 in the collection of the "Gr. Antipa" Museum.

Remarks. As already known, only three species of *Ceratocuma* are so far recorded : *C. horrida* Calman 1905 from the West of Ireland (approx. 690 m in depth) described from ♂♂ ad. and one immature, *C. amoena* Jones N. S. 1969 from Puerto Rico Trench, 2,840 m, that is from the West tropical Atlantic, described from 1 ♂ immature, incomplete, and *C. reyssi* Jones N. S. 1973 from Punta Delgada, Azores, 3,663 m, described from ♀♀ ad. and immature ♂♂. *C. panamensis* is from the West Atlantic. We were in doubt whether the type of this species — the second female found within the Ceratocumidae family — wouldn't belong to one of the above mentioned species of which we know the males only. It certainly presents more features in common with *C. horrida* (f. inst. the structure of uropoda) ; however, the distinct differences are far more exceeding the presumable dimorphic features the females of the previous species might have. It is enough to mention the distinctly larger size, the bifid apophyses on the carapace and on the peraeonite II, a different spiniform tubercles and another arrangement on the body, as well as the differring proportions of the peraeopod joints. We also underline the presence of the sensitive apophyses not only on the propodus (as in ♂ of *C. horrida*) but also on the distal portion of the carpus and merus, just like in *C. amoena* ♂. It differs from *C. reyssi* in its much larger size, in a different shape of its carapace as well as in the appearance of its plaeonites and peraeonites, and by the structure of Prp. I. The description of this *Ceratocuma* female allows for the addition of new features in the diagnose of the *Ceratocumidae* family, namely the vigurous structure of the Lampropid-type geniculate antennae (Fig. 2 B) and not of Pseudocumid or Bodotriid type, families to which it had been likened due to other characters. We feel that we must also consider as a family character the presence of the specific sensitive organs, at least on the propodus of Prp. I (*C. horrida*, *C. reyssi*) if not also on the carpus and merus, as in *C. amoena* and *C. panamensis* nobis. As for the genus diagnosis, according to Jones' observations and ours as well, we add the presence of only four peraeopods in both sexes and the presence of the exopodite in the first peraeopod only in the female.

2. **Cimmerius costlowi*** n. sp. (Fig. 3 and 4)

Description of adult female and immature male. Carapace and remaining tegument covered with scales, especially on the appendages. Carapace with a series of polygons bordered by faint ridges. Antennal notch distinct ; inferior margin glabrous, ending in infero-anterior angle, which is rounded, serrated. Carapace abruptly declining towards pos-

* Friendly dedicated to Dr. John D. Costlow, Director, Marine Laboratory — Beaufort, N. Carolina

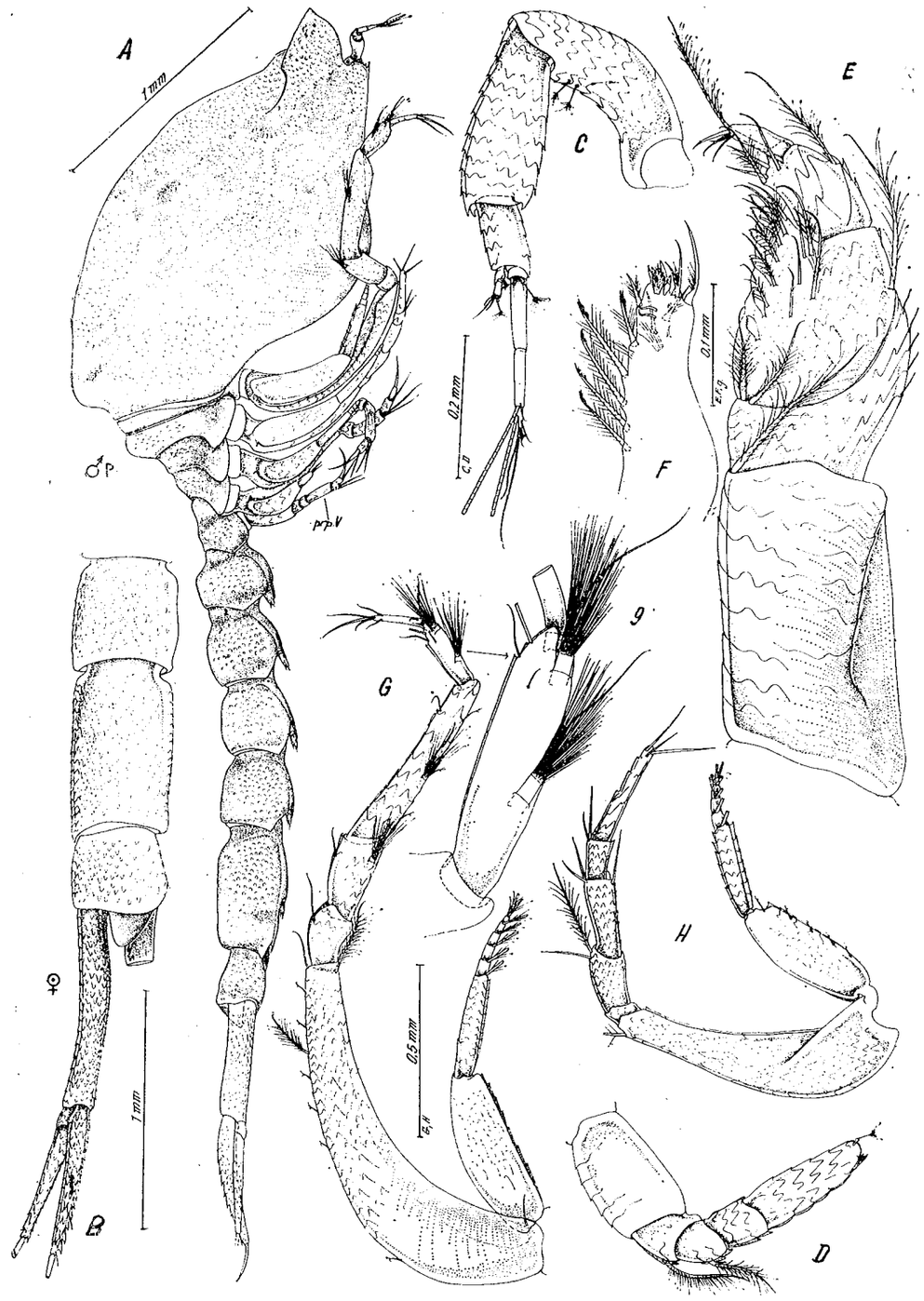

Fig. 3. — *Cimmerius costlowi* n.sp. A, E and F, ♂ P ; B—D, G and H, ♀M. *A*, ♂P. of 5.4 mm ; *B*, least abdominal segments, telson and uropods of 7 mm ♀ M. ; *C*, antennula ; *D*, antenna ; *E*, maxilliped I ; *F*, its endite ; *G*, peraeopod I ; g, magnified propodus, so that details of apophyses bearing sensitive hairs, may be seen ; *H*, peraeopod II. (Orig.).

tero-dorsal part, joining the first free short thoracomer. Next thoracomer forms a distinct gibbosity, followed by the other 3, without particular appearance (Fig. 3 *A*). Prp. I (Fig. 3 *G* and *g*) with two sensitive hair tufts on propodus, one on the carpus, and a fourth one on the merus. Prp. II (Fig. 3 *H*), III—IV and V (Fig. 4 *C—E*), of *Campylaspis* type, just like their whole morphology. All first four peraeopods have strong exopodite, the size of which slightly decreases towards Prp. IV, the flagellum of which practically disappears (Fig. 4 *D*), being reduced to a minute joint (*j*), only the basal joint keeps the size of the others. Plaeopods in preadult males biramous and with biarticulated exopodite (Fig. 4 *F*). Ratio between length of basis and foliaceous rami certainly changes in sexually mature males. Uropods in ♂ with basis slightly shorter than rami; in female the basis is much longer than rami (Fig. 3 *B*). *Length*: ♂ P, 5.4 mm, of which carapace, 1.7 mm; ♀ M., approx. 7 mm. *Material*: 2 ♂♂ P and 1 ♀ crushed, in St. 6238 Beaufort, 32°55′9″ N and 75°48′6″ W, 3,000 m depth; 1 ♀ without carapace in St. 6236 Beaufort, 33°18′5″ N and 75°46′0″ W, 3,020 m, N—E Florida. *Holotype*: ♀ P, no. 240 coll. "Gr. Antipa" Museum; allotype ♀ m, incomplete, ibid. no. 240 b.

Remarks. The main characteristic of this new genus (Jones, 1973) is the unique formula among the Cumacea consisting in the presence of 4 exopodites (the 4th only partially reduced in ♀) at the 5 peraeopods. We certainly found in many other families exopodites and rudiments

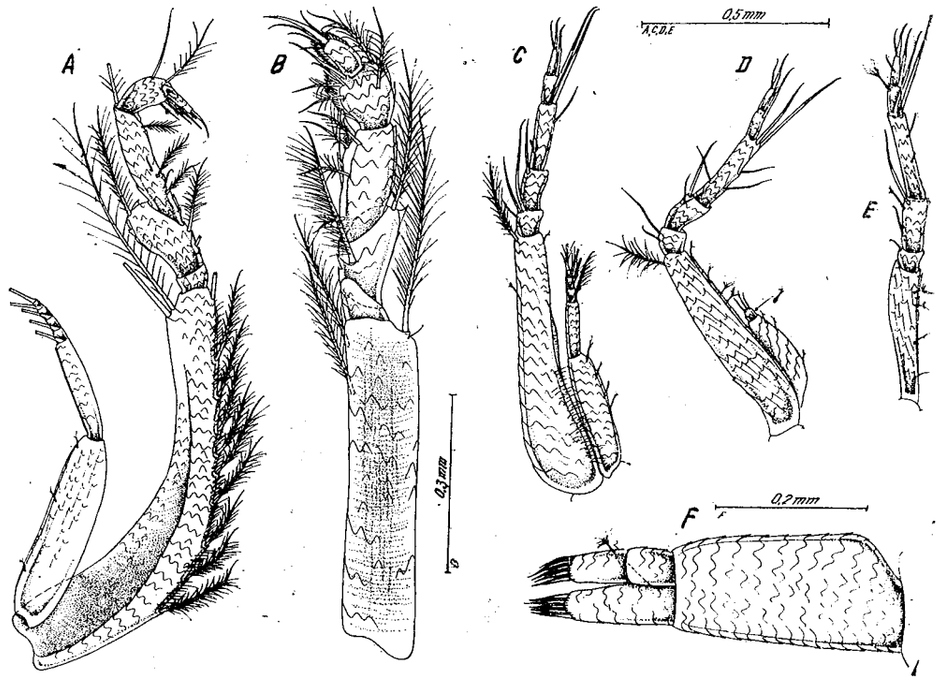

Fig. 4. — *Cimmerius costlowi* n. sp. A and C—E, ♀ M.; B and F, ♂ P. *A*, maxilliped III ♀ M.; *B*, maxilliped II ♂ P; *C—E*, peraeopods III—V ♀ M.; *F*, pleopod I ♂. P (Orig.).

of 1—2 exopodites in the first pairs of peraeopods (Bodotriidae, Pseudocumidae, Lampropidae and Diastylidae), but in none of these did we find them associated in the formula 3 + 1/2 r [8, pp. 59 and 183].

The present contribution was already in print when we received Jones'paper: Some new Cumacea from deep water in the Atlantic, *Crustaceana*, **25**, 1973, 297—319. We have also recognized that the above described crustacean belongs to a new genus, a genus we have differently named and which we do not mention here to avoid the introduction of additional name in the already quite complicated synonimies of the Cumaceans. We also doubted about the identity of the two, but by carefully comparing our figures 3 and 4 to those of *C. reticulatus*, as well as the respective descriptions, it clearly resulted that there were sufficient specific differences to leave as such both the description and the figures of *C. costlowi*. We emphasize but the differences established:

Cimmerius reticulatus Jones 1973	**Cimmerius costlowi** n.sp.
♀ ad. 5 mm; ♂ P 5.3 mm	♀ ad. 7 mm; ♂ P 5.4 mm
Pleonites are figured with a dorsal carena.	Pleonites without dorsal carena
Antennule provided with one single aesthetasca.	..with two aesthetascae.
Antenna three-segmented.	..five-segmented.
Carpus of Mxp. I cut ± straight.	..anteriorly prolonged.
Propodus of Mxp. II narrow.	..wide and ± discoidal.
Carpus of Mxp. III widened as the merus.	..not widened.
Ischium of Prop. I as long as half the length of the merus.	..a little longer than 2/3 of merus.
The dactylar claw of Prp. I twice as long as the dactylus.	..shorter than the dactylus.
Tufts of setae are found on the propodus and on the carpus of Prp. I	..On the propodus, carpus and merus.
Basis of Prp. III double figured in length in comparison with the distal segments together.	Basis 2/7 longer than the rest.
Carpus of Prp. III longer than the two anterior segments together and than the two next segments together.	..equal.
Uropodal endopodite in ♀ half the length of the peduncle (figure shows endopods as equal to peduncle).	This endopodite is 4/5 the length of the peduncle, while in the ♂ it is equal.
Uropodal endopodite provided with 7—8 short spines on the interior distal edge, ending in two unequal spines.	Edges of uropodal endopodite bordered with scales, more conspicuous all along the inner edge, ending in one strong apical spine and one single hair.

The study of the available material led us to the conclusion (recorded in advance) that the ♀ five-segmented antenna should be included in the diagnosis of the Ceratocumidae family. If the species described by Jones (1973) have indeed a three-segmented antenna in the females, then our statement would not remain valid.

3. **Petalosarsia declivis** (G. O. Sars) (Fig. 5—7)

This species is the only representative of the Pseudocumidae family occurring out of the European waters; even though it is one of the few Cumacea with large northern spreading (Fig. 7). *P. declivis* was nowhere found in high number. This may account for the lacunae and lack of precisions in its description. The species was described by Sars [6] mainly from a ♀ specimen, the data given for the ♂ are but compendious, as the author figured only the profile, A_1 and plaeopods. As in the Vema stations an abundant material belonging to this species of the N—E of Labrador is available, we deem some precisions as necessary, especially with regard to the morphology of males, namely:

Carapace ♀ (Fig. 5 *A*) with a lower number of ridges and less even than in the figure of Given [3, Fig. 5 *B*, p. 223]; it may be another apart subspecies from Point Barrow, N-Alaska. Abdomen ♂ (Fig. 5 *B*) with antennal notch just on the ventral-median line; flagelli longer than figured by Sars, reaching the tip of uropodal exopodite, are fixed in grooves by the crossing of 2 flagellated phanera of each abdominal somite, each lined by one pair of hairy seta at the first 4 plaeonites. Uropods ♂

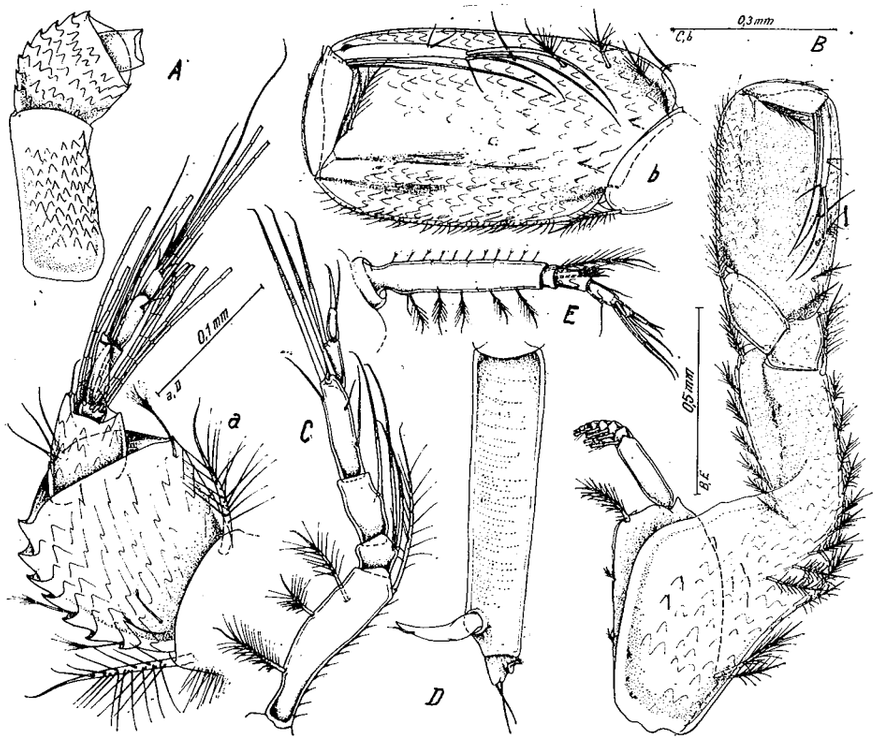

Fig. 6. — *Petalosarsia declivis* (Sars) *A*, antennula ♂ ad.; *a*, details of antennula ♂ ad.; *B*, peraeopod I ♂ ad.; *b*, details of peraeopod I ♂ ad.; *C*, peraeopod V ♂ ad.; *D*, pleopod II ♂ ad.; *E*, peraeopod III ♀ M (Orig.).

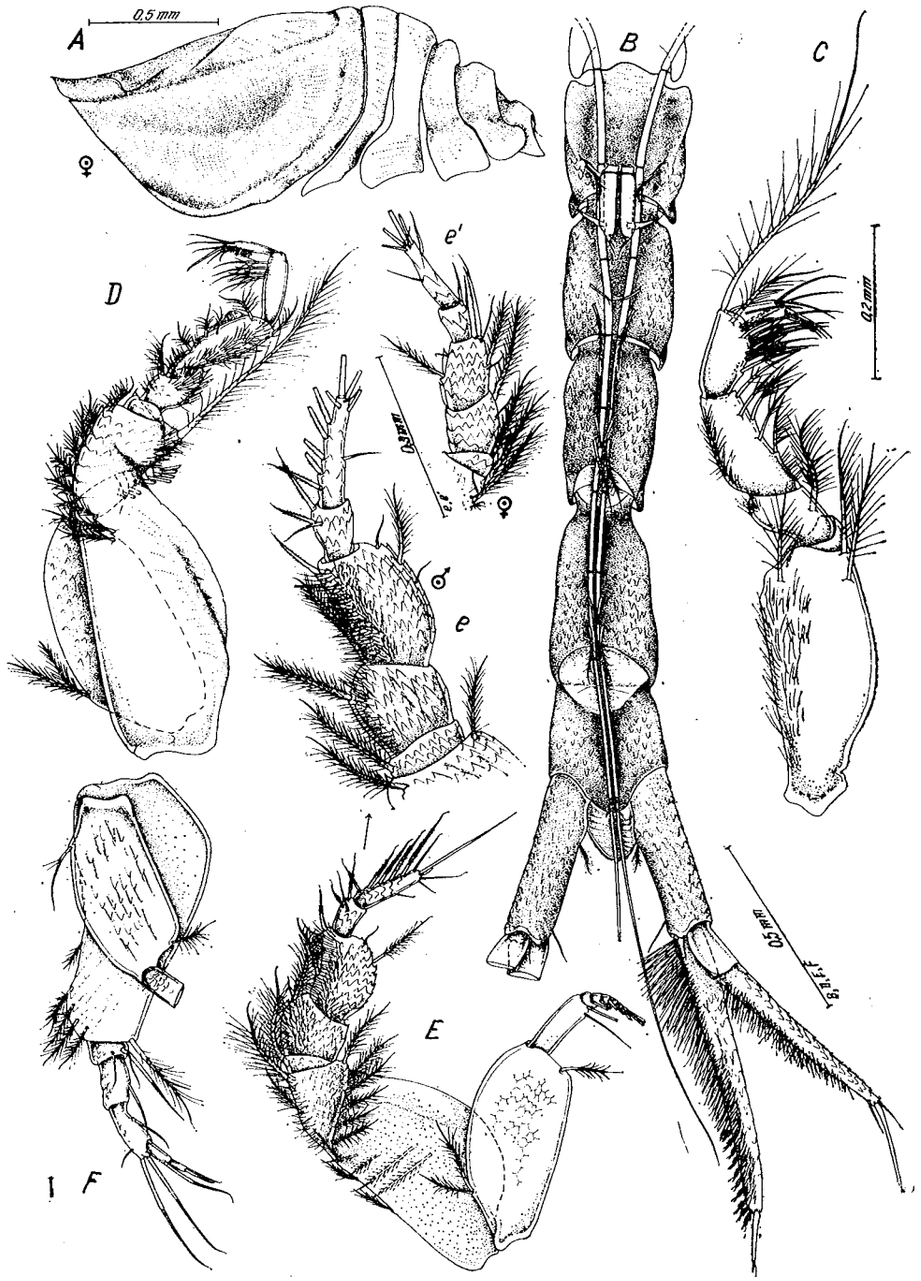

Fig. 5. — *Petalosarsia declivis* (Sars) *A*, carapace ♀ M. in lateral view; *B*, abdomen (starting with segment 2) and uropods, ♂ ad.; *C*, maxilliped II ♂ ad.; *D*, maxilliped III ♂ ad.; *E*, peraeopod II ♂ ad.; *e*, details of these peraeopod ♂ ad.; *e'* details of these peraeopod ♀ M.; *F*, peraeopod III ♂ ad. (Orig.)

(Fig. 5 *B*) with proportions as figured by Stappers [7], presenting a dense pilosity on the exopodite, too. A_1 ♂ (Fig. 6 *A*) with segment 2 shorter than figured by Sars; same proportions as in ♀. Details of Fig. 6a show that at segment 2, it is not a question od „3 blunt serrations" as given by Sars but there are 6—8 indententions resulting from the margins of the scales, which substantially cover the antennula; the hairs in Sars figure represent, in fact, the aesthetascae. Prp. II (Fig. 5 *E* and *e*) even though its merus and carpus differ in size and phanerotaxia from those in ♀, has in the main the same appearance, hence it is surely the ♂ of *P. declivis* and not of another species. The Prp. III ♀ (Fig. 6 *E*) on which Sars writes (as he does on peraeopod IV) that they have "a very small rudimentary exopodite" present, in fact, some apophyses and not rudiments of exopodites as those conventionally known in other species of Cumacea; in Prp. IV even these are absent. Plaeopod II ♂ (Fig. 6 *D*) differs from that figured by Sars and neither agrees with the type of *Pseudocuma*, as it had been given, having a rudiment of subapical articulation and 2 types of phanera on the apex.

Remarks. In the material collected by the 16th Vema Expedition we found nearly 100 specimens (4 ♂♂ ad., 60 ♀♀ M., 16 ♀♀ P., 16 ♀♀

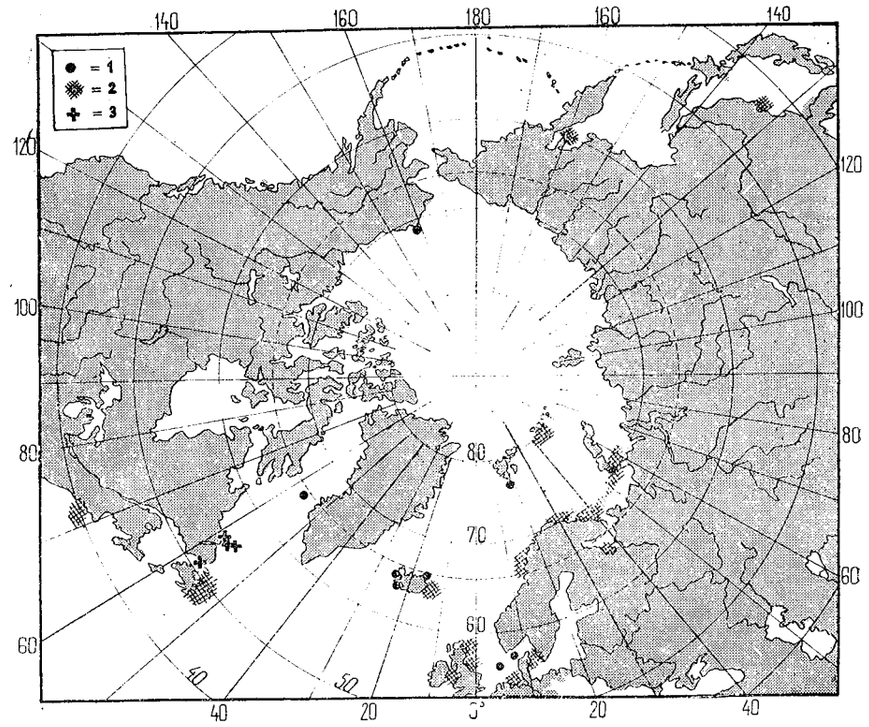

Fig. 7. — Map of spreading of species *Pelalosarsia declivis* (Sars) according to literature data and from our material: *1*, stations known; *2*, collecting areas known; *3*, stations of R/V Vema (Orig.).

juv.) all of them dredged N—E of Labrador (Fig. 7) namely: St. 12, 29/VIII/1960, 51°09′ N and 57°23′ W, 2,078 m; St. 58,59,63 (90—95 m), 1—6/IX/1960, 55°37′ N and 56°08′ W, 55°26′ N and 58°09′ W, 55°00′ N and 57°57′ W, respectively.

Petalosarsia declivis, as it distinctly appears from the known sites of its occurrence, which will certainly increase in the future, is a boreal-arctic species, with a large distribution between latitudes 50° and 70° N; only sporadically, it occurs beyond these latitudes at 40° N in the Okhotsk Sea, U.S.A. coast, as well as at about 80° N at Spitzberg and at Franz Joseph Islands. The species also seems to present a large eurybathya as it is currently found between 18 m (Franz Joseph Islands) and 450 m (Skagerak, Norwayan coast Islands, a.s. o.); the Vema material established an exceptional depth record for this species, of 2,078 m, between Labrador and Newfoundland.

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LA STRUCTURE FINE DU NÉPHROCYTE DE L'ÉCREVISSE *ASTACUS LEPTODACTYLUS*

PAR

MIRCEA IONESCU-VARO

Electron optic studies were carried out on the fine structure of the crayfish nephrocyte originating in the green and the white labyrinth. The existence of the striated border (microvilli), of the lysosomes (green granules) and of the basal labyrinth of the plasmolema was observed in the frist type of cells. The second type of cells was found to contain only basal infolding which were made of fairly regular vesicles belonging to the basal plasmolema. These findings are consistent with the view that the cellular configuration of these nephrocytes closely ressembled the ultrastructural pattern alreaay described in the nephrocytes of the vertebrates.

Les études de microscopie électronique ont prouvé toute une série de particularités en ce qui concerne le néphrocyte des vertébrés. Ainsi, on a décrit les microvilli de la bordure en brosse, les invaginations du plasmolemme basal qui constituent un véritable « labyrinthe basal » et le phénomène de pinocytose qui détermine l'apparition de lysosomes dans le cytoplasme [1], [6].

Les recherches entreprises sur les néphrocytes des invertébrés ont été réalisées à une époque quand les méthodes de fixation n'étaient pas encore pratiquées.

Dans la présente étude nous nous proposons d'analyser la structure fine du néphrocyte de la glande verte d'*Astacus leptodactylus*, étant donné que la seule recherche entreprise sur cette glande date de 1956 et a eu comme matériel d'étude un seul crabe *Cambarus*.

MATÉRIEL ET MÉTHODES

Les écrevisses de rivière *Astacus leptodactylus* sont dissequées immédiatement après leur capture et la glande verte a été fragmentée dans un mélange de glutaraldéhyde 3% et paraformaldéhyde 2%, en tampon cacodylate 0,1 M pH 7,4. La préfixation a duré 1 heure et, après un lavage d'une heure dans le même tampon, les pièces ont été transférées dans Os O_4 en tampon de collidine. Des fixations ont été faites aussi dans le mélange de Palade avec 7% sucrose, et ensuite déshydratées avec de l'acétone et incluses dans du Vestopal ou Durcupan. Les coupes ont été faites au moyen de l'ultrotome LKB, muni de couteaux de verre et doublement colorées à l'acétate d'uranyle, puis au citrate de plomb. Elles ont été observées au microscope électronique Hitachy de l'Institut « Dr. I. Cantacuzino ».

RÉSULTATS

Les dernières études amples sur la glande verte des Décapodes ont été faites par Peters [5], Maluf [2] et Parry [4] qui ont déterminé les fonctions des différentes parties de ce rein céphalique avec les méthodes

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dont disposait alors la cytophysiologie. On sait depuis Marchal [3] que chaque glande verte est constituée d'un saccule, d'un labyrinthe vert, d'un labyrinthe blanc et d'une vessie collectrice de l'urine produite. Certains auteurs considèrent le saccule comme un cœlomosac et le labyrinthe a une structure compacte totalement distincte du labyrinthe blanc, qui est considéré comme un véritable tubule néphronique [1], [5].

Le labyrinthe vert consiste en fait en néphrocytes typiques, semblables au segment principal du néphron des mammifères (fig. 1). Au pôle apical des cellules il y a un plateau de microvilli, qui cependant n'est pas toujours continu, parce que sur certaines portions la plasmolemme forme par ses protrusions des « bouchons » dans le plateau microvillaire. A la base des microvilli le cytoplasme forme en permanence des invaginations vésiculaires, qui suggèrent une pinocytose active de liquide. Le plasmolemme basal forme des invaginations continues, rarement vésiculaires, qui enferment entre les compartiments interlamellaires des mitochondries et des lysosomes. Les mitochondries, qui sont filamenteuses sur les sections épaisses pour le microscope photonique, apparaissent avec un contur ovale ou circulaire sur les sections ultrafines. Elles ont une matrice claire, finement granulée, et sont riches en cristae et tubules. La moitié inférieure de la cellule est remplie de lysosomes typiques, sphériques, entourés d'une seule membrane et ayant un contenu finement granulaire (fig. 2). Parfois ils contiennent aussi un corpuscule opaque. Au milieu de la cellule on peut remarquer de grandes formations lysosomiales, d'aspect autophagosomique, qui ont inclus des restes de mitochondries et des figures myéliniques. L'ensemble lysosomial qui entoure le noyau dans sa moitié inférieure représente en fait les granules verts décrits par les anciens auteurs.

L'appareil de Golgi est représenté par de petits dictyosomes qui sont situés pour la plupart dans le tiers supérieur de la cellule. Ils sont formés de quelques citernes aplaties et de vésicules de grandeur diverse, accumulées en ordre dans une portion de l'hyaloplasme privée d'ergastoplasme. Le reste de l'hyaloplasme est occupé par des vésicules sphériques et ellipsoïdales du réticule endoplasmique lisse. Parfois on trouve sur elles des ribosomes. Au long de la cellule, parallèlement à son grand axe, se trouvent des microtubules.

Du point de vue histochimique, les microvilli ont une réaction à la phosphatase alcaline par la méthode Gömöri ou par couplage diazoïque (naphtol AS—TR et Fast blue BB), le labyrinthe basal à l'ATP^{ase}, les lysosomes ont une phosphatase acide mise en évidence par la méthode du couplage diazoïque (naphtol AS—TR et Fast red LB) et des estérases non spécifiques (naphtol AS—D et Fast garnet GBC).

Le noyau occupe une bonne partie de la cellule. Il a une forme ellipsoïdale avec toutes les caractéristiques bien connues : membrane à pores denses, désoxyribonucléoprotéines filamenteuses formant des mottes plus ou mois étendues, un nucléole compact granulaire et une série de granules dispersés avec des ribosomes dans le cytoplasme.

Le labyrinthe blanc est formé par des tubules néphrotiques caractérisés par l'absence de la bordure en brosse et par l'existence d'un labyrinthe basal formé non seulement de lames continues, mais aussi de vésicules. En effet, sur le glycocalixe basilaire, dont les filaments sont dirigés

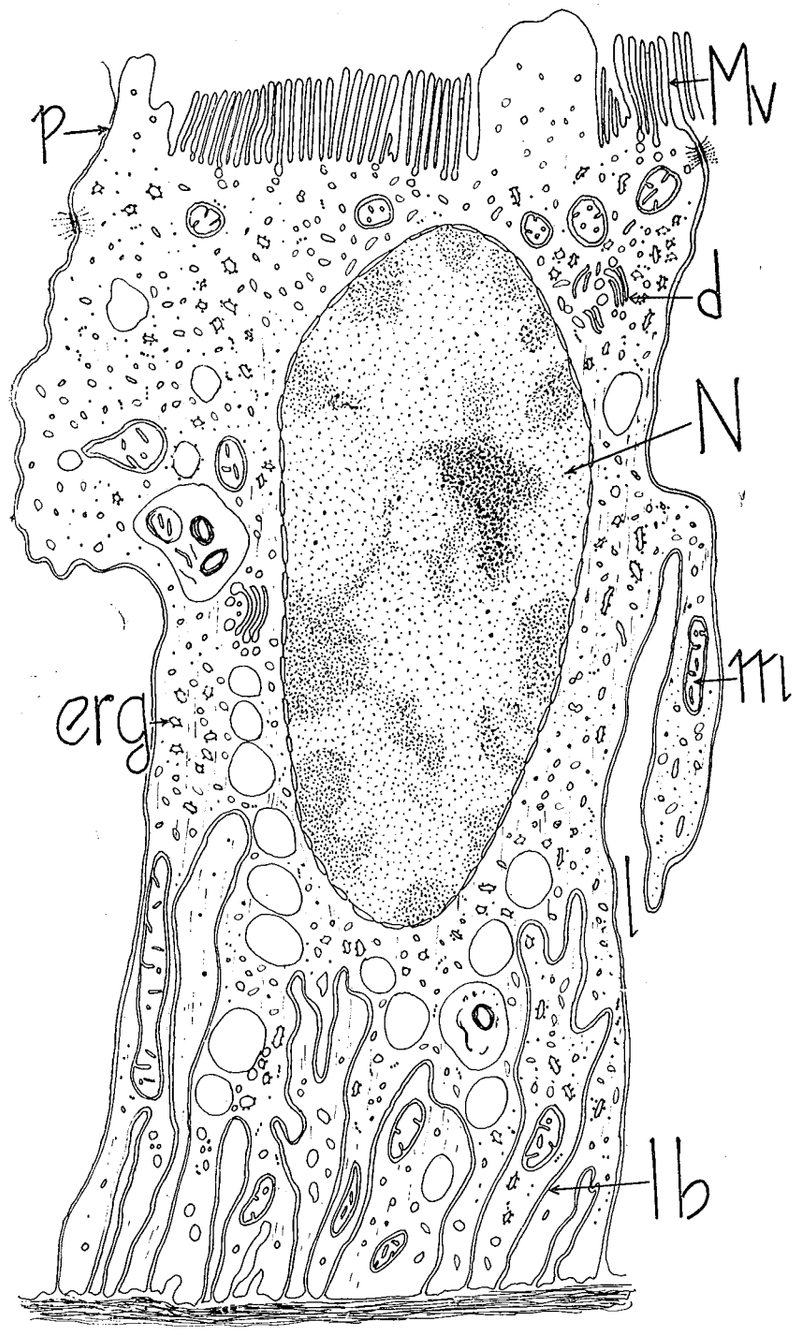

Fig. 1. — Néphrocyte du labyrinthe vert. *MV*, microvilli de la bordure en brosse ; *p*, plasmolemme ; *N*, noyau ; *l*, lysosomes ; *lb*, labyrinthe basal ; *g*, glycocalix basal.

en trois directions, la membrane est invaginée en lames doubles continues, qui sur certaines portions deviennent vésiculeuses, en réalisant une amplification de la surface d'échange cellulaire (fig.3). On peut voir ainsi des chapelets de vésicules qui décrivent des trajets sinueux, changent de direction, se courbent, sur certaines portions se reconstituent en lames, pour revenir à la structure lamellaire. Entre les compartiments de ce genre de labyrinthe basal on aperçoit les mêmes mitochondries avec peu de cristae et de tubules (fig. 4). Les dictyosomes sont petits et localisés dans la partie supérieure de la cellule. Ils sont beaucoup plus petits qu'une mitochondrie filamenteuse et sont formés de 6 à 7 citernes aplaties, du bord desquelles se séparent de grandes vacuoles sphériques. Les saccules ont une structure réticulée, car sur des sections transversales ils paraissent interrompus par endroits. Les vésicules transporteuses, détachées de l'ergastoplasme, s'insinuent à la limite supérieure du dictyosome, entre celui-ci et une mitochondrie voisine dont elle peut emprunter du matériel énergétique transportable.

Le noyau sphérique présente souvent des septums qui le séparent presque en 2 noyaux. D'ailleurs chaque moitié possède un nucléole typique, compact et granulaire (fig. 5), étant en fait une cellule endoploïde. Le labyrinthe basal contient de l'ATP^{ase} et quelques phosphatases acides.

La structure du néphrocyte de la glande verte est typique, dans le sens qu'on la trouve dans les tubules néphrotiques de toute la série animale. L'existence d'un plateau apical de microvilli et d'un labyrinth basal doit être attribuée à la résorbtion de l'eau et à la concentration de l'urine. Par ailleurs, l'existence des lysosomes basaux suggère la pinocytose de l'extérieur d'une série de produits du catabolisme qui sont détruits ou rendus inoffensifs dans les lysosomes. On sait que l'activité de résorption caractéristique du labyrinthe des Crustacés dulçaquicoles a comme conséquence la production de l'urine définitive. D'autre part la présence de l'ATP^{ase} dans le labyrinthe basal indique l'apport de l'ATP nécessaire au transport actif par le plasmolemme du pôle inférieur. Si on ajoute aussi l'augmentation de la surface d'échange par la vésiculation de membranes du labyrinthe basal, on comprend pourquoi celui-ci représente le maximum des échanges à travers la membrane.

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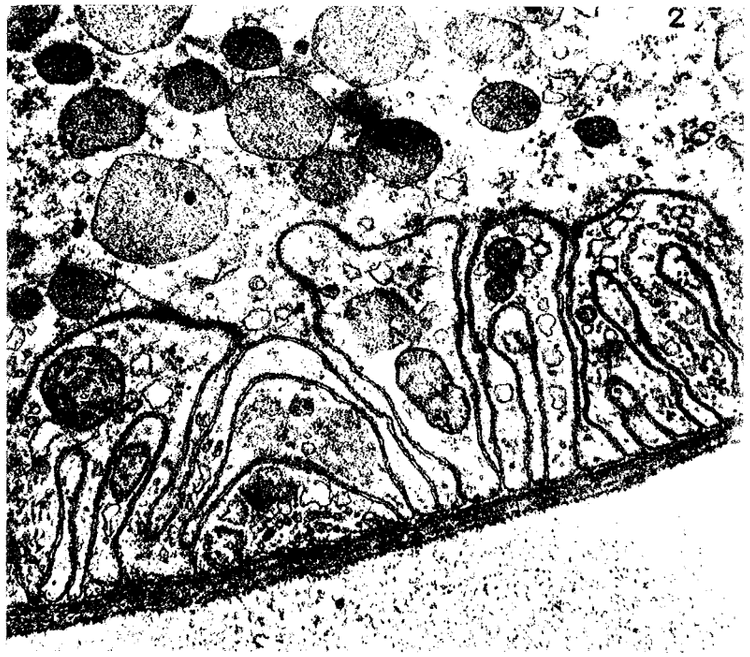

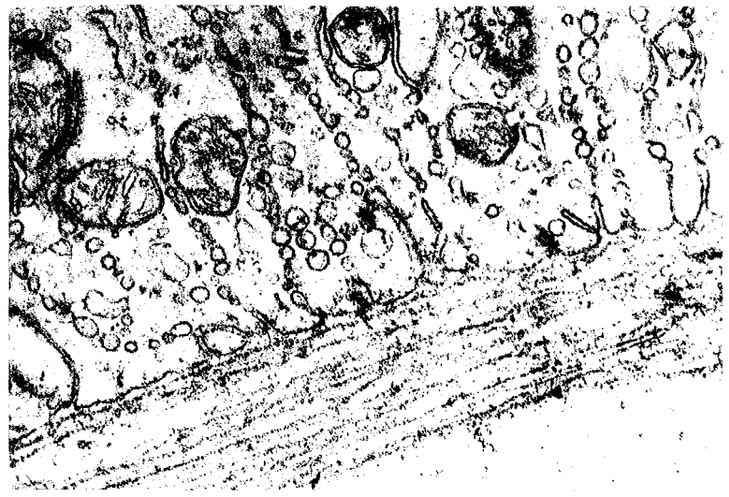

Fig. 2.—Labyrinthe basal des néphrocytes du labyrinthe vert. *m*, mitochondries ; *l*, lysosomes ; *co*, compartiments du labyrinthe ; *l.b.v.*, labyrinthe basal vésiculaire ; *g*, glycocalix basal. 11.600

Fig. 3. — Labyrinthe basal des néphrocytes du labyrinthe blanc. *l.b.v.*, labyrinthe basal vésiculaire ; *m*, mitochondries ; *g*, glycocalix. 12.800

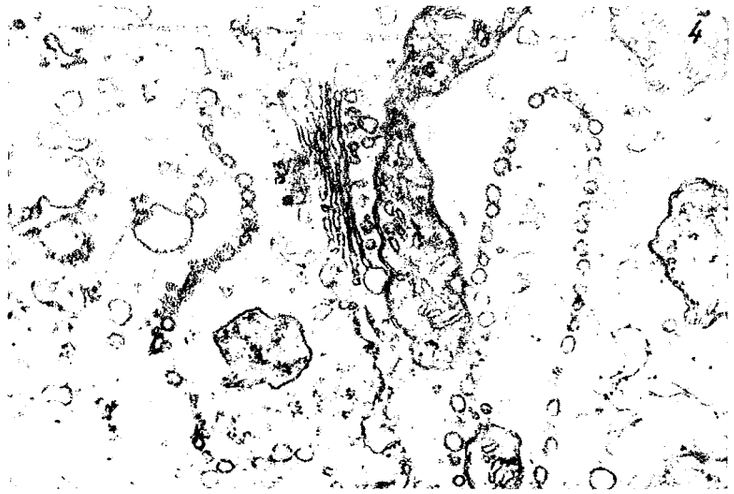

Fig. 4. — Cytoplasme d'un néphrocyte du labyrinthe blanc. *d*, dictyosome ; *m*, mitochondrie ; *vt*, vésicule transporteuse ; *lbv.*, labyrinthe basal vésiculaire ; *mt* microtubules 16.000.

Fig. 5. — Portion du noyau d'une néphrocyte du labyrinthe blanc. *mn*, membrane nucléaire ; *cr*, chromatine ; *nu*, nucléole ; *crp*, chromatine périnucléaire. 14.400.

THE FINE STRUCTURE OF PREVITELLOGENETIC OVOGONIA AND OVOCYTES IN *CYPRINUS CARPIO*

BY

MARIA CALOIANU-IORDĂCHEL and LILIANA BABEŞ

The previtellogenetic development of the female sexual cells in *Cyprinus ccrpio* was studied. Three characteristic stages concerning the modification of the cell organelles are described.

The study of ovogenesis has been the object of many works for almost a century. These tried to describe and elucidate modifications occuring in the female sexual cells in various steps of their evolution to the fecundation-apt stage. In recent years, to cytological, cytochemical and biochemical studies were added numerous works which analysed the structure of sexual elements under the electron microscope. New details have thus been revealed, contributing to point out some essential processes carried during ovogenesis. These are : auxocytosis, nucleus-plasma exchanges, vitellogenesis, etc.

The many studies devoted to ovogenesis in the fishes dealt with different aspects of this process, such as : the structure of the various organites in young ovocytes [3] [4] [18] [25] [28] [29], the question of the nucleolar material transfer [30], the formation of the pellucid membrane [1] [9] [10] [29], the vitellogenesis process [13] [22], the hystochemistry of the different categories of substances from the ovocytes [11] [12] [19], etc. All those works especially referred to certain development stages of the ovocytes. We have considered it useful, therefore, to study alterations occurring in the female sexual cells, starting with ovogonia (a very little studied stage in literature) and ending with ovocytes in previtellogenesis. The synthesis study on ovogenesis, carried out by J. Bruslé in 1972 [6] for the whole animal series, was a landmark for us.

MATERIAL AND METHOD

The material was represented by previtellogenetic ovogonia and ovocytes, taken from young and adult specimens of *Cyprinus carpio* L. For study under the electron microscope, the samples were prepared according to the routine method, viz. prefixation in glutaraldehide 2% in phosphate or cacodylate buffer, fixation in OsO_4 1% and then inclusion in Durcupan, after a prev dehydration in ethyl alcohol of increasing concentration and propylene oxide. Ultrafine sections were cut in a Tesla ultramicrotome and stained with uranyl acetate and lead citrate, a according to Reynold's method (1965). Samples were examinated under a JEM-7 electron microscope.

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OBSERVATIONS AND DISCUSSIONS

To point out stages we intended to study, we made use of the research-work on ovogenesis in *Cyprinus carpio* carried out by Steopoe et al. [24] in light microscopy.

A. *Ovogonia* (Stage I, according to Steopoe) are cells with irregular outline, little cytoplasm but with a bulky nucleus (Fig. 2). Ovogonia come into contact with one another, without evincing continuity at those levels (Fig. 2). At the contact level there appear no cell junctions or membrane thickening. In *Cyprinus*, the syncytial aspect of ovogonia which is a rule in mammals [14] [15] [23] [26] is therefore not observed. It is met in other species of vertebrates and invertebrates. Those bridges, resulting from incomplete cytogenesis account for the synchronous evolution of ovogonia in these species, according to some authors. In Teleosts, their absence may be linked to the fact that no exchanges of organelles and information occur, as there is no simultaneous evolution.

The nucleus, as mentioned before, is bulky, representing about half of the cell volume. Its outline is sphaerical, regular and only sometimes with incisions (Fig. 2) In some nuclei, chromatin is uniformly distributed, while in others agglomerations of heterochromatin are visible especially at the periphery. The nucleus generally has a single central or excentric nucleolus, made up of its characteristic components (Fig. 2). The nuclear membrane has very few pores. Their number considerably increases in ovocytes, especially during the passage of the nuclear substance to the cytoplasm. Anderson [1] [2] pointed out that in the Teleosts *Fundulus* and *Syngnathus* the material of nucleolar origin passes into the cytoplasm, as early as the ovogonia stage.

The cytoplasm of ovogonia has the aspect of a thin perinuclear film. It has very few organites : few mitochondria, some tubules of the granular endoplasmic reticulum and, sometimes, lysosomes (Fig. 2). No components of the smooth endoplasmic reticulum are visible and the Golgi elements are poorly represented. Cytoplasm has a great amount of ribosomes, which grant it intense basophilia and it also has glycogen granules, pointing to a high energy potential. In the cytoplasm are often met myelinic figures or residual bodies which are secondary lysosomes and sometimes seem to originate from the transformation of elements of the Golgi complex. Ovogonia do not present yet organite associations pointing to the presence of a vitelline nucleus (Yolk nucleus, Dotterkern, Balbiani körper). This corroborates the opinion of some authors who consider that this formation appears only in the ovocyte differentiation.

B. *First order ovocytes* (stages II and III according to Steopoe). The transition from ovogonia to first order ovocytes can be evinced morphologically by the contact with follicular cells and by microvilli formation. The two mentioned criteria cannot be applied simultaneously to the carp, as there often appear very young ovocytes surrounded by flattened follicular cells, not yet showing microvillosities (Fig. 3). It is worthy of note that the cytoplasm of these ovocytes is very poor in constituents, excepting the ribosomes which are in great amount. In this basophylic cytoplasm few mitochondria appear, almost devoid of cristae as well as remnants of vesicles, probably belonging to the Golgi complexes or to

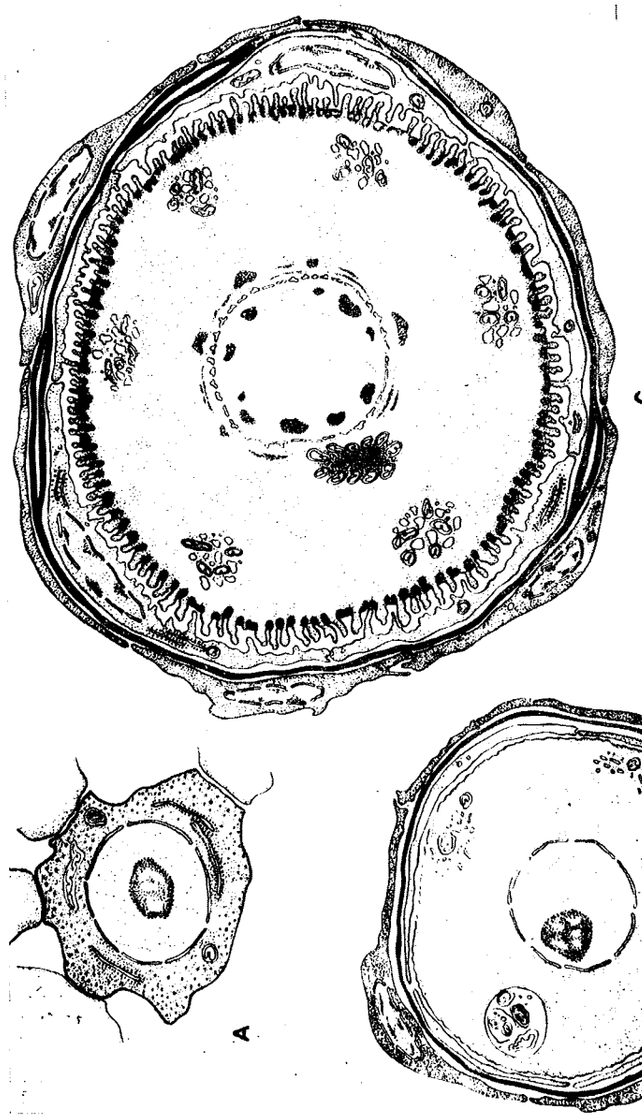

Fig. 1. — Initial stages in the evolution of female sexual elements in *Cyprinus carpio*. *A*, ovogonia ; *B*, *C*, first order ovocytes.

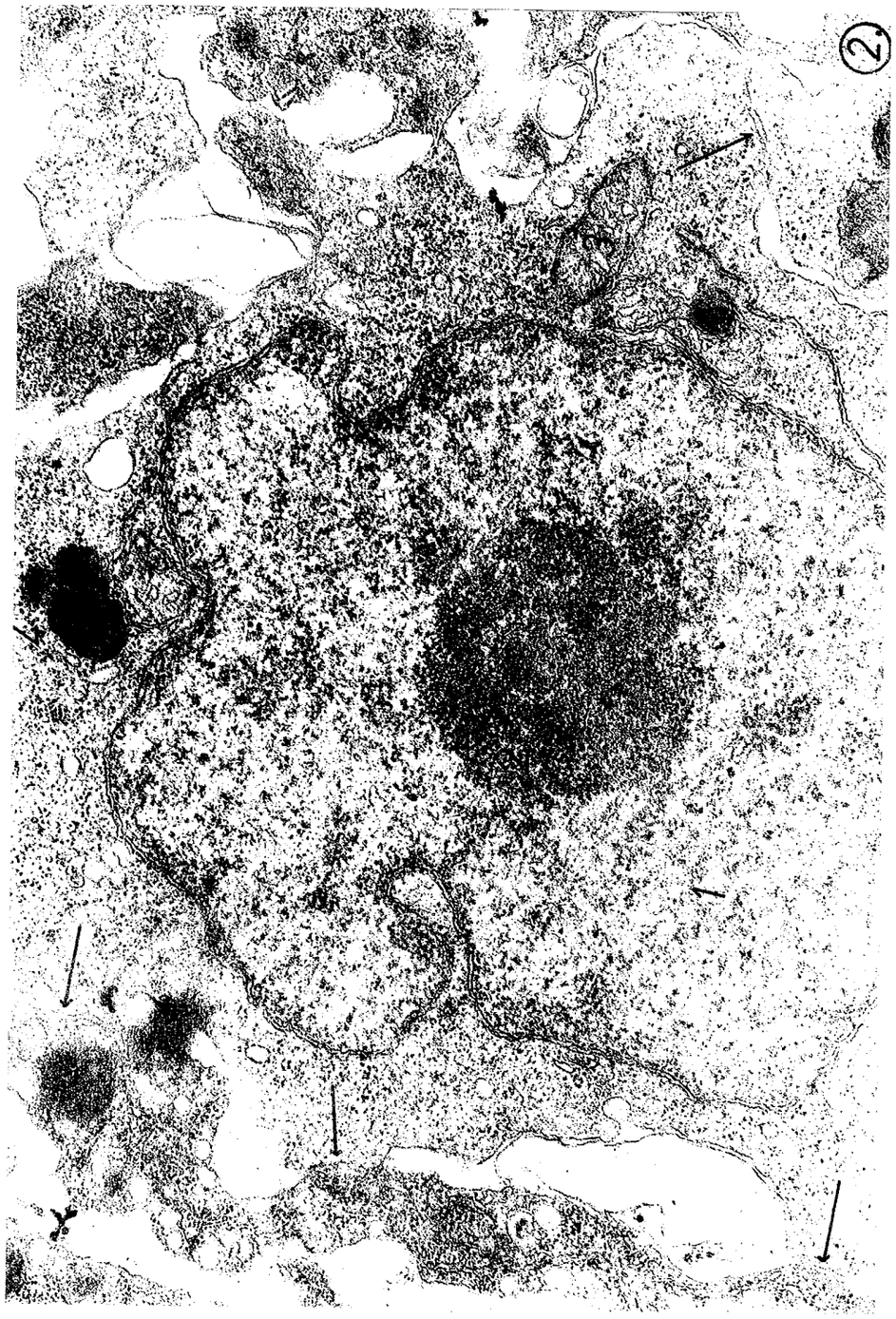

Fig. 2. — Ovogonia in contact (arrows) with neighbouring cells. *1*, nucleus; *2*, nucleolus; *3*, mitochondria; *4*, myelinic figures. 32.180 ×.

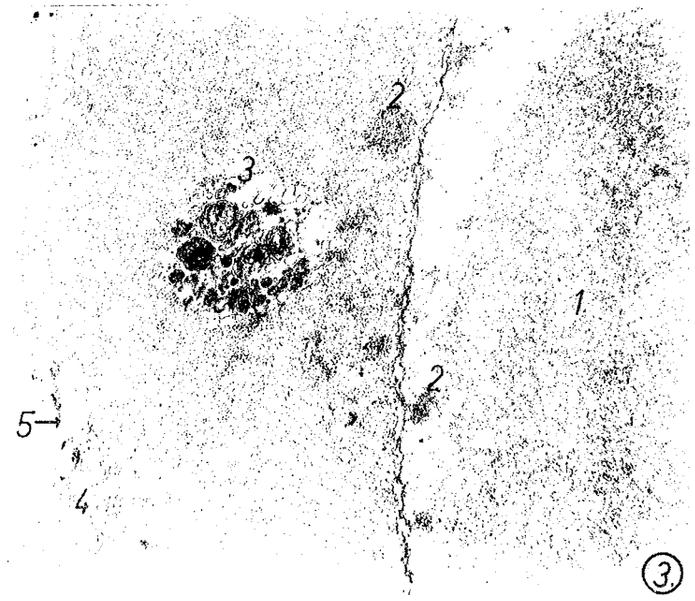

Fig. 3. — First order ovocyte. *1*, nucleus; *2*, nucleolar material; *3*, autophagosome; *4*, follicular cell; *5*, basal membrane. 26.250 ×.

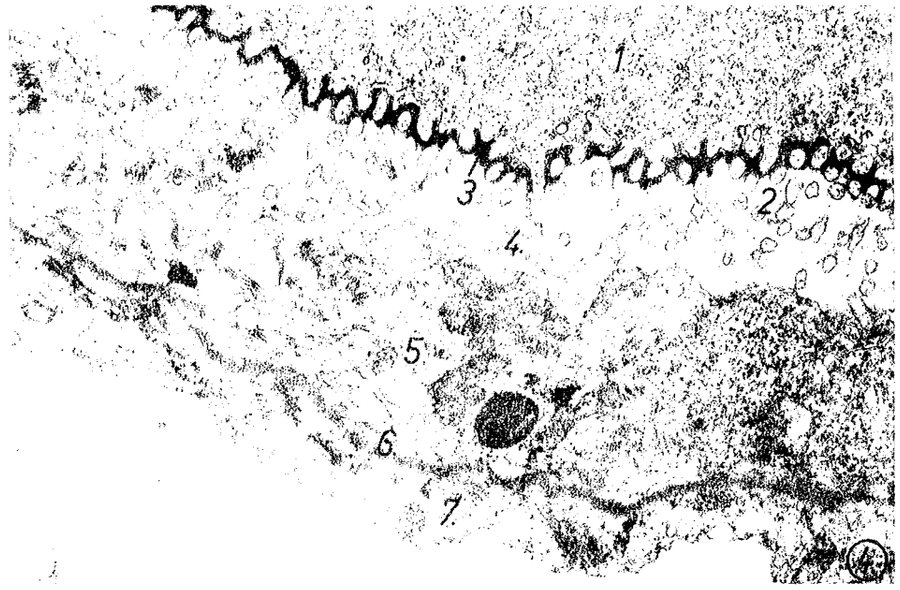

Fig. 4. — First order ovocytes. *1* ovocyte; *2*, microvilli of the ovocyte; *3*, pellucid membrane; *4*, microvilli of the follicular cell; *5*, follicular cell; *6*, basal membrane; *7*, conjunctival cell 15.600 ×.

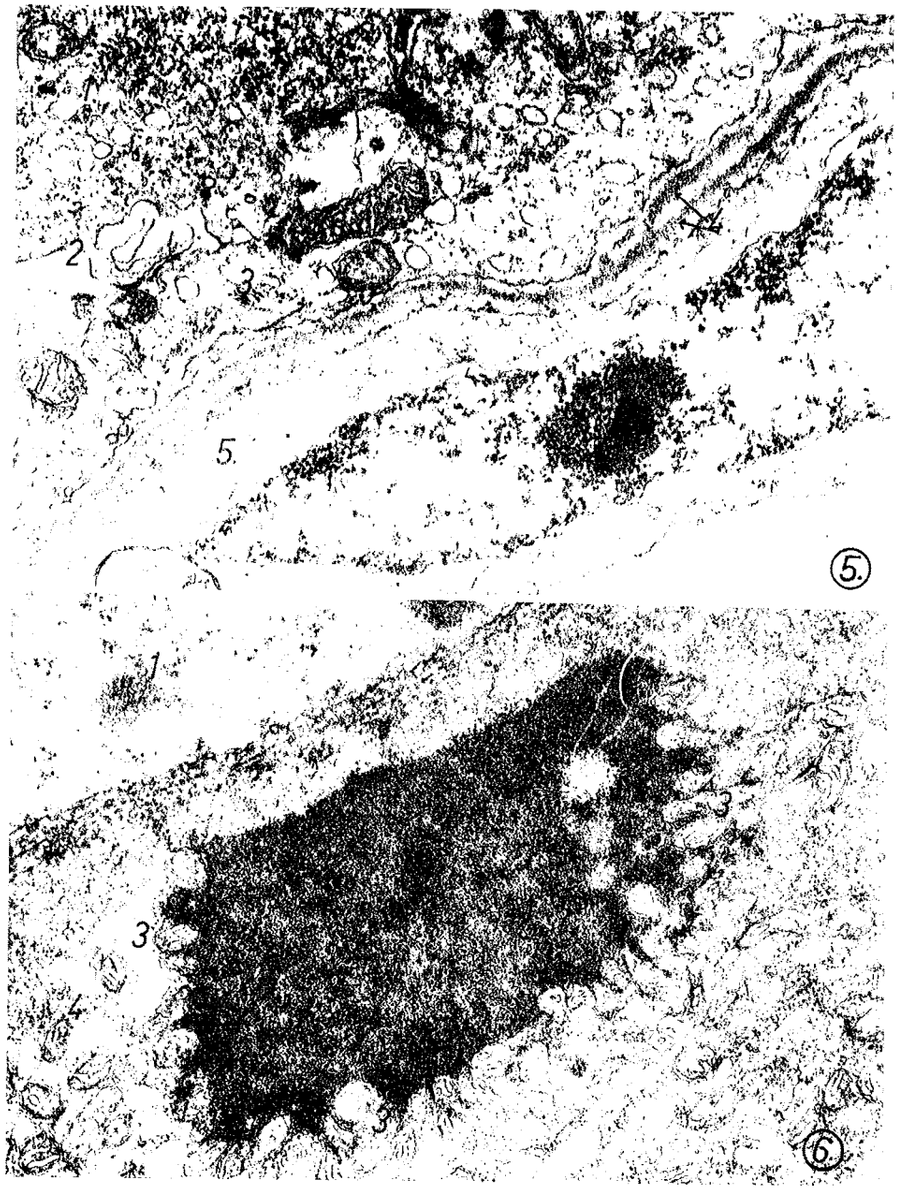

Fig. 5. — First order ovocyte (*1*); *2*, microvillosity of the ovocyte; *3*, follicular cell; *4*, double basal membrane; *5*, conjunctival cell. 20.500 ×.

Fig. 6. — Homologous formation with the vitelline nucleus in Amphibia. *1*, nucleus; *2*, complex formed of nucleolar-origin material; *3*, mitochondria; *4*, vesicles of RER. 29.250 ×

the smooth endoplasmic reticulum (Fig. 3). Characteristic of this transition stage from ovogonia to first order ovocyte is also the presence in the cytoplasm of a very large autophagic vacuole. Remnants of cell organelles (Fig. 3) can be easily distinguished there, which may lead to the idea of a change of cell organelles, followed by their restoring during the growth period. We point out the fact that the passage of nucleolar material into the cytoplasm starts as early as this stage (Fig. 3).

In a more advanced stage, microvilli start appearing (Fig. 4 and 5). They come in contact with follicular cells which also send cytoplasmic processes to the ovocyte. Subsequently, between the microvillosities of the ovocyte, an intensely osmiophilic material is deposited, forming the pellucid zone (Fig. 4). The origin of this membrane is difficult to establish. It seems to belong especially to the ovocyte, but it is not impossible that follicular cells should participate in their formation. Their processes favour a substance contribution [7] [8].

The cytoplasm of growing ovocytes is still populated by many ribosomes. Organelles become numerous and their grouping is characteristic, viz. clusters of mitochondria associated to the dilated vesicles of the smooth endoplasmic reticulum. They are usually distributed at the periphery of the ovocyte but, as already mentioned, can be met in contact with the perinuclear nucleolar material (Fig. 6). These characteristic associations probably represent the place of enzymatic synthesis playing a role in the synthesis of proteins that precede the vitellus, and the generation centre of membranes meant to be used by growing ovocytes.

The Golgi complex is poorly represented, especially placed in the peripheral zone and formed of a few sacs and vesicles. In these previtellogenetic stages, in the ovocytes of the species studied by us lipidic inclusions or glycogen, or annulate lamellae are not yet visible. Pinocytic-vesicles are rare, pointing to a poor and inconstant pinocytic activity in these early stages.

These previtellogenetic ovocytes are surrounded by a layer of follicular cells that increase in size as the ovocyte grows. Follicular cells send cytoplasmic processes on the surface of contact with the ovocyte. In their cytoplasm, mitochondria, granular endoplasmic reticulum and free ribosomes can be noticed (Figs 4 and 5). The layer of follicular cells is surrounded by a basal membrane which sometimes gets double (Fig. 5). Beside this basal membrane, there is a layer of conjunctival cells with dense cytoplasm, making up the follicular sheath.

CONCLUSIONS

Previtellogenetic stages in the evolution of female sexual elements were studied in the species *Cyprinus carpio*, in order to determine the modifications of the cell organelles, connected to these functional phases:

1. Ovogonia with bulky nucleus, little and intensely basophilic cytoplasm, very few organelles, come in contact one with the other, having no continuity relations as in species with synchronous evolution (Fig. 1 *A*).

2. The transition stage from ovogonia to first order ovocytes is pointed out by a change in cell organelles, which undergo lysis from huge autophagic vacuoles (Fig. 1 *B*).

3. First order ovocytes in the growth period, start sending microvillosities, between which the pellucid membrane begins to appear. Nucleus-plasma exchanges are remarkable. The number of organelles increases. Characteristic associations of mitochondria and vesicles of the smooth endoplasmic reticulum with nucleolar-origin material can be observed. This distribution is doubtlessly connected to the onset of the synthesis of lipoproteic yolk (Fig. 1 *C*).

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MORPHO-HISTOCHEMICAL STUDY OF SMALL SUBLINGUAL GLANDS IN A FEW RODENTS

BY

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This work deals with small sublingual glands, morphohistochemically studied in *Rattus norvegicus*, *Mesocricetus auratus*, *Microtus arvalis* and *Citellus citellus*. The glands, morphologically similar, are different by the properties of the secreted mucin.

In *R. norvegicus*, *M. auratus* and *M. arvalis*, the gland produce sulfomucin and sialomucin, either within the same cell, or in different cells, whereas in *C. citellus*, sialomucins are secreted exclusively.

The small sublingual glands in *R. norvegicus* are characterized by a strong histochemical polymorphism.

In this work we have morpho-histochemically studied the small sublingual glands in two laboratory rodent species, *Rattus norvegicus* (Berkenhout, 1796), the white rat, and *Mesocricetus auratus* (Waterhouse, 1839], the golden hamster, as well as in two species of wild rodent, *Citellus citellus* (Linnaeus, 1758), the ground squirrel, and *Microtus arvalis* (Pallas, 1779), the field mouse.

The small sublingual glands are polystomatic glands, placed under the mucous membrane of the floor mouth, before the lingual nerve, in the proximity of the retrolingual gland duct and the submaxilary duct.

The small sublingual glands in *R. norvegicus*, the macro- and microscopic anatomy is mentioned in the literature [3] are histochemically presented in this work. The small sublingual glands in *M. auratus* and *C. citellus* and *Microtus arvalis* are morpho-histochemically presented for the first time.

MATERIAL AND TECHNICS

For each species, glands from 3–6 individuals (males and females) were set apart.

They were fixed in Carnoy, Bouin-Hollande and neuter formol. The samples were embedded in paraffin and sectioned at 7 μ.

For histological study, hemalaun-erythrosin and azan stainings were used.

As for the histochemical study, the following histochemical technics were applied:

– to render evident the acid mucosubstances: alcian blue [AB] pH 1[4]. AB pH 2.5[8], methylation + saponification + AB pH 2.5 [14], mild acid hydrolysis + AB pH 2.5 [10], AB pH 1 + alcian yellow [AY] pH 2.5 [12] [20]

– to render evident the neutral mucosubstances: PAS[5], acetylation + PAS, acetylation + saponification + PAS [6]

– to render evident the neutral and acid mucosubstances: AB pH 2.5 + PAS [7], AB pH 1 + PAS [18]

In the working technics, the AB pH 2.5 + PAS reaction has been preceded by mild acid hydrolysis.

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To indicate the sulfated acid mucosubstances with alcianophilia at pH 1 in the AB pH 1, AB pH 1 + PAS, AB pH 1 + AY pH 2.5 technics and showing alcianophilia at pH 2.5 after methylation + saponification + AB pH 2.5 we used the denomination of sulfomucins [19]. We called the non-sulfated mucosubstances carboxymucins [2] or sialomucins, when the mild acid hydrolysis was effected to reveal the sialic acid.

RESULTS

If histologically the small sublingual glands are mucous of aciniform type in all studied species, they are different by their histochemical pecularities.

In *R. norvegicus*, the glandular cell get ununiformly stained with AB pH 1, most of them being moderately to intensely alcianophile, which indicates the presence of sulfated acid mucosubstances (sulfomucins), and a few cells do not present a positive reaction (Fig. 1). With AB pH 2.5, the secretion turns blue in all cells, the pH 1 negative ones presenting, in this case, a more reduced depth (Fig. 2).

The methylation removes the affinity for AB pH 2.5; this affinity is restored by saponification partially in cells, in the secretion of which both sulfomucins and sialomucins are contained, and wholly in a small number of cells, the secretion of which only contains sialomucins. Instead, the saponification does not restore the affinity for the AB pH 2.5 in the cells that secrete sulfomucins exclusively.

The mild acid hydrolysis diminishes the intensity of the AB pH 2.5 reaction. Following the AB pH 1 + AY 2.5 method, a significant number of cells get stained in greenish blue and, relatively few cells in blue or yellow (Fig. 3). By this technics, both the presence of sulfated and non-sulfated acid mucosubstances was identified in most glandular cells, while in a reduced number of cells, either sulfated or non-sulfated acid mucosubstances were only put into evidence.

The PAS reaction is intensely positive in some cells, moderately or weakly positive in others. The methods used for increasing the specificity of the reaction (acetylation and saponification) demonstrated the glucidic character of the positive PAS material.

In AB pH 1 + PAS, the cells react differently: some are PAS positive, or AB and PAS positive (Fig. 4).

Likewise, after AB pH 2.5 + PAS, the cells show a strong histochemical polymorphism: numerous cells are intensely PAS positive, indicating the presence of neutral mucosubstances, some others have an affinity for both dyes, whereas a series of cells are only alcianophile (Fig. 5).

The mild acid hydrolysis diminishes the AB intensity in the AB pH 2.5 + PAS staining.

With *M. auratus*, the mucous secretion in glandular cells is similar, from the histochemical point of view, to that of the cells in *R. norvegicus*. As a pecularity, we mention, however, the increase of the staining intensity of cells at AB pH 1, as well as the presence of a very reduced number of cells that are positive with this dye.

After AB + AY, the cells are stained in blue or green, yellow cells non existing.

Fig. 1. — Section through the sublingual gland in *Rattus norvegicus*. The negative and AB pH 1 cells are intensely positive. AB pH 1 + carmine alaun Grenacher staining. × 200.

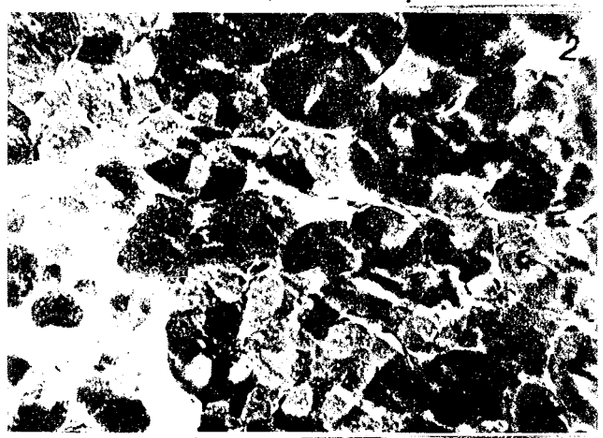

Fig. 2. — Section through the small sublingual gland from *Rattus norvegicus*. Moderately and intensely AB pH 2.5 positive cells. Staining by AB pH 2.5 + carmine alaun Grenacher. × 200.

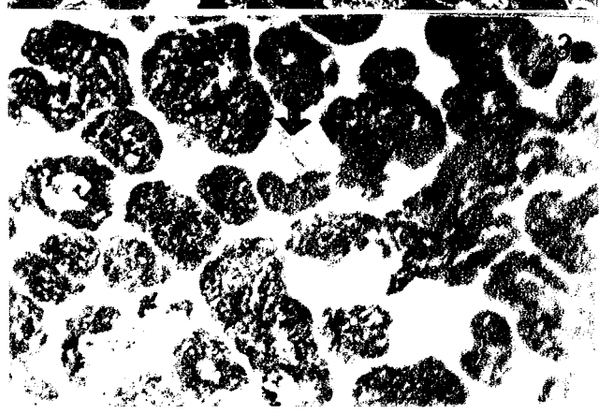

Fig. 3. — Section through the small sublingual gland from *Rattus norvegicus*. In an acinus one can state a strong histochemical polymorphism: cells with affinity for AY pH 2.5 and. cells with affinity for AB pH 1+AY pH 2.5. The arrow points to cells with affinity for AY. Staining by AB+AY × 200.

Fig. 4. — Section through the small sublingual gland from *Rattus norvegicus*. A strong histochemical polymorphism to AB pH 1 + PAS is stated. × 200.

Fig. 5. — Section through the small sublingual gland from *Rattus norvegicus*. A strong histochemical polymorphism is stated to AB pH 2.5 + PAS × 200.

Fig. 6. — Section through the small sublingual gland from *Citellus citellus*. Glandular cells uniformly yellow, positively alcian yellow to AB + AY × 200.

The cell secretion in small sublingual glands in *C. citellus* is histochemically different from that in the glandular cells of the two species above studied.

After AB pH 1 the reaction is negative, in glandular cells rarely insignificantly positive; AB pH 2.5 intensely stains all glandular cells.

The mild acid hydrolysis cancels the alcianophilia at a pH 2.5.

With the AB pH 1 + AY 2.5, all cells get stained in yellow, corroborating the exclusive presence of sialomucins (Fig. 6).

After AB pH 1 + PAS, all cells are intensely magenta stained.

The secreted mucin gets uniformly stained in purple blue with AB pH 2.5 + PAS. After mild acid hydrolysis, the staining is uniformly red, revealing the presence of neutral mucosubstances.

In *Microtus arvalis*, the glandular cells present characteristic histochemical properties: all cells get uniformly stained in moderate blue with AB pH 1 and uniformly intense blue with AB pH 2.5.

The alcianophilia intensity diminishes after mild acid hydrolysis. The methylation cancels the glandular cell basophilia and the saponification restores it partially.

With the AB pH 1 + AY pH 2.5 technics, all cells get stained in greenish blue indicating the presence of sulfo- and sialomucins.

After AB pH 1 + PAS, all cells are alcianophile and PAS positive. In the AB pH 2.5 + PAS staining, the AB reaction increases its intensity, whereas the mild acid hydrolysis followed by AB pH 2.5 + PAS decreases its staining depth with alcian blue.

CONCLUSIONS AND DISCUSSIONS

Numerous research works were assigned to study the acid mucosubstances in salivary glands.

In the submandibular gland of the golden hamster [13], of the rat [10] and in the retrolingual gland of the rat [10] the presence of sialomucin was put into evidence. Sulfomucins were identified in the glossopharingeal glands of rabbits [17] and in the mucous glossal glands of mice [16]. Sulfomucins and sialomucins were identified in the sublingual gland of man [12], in those of the dog [11], in the submandibular gland of the rat [9] and in the small sublingual gland of the cotton rat [1].

Our study results in stating that the small sublingual glands, morphologically similar in the studied species, are different, however, by the histochemical properties of the secreted mucus.

In *R. norvegicus*, *M. auratus* and *Microtus arvalis*, the glands produce sulfo- and sialomucins, either in different cells or in the same cell, whereas in *C. citellus*, the glands secrete sialomucins exclusively.

The small sublingual glands in *R. norvegicus* are characterized by strong histochemical polymorphism. Most cells secrete both sulfo- and sialomucins, a reduced number of cells only producing either sulfo- or sialomucins. Besides these cells, some others appear, only secreting neutral mucosubstances.

From the research effected in *M. auratus*, only the fact that there are not any cells to secrete exclusively sialomucin is corroborated.

With the glands of *Microtus arvalis*, in all cells sulfo- and sialomucins were put into evidence.

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AFFRONTEMENT MULTIPLE *IN VITRO* ENTRE LE FOIE ADULTE NORMAL ET RÉGÉNÉRATIF, LE FOIE EMBRYONNAIRE ET LE MESONEPHROS DU RAT

PAR

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Dans certains de nos travaux antérieurs [1–6], nous avons étudié la possibilité de culture locale *in vitro* du foie adulte normal, du foie adulte régénératif et du foie embryonnaire du rat, ainsi que la culture en affrontement entre deux de ces partenaires, établissant leurs influences réciproques. Dans le cadre des mêmes préoccupations nous avons étudié, cette fois-ci, l'affrontement entre 3 partenaires, différents du point de vue fonctionnel et ontogénétique, ainsi que l'affrontement entre ces 3 partenaires et le mesonephros embryonnaires.

MATÉRIEL ET MÉTHODE

On a utilisé des rats blancs de la lignée Wistar–Bratislava, pesant en moyenne 80 g. On a récolté – en vue de l'affrontement – des fragments de *foie normal* chez le rat adulte; des fragments de *foie régénératif* sur le foie adulte de rat ayant subi une hépatectomie sous-totale (technique de Higgins-Anderson), en prélevant 75% de la masse hépatique (fragments récoltés 24 heures après l'opération, lorsque se succèdent les phénomènes de restitution de la plus grande ampleur); des fragments de *foie embryonnaire* de rat de 18 jours de gestation et du *mesonephros* de l'embryon de poulet de 12 jours.

Les fragments furent cultivés – en affrontement – sur le milieu organotypique de Wolff–Haffen, l'extrait embryonnaire du milieu provenant de l'embryon de rat homoplastique. L'on a effectué deux variantes d'affrontement, c'est-à-dire: *un triple affrontement* (entre le foie normal, le foie régénératif et le foie embryonnaire), ainsi qu'*un quadruple affrontement* (entre le foie normal, le foie régénératif, le foie embryonnaire du rat et le mesonephros de l'embryon de poulet). Ces affrontements furent cultivés pendant 7 jours sur le même milieu de culture ou bien 14 jours, avec un seul repiquage sur le même milieu.

Les affrontements ont très bien réussi du point de vue technique, les fragments affrontés se soudant parfaitement et formant des blocs communs.

Après culture, les blocs furent prélevés, fixés dans le liquide de Carnoy, inclus à la paraffine, sectionnés à 7 microns et colorés à l'hématoxyline-éosine.

RÉSULTATS

A. TRIPLE AFFRONTEMENT. 7 JOURS DE CULTURE

a) *Aspect du fragment du foie normal inclus dans le bloc d'affrontement.* La structure générale est bien conservée, mieux encore que dans le cas du foie normal cultivé isolément. Les travées cellulaires sont tout de même effacées et le cytoplasme des cellules parenchymateuses présente un fort caractère éosinophile. Les noyaux sont plus condensés,

dans certains cas pycnotiques. On ne remarque pas d'infiltrations de cellules embryonnaires ou du foie régénératif dans le foie normal (fig. 1).

b) *Aspect du fragment de foie régénératif inclus dans le bloc d'affrontement.* La structure générale est bien conservée, mieux encore que dans le cas du foie régénératif cultivé isolément. Les travées cellulaires sont maintenues, les capillaires sont dilatés, les noyaux et les nucléoles sont intègres. Les signes de dégénérescence graisseuse sont absents. L'on constate la présence de multiples îlots d'infiltration, constitués par des cellules provenant du foie embryonnaire (fig. 2).

c) *Aspect du fragment de foie embryonnaire inclus dans le bloc d'affrontement.* L'on constate la présence de petites cellules parenchymateuses disposées en cordons, fortement dissociées par des nombreux éléments hématoformateurs. La structure cytologique des hépatocytes est intègre. On remarque que le foie embryonnaire s'insinue (par prolifération) dans l'espace situé entre le foie normal et le foie régénératif (lieu de moindre résistance).

B. TRIPLE AFFRONTEMENT. 14 JOURS DE CULTURE

a) Le *foie normal* présente un faible degré de dystrophie. L'on constate aussi une faible infiltration de cellules embryonnaires à l'intérieur de l'organe (fig. 3).

b) Le *foie régénératif* est très bien conservé. L'infiltration par les cellules embryonnaires est plus forte qu'à 7 jours de culture.

c) Le *foie embryonnaire* est parfaitement conservé et présente un développement continu dans le sens de l'apparition d'une nette structure de cordons hépatiques. Les cellules hématopoïétiques sont réduites en nombre et assemblées en îlots. Les noyaux prennent une teinte basophile.

C. QUADRUPLE AFFRONTEMENT. 7 JOURS DE CULTURE

a) Le *foie normal* garde l'aspect qu'il a eu dans le triple affrontement. L'infiltration par des cellules hépatiques ou par des cellules du mesonephros est absente.

b) Le *foie régénératif* garde le même aspect qu'il a eu dans le triple affrontement, étant infiltré par des cellules de provenance mésonephrotique (fig. 4), ainsi que par des cellules du foie embryonnaire (fig. 5). L'infiltration par les cellules provenant du foie embryonnaire est prépondérante.

c) Le *foie embryonnaire* ne présente pas une évolution ontogénétique similaire à celle apparaissant dans le triple affrontement. L'on constate des processus prolifératifs vers le mesonephros et vers le foie régénératif. L'on constate une forte infiltration du foie embryonnaire par des cellules mésenchymateuses indifférentes provenant du mesonephros.

d) Le *mesonephros* garde une structure normale, présentant certaines infiltrations de cellules hépatiques provenant du foie embryonnaire.

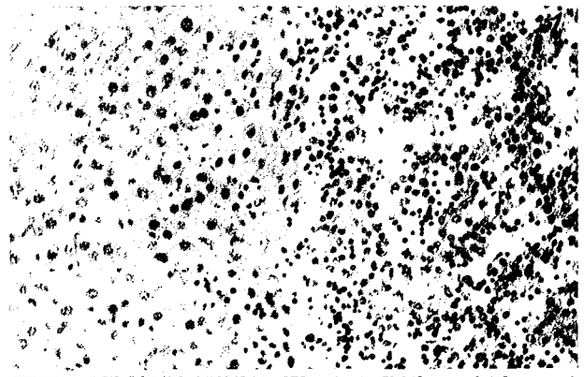

Fig. 1. — Triple affrontement, 7 jours de culture. Le foie normal reste indemne à l'infiltration des cellules du foie embryonnaire.

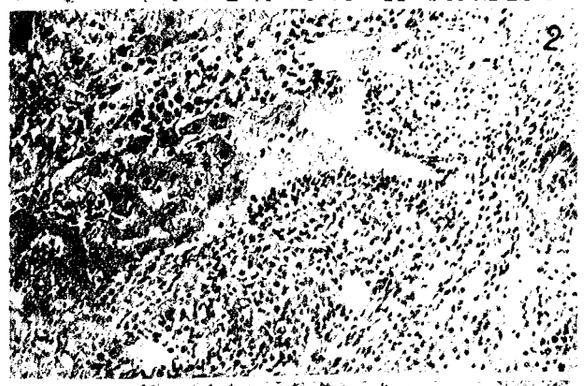

Fig. 2. — Triple affrontement, 7 jours de culture. Le foie régénératif présente des îlots d'infiltration de cellules du foie embryonnaire.

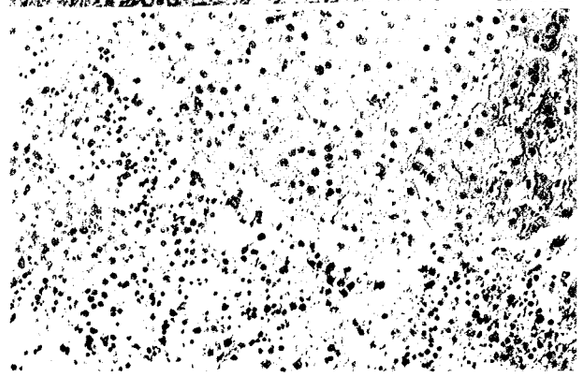

Fig. 3. — Triple affrontement, 14 jours de culture. Le foie normal est faiblement infiltré par le foie embryonnaire.

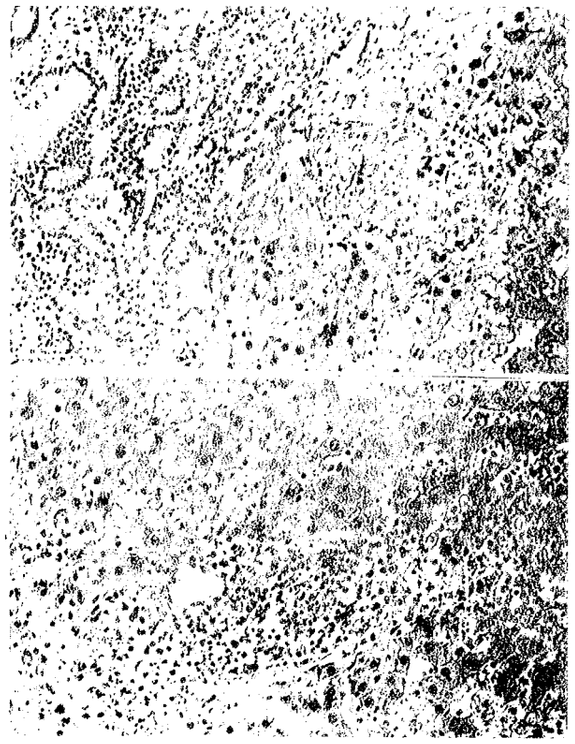

Fig. 4. — Quadruple affrontement, 7 jours de culture. Le foie régénératif est infiltré par des cellules provenant du mesonephros.

Fig. 5. — Quadruple affrontement, 7 jours de culture. Le foie régénératif est infiltré par des cellules provenant du foie embryonnaire.

L'on constate donc une infiltration réciproque entre le foie embryonnaire et le mesonephros, l'infiltration mésonéphrotique étant plus forte dans le foie embryonnaire que l'infiltration des hépatocytes embryonnaires dans le mésonephros.

D. QUADRUPLE AFFRONTEMENT. 14 JOURS DE CULTURE

La situation est la même qu'à 7 jours de culture, le foie embryonnaire présentant tout de même une évolution ontogénétique, fait que l'on ne constate pas dans le foie de 7 jours de culture.

DISCUSSIONS

En analysant les résultats obtenus, on constate que les éléments affrontés réalisent un bloc, mais que ces éléments ne s'unissent pas au même degré. Ainsi, le foie embryonnaire se soude intimement au mesonephros, l'affrontement étant très puissant sur toute la surface de contact. Dans les relations entre le foie régénératif et le foie embryonnaire, l'intimité est plus légère, étant effectuée par des ponts de liaison qui, d'une part, font le service mécanique de liaison des éléments affrontés et, d'autre part, le service de ponts de transit des substances entre les deux partenaires.

On constate que la structure des organes affrontés, provenant soit de l'embryon, soit de l'adulte, est incomparablement mieux maintenue que la structure des organes cultivés isolément. Au cas où les explants de foie régénératif et du foie normal n'ont pas, dans leur voisinage immédiat, des explants embryonnaires, l'on constate leur faible dégradation. Il s'ensuit que le milieu de culture est insuffisant pour le développement complet de ces organes. La présence des extraits embryonnaires offre les tréphons nécessaires à la meilleure survie. Il est vrai que le milieu de culture contient des tréphones nécessaires, mais la qualité de ces tréphones extra-cellulaires diffère de celle des tréphones intra-cellulaires fournis par les organes embryonnaires. Ces derniers tréphones exercent leur action en spécial à la suite de leur filtration à travers les membranes cellulaires des organes accolés (organes embryonnaires et organes adultes). Leur diffusion, par la voie du milieu de culture, n'a pas le même effet. Leur passage à travers les membranes cellulaires, sélectives à leur réception (les organes adultes), augmente leur efficacité.

Dans le foie régénératif, l'infiltration des cellules du foie embryonnaire est plus massive à 14 jours de culture qu'à 7 jours, ce qui démontre une activité proliférative continue. Cette infiltration confère au foie régénératif une plus grande quantité de tréphones, ce qui augmente la survie du dernier. D'autre part, l'infiltration du foie embryonnaire dans le foie régénératif est plus massive que l'infiltration mésonéphrotique. Il paraît donc que la similitude de fonctionnalité et de biochimisme entre le foie régénératif et le foie embryonnaire est plus grande que celle entre le foie régénératif et le mesonephros.

La viabilité plus exprimée du partenaire régénératif s'explique non seulement par l'addition de tréphones, mais aussi par la similitude fonctionnelle entre le foie régénératif et le foie embryonnaire, la régénération étant une réactualisation de certaines potentialités embryonnaires.

Dans le triple affrontement, la durée de 14 jours de culture donne au foie embryonnaire la possibilité de continuer son activité prolifératives qui, cette fois-ci, affecte même le foie normal.

Le mesonephros et le foie embryonnaire établissent entre eux une intimité parfaite, en dépit de la différence d'organe et leur provenance d'espèces différentes (rat et poulet). Leur qualité d'organes embryonnaires les rapprochent en absence de leur spécificité biochimique.

La présence du mesonephros permet aussi une prolifération plus forte de la part du foie embryonnaire.

Les deux organes embryonnaires s'infiltrent réciproquement prouvant leur compatibilité dans les conditions de leur non-différenciation biochimique.

La prolifération histochimique du foie embryonnaire, dans le quadruple affrontement (14 jours de culture), se fait au détriment de son évolution ontogénétique, fait différent au cas du triple affrontement où le foie embryonnaire continue son développement in vitro.

On a établi que le foie normal reste indemne à l'infiltration par les cellules embryonnaires (hépatiques ou mésonéphrotiques) car il dispose d'une « individualité biochimique » qui le rend inapte à établir des jonctions avec d'autres éléments. Mais cette individualité biochimique est affaiblie après 14 jours de culture, rendant le foie réceptif à l'infiltration par d'autres éléments. Il en résulte donc que la prolongation de la durée de culture induit un état de plasticité biochimique du foie normal adulte.

Le foie adulte régénératif est réceptif aux infiltrations, car il a déjà in vivo une labilité biochimique induite par l'exérèse. Cette labilité assure au foie régénératif, d'une part, la possibilité de jonction avec le foie embryonnaire et, d'autre part, d'une capacité plus grande d'adaptation aux conditions de culture.

L'on constate, enfin, que la capacité maximum de prolifération est réservée aux éléments les moins différenciés du mesonephros (les cellules mésenchymales non différenciées).

CONCLUSIONS

1) En effectuant, dans les cultures d'organes (pendant 7 ou 14 jours), un triple affrontement entre des fragments de foie normal adulte, de foie adulte régénératif (à 24 heures après l'exérèse) et de foie embryonnaire (18 jours de gestation) de rat, l'on constate qu'à 7 jours de culture, le foie normal garde sa structure, dans le foie régénératif apparaissent des infiltrations de cellules embryonnaires provenant du foie embryonnaire, tandis que le foie embryonnaire devient fortement prolifératif. À 14 jours de culture, l'on constate une très faible infiltration du foie normal par des cellules embryonnaires, l'accroissement du nombre des îlots prolifératifs de cellules embryonnaires dans le foie en régénération, ainsi que

le développement ontogénétique et la prolifération continue du foie embryonnaire.

2) Dans le quadruple affrontement entre les fragments de foie adulte, de foie adulte régénératif, de foie embryonnaire de rat et de mesonephros de l'embryon de poulet (12 jours), l'on constate que tandis que le foie normal garde sa structure, le foie régénératif présente des infiltrations cellulaires provenant du foie embryonnaire (avec prédilection) et de cellules provenant du mesonephros. Dans le foie embryonnaire l'on constate une abondante infiltration de cellules mésenchymateuses provenant du mesonephros. Dans le mesonephros l'on constate des infiltrations de cellules parenchymateuses provenant du foie embryonnaire.

3) On souligne le fait que — en général — le foie normal est indemne à son envahissement par des cellules prolifératives, à l'encontre du foie régénératif. Le fait est mis sur le compte de l'existence d'une individualité biochimique fixe du foie normal et d'une labilité biochimique du foie régénératif, labilité induite par l'exérèse.

4) Le mesonephros et le foie embryonnaire établissent, entre eux, une intimité parfaite en dépit de la différence d'organe et d'espèce, intimité réalisée par des infiltrations réciproques.

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HISTOENZYMOLOGICAL STUDIES ON FOLATE METABOLISM IN RAT SALIVARY GLANDS

BY

DOINA ONICESCU, LETIȚIA MISCHIU and AURELIA MARIN

Histoenzymological dihydrofolate dehydrogenase activity in rat large salivary glands was studied. In parotid, sublingual and submandibular glands, enzyme activity was very low in the secreting acini, but very high in excretory ducts and in fibrous connective tissue septa. The more intense enzyme activity was present in submandibular gland. The intercalated and secretory ducts of the submandibular gland differently reacted in the cells of the same duct and with regard to the sex.

The structural components of salivary glands are differentiated by specific functionality [1] [4] [8] [11], by characteristic histoenzymological aspects [3] [9] [12] each of them depending on the biostimulators concentration [2] [8.]

The purpose of the present paper is to describe the characteristics of folate metabolism in parotid, sublingual and submaxillary gland not yet studied.

MATERIAL AND METHOD

Cryostat-cut sections of rat large salivary glands, were fixed at 4°C in buffered 2.5% glutaraldehyde, pH 7.2 for 10 min. After being washed in distilled water the sections were incubated for 20 min. at 37°C in the incubation medium [12], for the localization of dihydrofolate dehydrogenase activity. In parallel, sections fixed in neutral formalin, were stained with hematoxylin — eosin.

RESULTS

Parotid and sublingual gland. A weak enzyme activity was present in the serous cells, weaker in the mucous cells of the secreting acini (Fig. 1), but strong in the basket cells. In the excretory ducts the reaction stronger than in acini, was located in the bazal cytoplasm of the luminal cells (Fig. 2).

Submandibular gland. In males the serous and mixed acini displayed a weak enzymic reaction in the acinar cells, the myoepithelial cells a strong reaction (Fig. 3). *Intercalated ducts* has a positive strong enzyme activity in one or two of their periluminal cells, but very weak in other cells. In the *granular ducts* (intralobular secretory ducts), the epithelial cells are characterized by different degrees of enzymic activity. One can observe three cellular reactivities: a). strongly positive cells

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with basal localization of formazan granules; b). moderately positive cells with reactive granules in the whole cytoplasm; c). areactive cells (Fig. 4).

In the *striated* (*secretory*) *ducts*, (not so numerous), an intense reaction appeared perinuclear or in the basal portions of the simple columnar epithelium cells (Fig. 5).

The main *excretory ducts* (interlobular ducts) displayed a very strong enzymic activity in basal cytoplasm of periluminal cells. A very strong reaction appeared also in the surrounding connective cells and nerve fibres.

In *females* was found the same localization of the diphydrofolate dehydrogenase activity as in males but with different degrees of enzymic reaction in some ducts. In the intercalated, secretory, and striated ducts (Fig. 6), the enzymic activity is very weak, the difference in cells reactivity was not so distinct.

DISCUSSIONS AND CONCLUSIONS

Our histochemical results indicate a positive folate metabolism in the rat major salivary glands, but in various degrees of intensity regarding the gland type, the structural components of each gland (acini and ducts) and the sex of animal.

The *parotid* and *sublingual* gland, despite of their morphophysiological differencies present a similar folate turnover with weak enzymic activity in acinar cells, and stronger in ducts.

The *submandibular* gland, detaching from the two others displays a greater dihydrofolate dehydrogenase reaction with evident enzymic variability in their structures, in males and in females.

The secretory acinar cells have a weak folate activity. The myoepithelial cells surrounding the acini and the intercalated ducts have an increased folate metabolism, in this way proving the common origin of this two components of salivary parenchyme mentioned also by other's[4] [10] [16] [17] [18]. The excretory ducts displayed a strong positive reaction, more intense in males than in females.

The mitotic cells of the intercalated ducts implicated in the regeneration of the acini and incipient ducts [15], with an increased DHFD activity, need this enzyme for the synthesis of nucleic acids and some aminoacids, required in this metabolic process.

In the granular ducts, the folate turnover very high in males appeared in various degrees from one periluminal cell to another, confirming thus histochemically the morphological data of Tamarin and Sreebny [15], describing three cellular types in this secretory ducts, each of them with peculiar functions. The greater enzymic activity in granular ducts can be corelated also with the important transfer present at this level between the blood plasma and the lumen saliva [3] [5].

Striated ducts very active in the transport across the basement lamina, continually reshuffling their mitochondria, display a great folate metabolism, the dihydrofolate dehydrogenase beeing necessary for the permanent regeneration of these organelles. In the same time, the presence

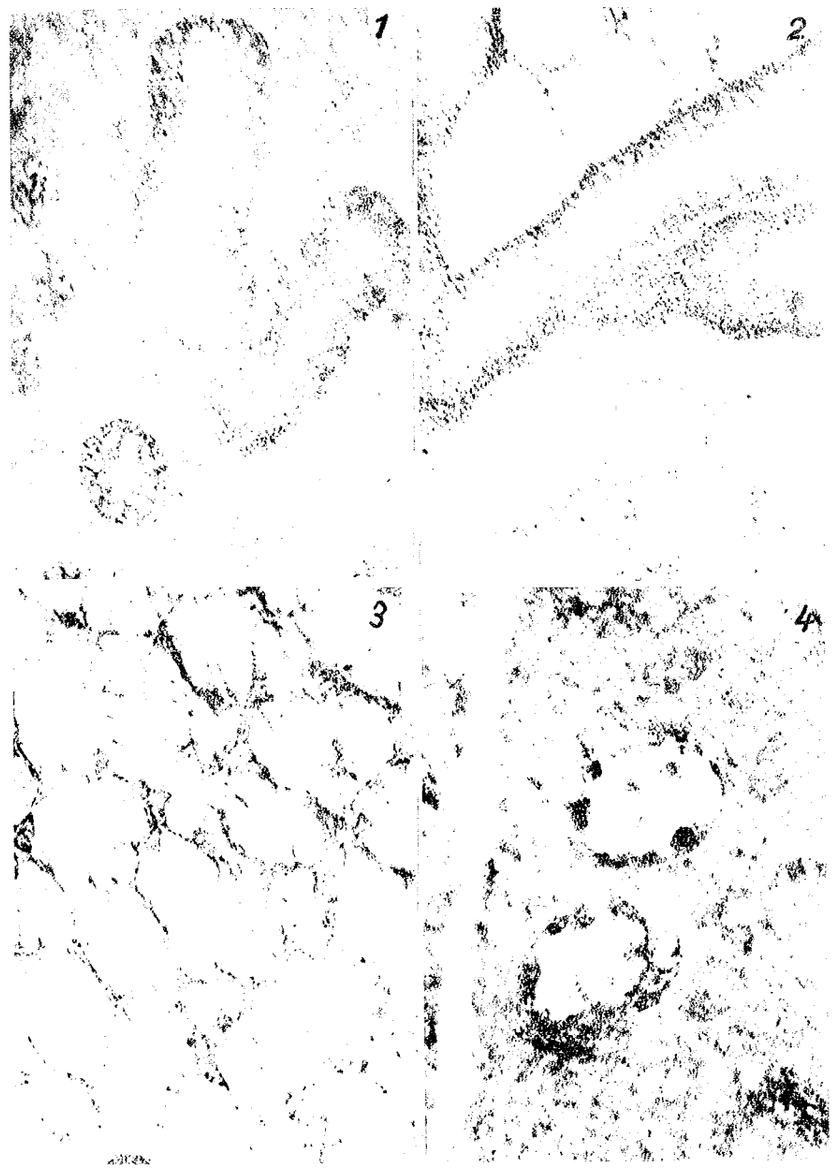

Fig. 1. — Parotid gland. Dihydrofolate dehydrogenase activity weak positive in the acinar cells and intense in the excretory ducts.

Fig. 2. — Sublingual gland. Folate enzyme appeares very weak in the secreting acini of mean intensity in the myoepithelial cells and very strong in the excretory ducts.

Fig. 3. — Submandibular gland. Enzymic activity of dihydrofolate dehydrogenase weak in the acini and intense in the myoepithelial cells.

Fig. 4. — Male submandibular gland. A strong dihydrofolate dehydrogenase reaction is present in different degrees in the cells of granular ducts.

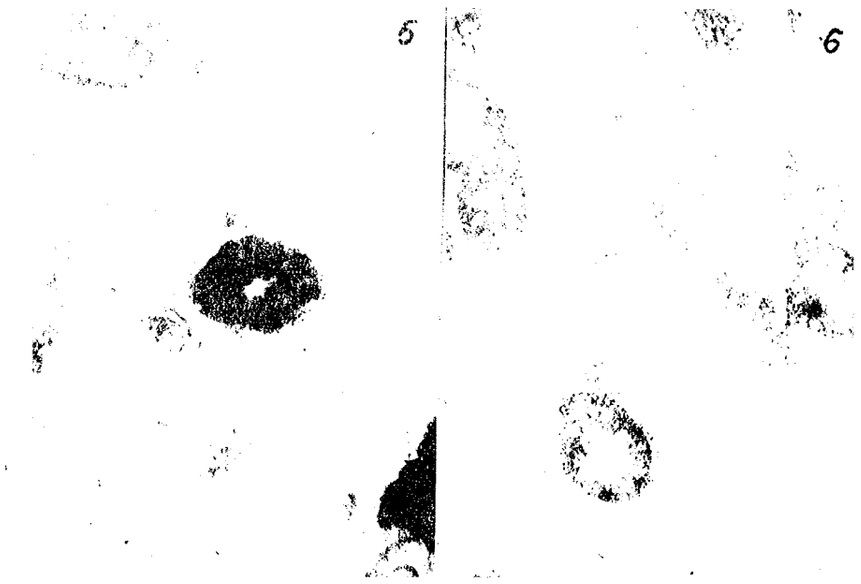

Fig. 5. — Male submandibular gland. Very intense folate enzyme activity appears in the cells of striated ducts.

Fig. 6. — Female submandibular gland. The folate enzyme is weaker in intensity than in male salivary gland especially in the granular and striated ducts.

at the basal membrane level of the iodide ions (well known as activators of folate enzyme) in grater concentration in males than in females, explains evidently the enhanced folate turnover in striated ducts.

The different degrees of folate enzymic activity in all these ducts (intercalated, secretory and striated) in males and in females adds a new aspect in the problem of sexual salivary dimorphisme.

The presence of a strong folate metabolism in the main excretory ducts in all major salivary glands, and in their surrounding connective or nervous structures, attract the attention to the principal role of these areas in the metabolization of folates. It is possible that in these regions of salivary glands do takes place in principal the turnover of folates.

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DEVELOPMENT OF THE FOETAL MEMBRANES IN THE INDIAN LEAF-NOSED BAT, *HIPPOSIDEROS FULVUS FULVUS* (GRAY). I. EARLY DEVELOPMENT

BY

K. B. KARIM

Van der Sprenkel [11] described the yolk sac at about mid-pregnancy of *Hipposideros* (*sp*?). A few advanced stages of development of *Hipposideros bicolor pallidus* were described by Gopalakrishna [2] and Gopalakrishna and Moghe [4]. Karim [5] gave a description of the arrangement of the foetal membranes in *Hipposideros fulvus fulvus* at an advanced stage of gestation. From the foregoing it is apparent that there is practically no information concerning the early embryology and the details of the development of any member of the family Hipposideridae. The present study on the embryology of *Hipposideros fulvus fulvus* is intended to fill this lacuna.

MATERIAL AND METHODS

63 specimens of *Hipposideros fulvus fulvus* at various stages of pregnancy were examined for the present report. The specimens were collected from old houses in and around Nanded in Maharashtra, India.

The genitalia were fixed in various fixatives such as Bouin's, Rossman's, Carnoy's fluid and neutral formalin. In cases of advanced pregnancy the uterine wall was punctured or slit open with a view to allowing the penetration of the fixative. The fixed material was preserved, where necessary, in 70% ethanol. After the usual procedure of dehydration through graded ethanol, the tissues were embedded in paraffin, and cut at various thicknesses. For routine histological study the sections were stained with Ehrlich's haematoxylin, counterstained with eosin, and mounted in DPX after clearing in xylol. A few sections in each series were also stained by the periodic acid-Schiff procedure [10], some with and some without prior salivary digestion, and some by Heidenhain-azan technique.

OBSERVATIONS

1. General remarks on the breeding habits

Hipposideros fulvus fulvus breeds once a year in a sharply defined season. Copulation occurs during the second half of November, and is immediately followed by pregnancy. During each cycle only one ovary

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releases an ovum, and the pregnancy is carried in the corresponding uterine cornu. Although the female genitalia is morphologically bilaterally symmetrical, the left side exhibits pronounced physiological dominance, and about 70% of the ovulations and pregnancies occur on this side [9].

2. The female reproductive organs

Although externally the genitalia resemble those of other bats possessing bicornuate uterus, examination of serial sections reveals that the vagina in *Hipposideros fulvus fulvus* is bifid at its cranial region, and each branch of the vagina actually forms the caudal half of the respective uterine cornu, only the cranial half of the cornu representing the uterus proper [6]. The uterine segment of each cornu is sharply bent towards the dorsal aspect of the respective cornu.

The nonpregnant uterus is slightly oblong in outline in transverse sections, buf the uterine lumen is slit-like with its long axis lying along the mesometrio-antimesometrial axis (Pl. I, fig. 1). On the approach of the breeding season the uterine lumen undergoes slight enlargement, and the uterine glands increase in number and length and become coiled, and their lumina become wide and contain an eosinophilic secretion. The gland epithelium is composed of columnar cells with basally situated nuclei and vacuolated cytoplasm.

3. Bilaminar blastocysts

Stage I : *Early implanted bilaminar blastocyst.* Nine early implanted bilaminar blastocysts are available for the present study. During this stage of development the blastocyst enlarges rapidly in size although the embryonic mass does not undergo appreciable change. The blastocyst fills a large implantation chamber formed by the expansion of the antimesometrial part of the uterine luminal slit, the rest of the uterine lumen towards the mesometrial side remaining slit-like. The wall of the blastocyst is in contact with the endometrium on all the sides of the implantation chamber the uterine epithelium being absent from the implantation chamber. In all the specimens the embryonic mass is in the form of a concavo-convex disc made up of compactly arranged, large polygonal cells, each with a large vesicular nucleus. In seven of the specimens studied here the embryonic mass is oriented laterally with respect to the morphology of the uterus (Pl. I, figs 2 and 3), while in the other two specimens it lies towards the antimesometrial side of the uterus (Pl. I, figs 4 and 5). The endodermal layer is composed of squamous cells which form a complete inner lining to the trophoblast.

The trophoblast has undergone proliferation and the cells have entered the endometrium on all the sides of the implantation chamber thus forming a two or three layered band of trophoblast surrounding the implantation chamber. In some places the trophoblast cells are several layers thick, and form broad conical projections into the endometrium

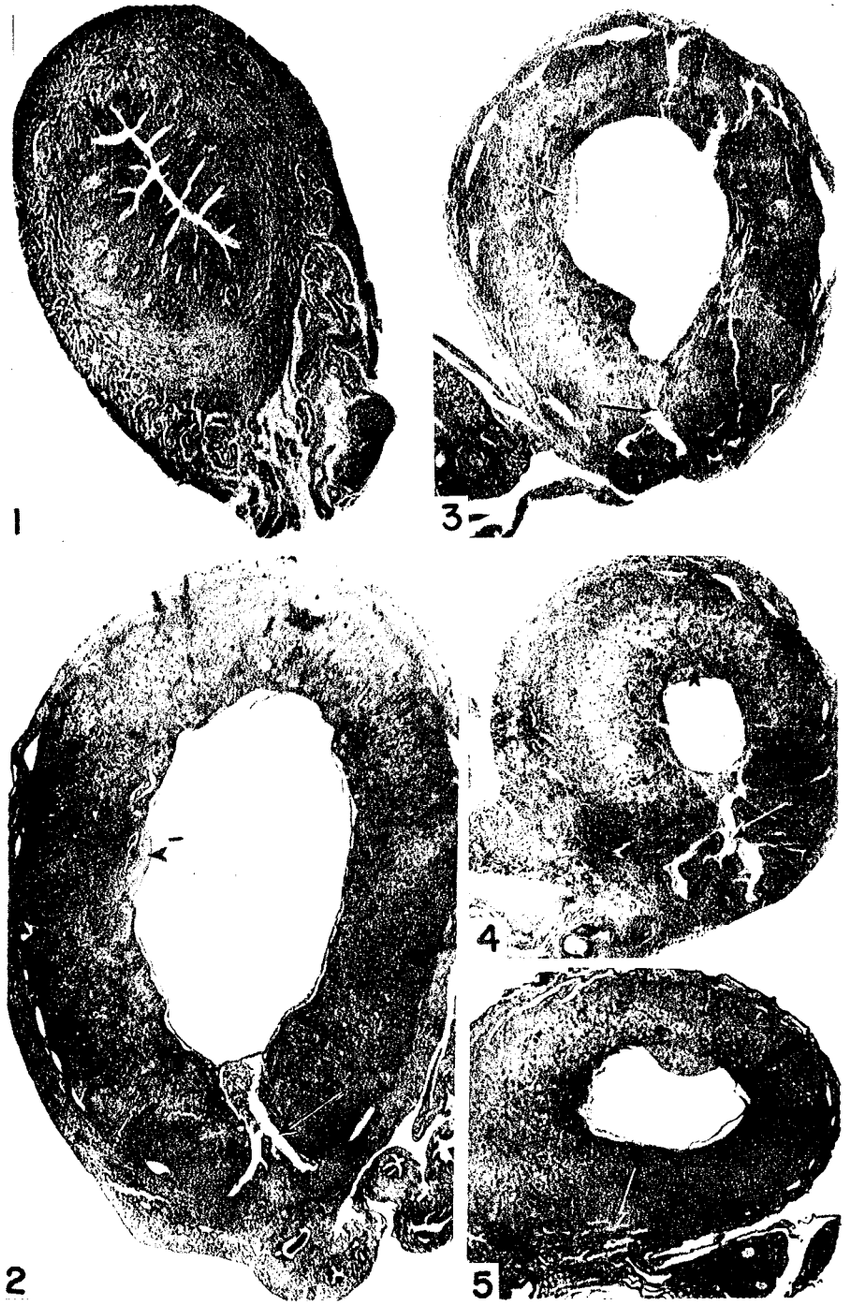
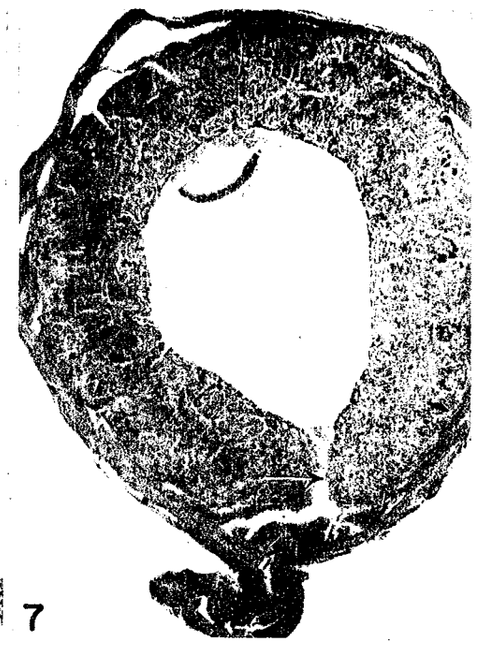


Fig. 1. – Transverse section of the nonpregnant uterus of *Hipposideros fulvus fulvus*. × 44.
Fig. 2. – Transverse section of the uterus containing a bilaminar blastocyst with the embryonic mass (arrow head) directed towards the lateral side of the uterus. Arrow points towards the luminal slit of the uterus towards the mesometrial side. × 60.
Fig. 3. – Transverse section of the uterus containing a bilaminar blastocyst with the embryonic mass (arrow head) directed towards the lateral side of the uteurs. Note the large implantation chamber formed by the expansion of the uterine luminal slit on the antimesometrial side of the uterus while the rest of the uterine lumen towards the mesometrial side remains slit-like (arrow). × 55.
Fig. 4. – Transverse section of the uterus containing a bilaminar blastocyst with the embryonic mass (arrow head) oriented antimesometrially. Arrow points towards the luminal slit of the uterus towards the mesometrial side. × 50.
Fig. 5. – Transverse section of the uterus containing a bilaminar blastocyst with the embryonic mass directed towards the antimesometrial side of the uterus. Arrow points towards the mesometrial side of the uterus. × 30.



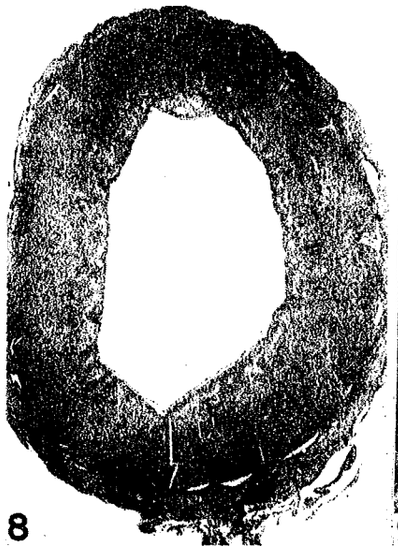
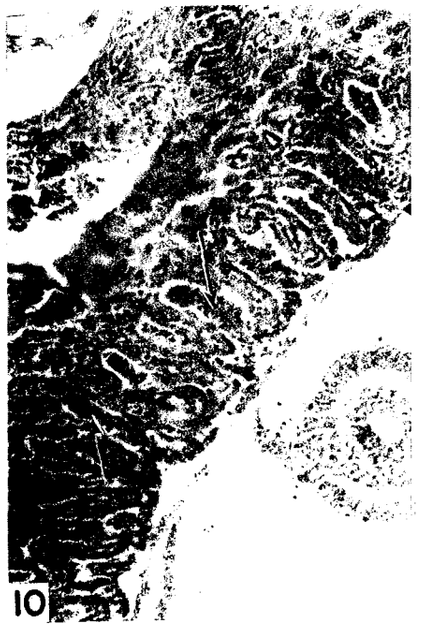


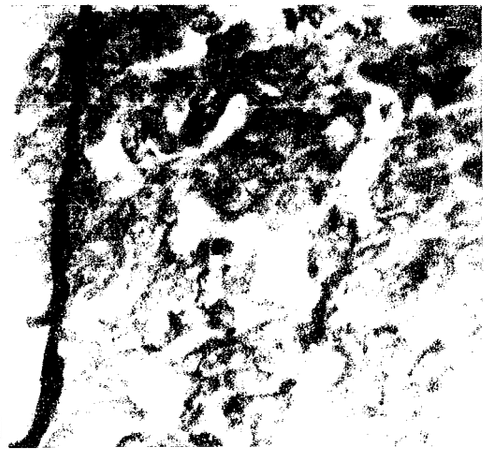
Fig. 6. — Part of the figure 4 magnified. Note the broad conical projections (arrow head) of the trophoblast entering the endometrium. Arrow points towards the endodermal lining. × 450.

Fig. 7. — Transverse section of the uterus containing a late implanted bilaminar blastocyst with the embryonic disc directed antimesometrially. Arrow points towards the slit-like uterine lumen towards the mesometrial side of the uterus. × 35.

Fig. 8. — Transverse section of the uterus containing a late implanted bilaminar blastocyst with the embryonic mass oriented towards the antimesometrial side of the uterus. Arrow points towards the mesometrial side of the uterus. × 30.

Fig. 9. — Part of the figure 8 enlarged. Note the presence of a small primitive amniotic cavity in the centre of the embryonic mass. × 140.





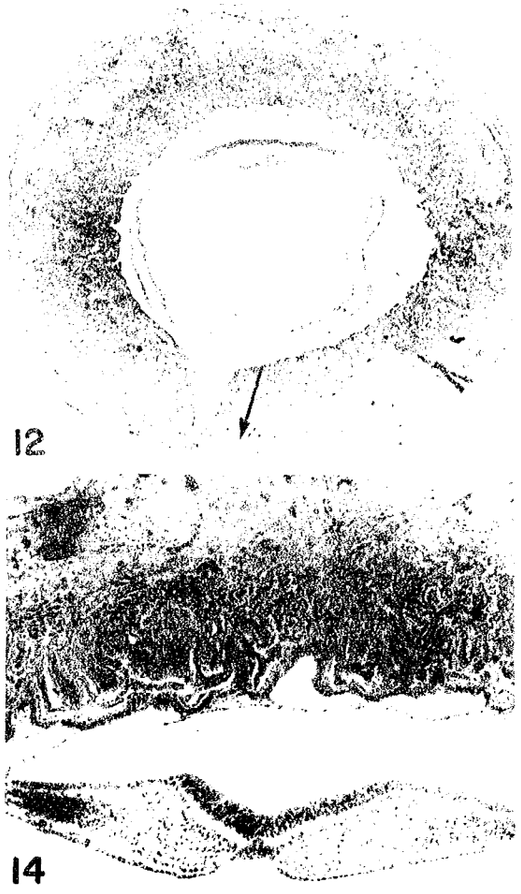


Fig. 10. — Part of the trophoblastic placenta at the late implanted bilaminar blastocyst stage. The syncytiotrophoblast has formed a thick mantle on all the sides of the implantation chamber. The basal layer of cytotrophoblast has pushed into the syncytiotrophoblastic zone in the form of solid columns of cells (arrow). × 180.

Fig. 11. — Part of the yolk-sac wall at the bilaminar blastocyst stage. Note the presence of the Reichert's membrane (PAS staining). × 560.

Fig. 12. — Transverse section of the uterus containing a trilaminar blastocyst with the embryonic disc oriented antimesometrially. Arrow points towards the mesometrial side of the uterus. × 35.

Fig. 13. — Part of the figure 12 enlarged. Some of the cytotrophoblastic cords have become hollow near the foetal border of the placenta so that the maternal capillaries invested by the syncytiotrophoblast (arrow) appear like tubules. × 180.

Fig. 14. — Part of the chorio-amniotic placenta at the late neural groove stage of development of the embryo. Please see text for description. × 88.

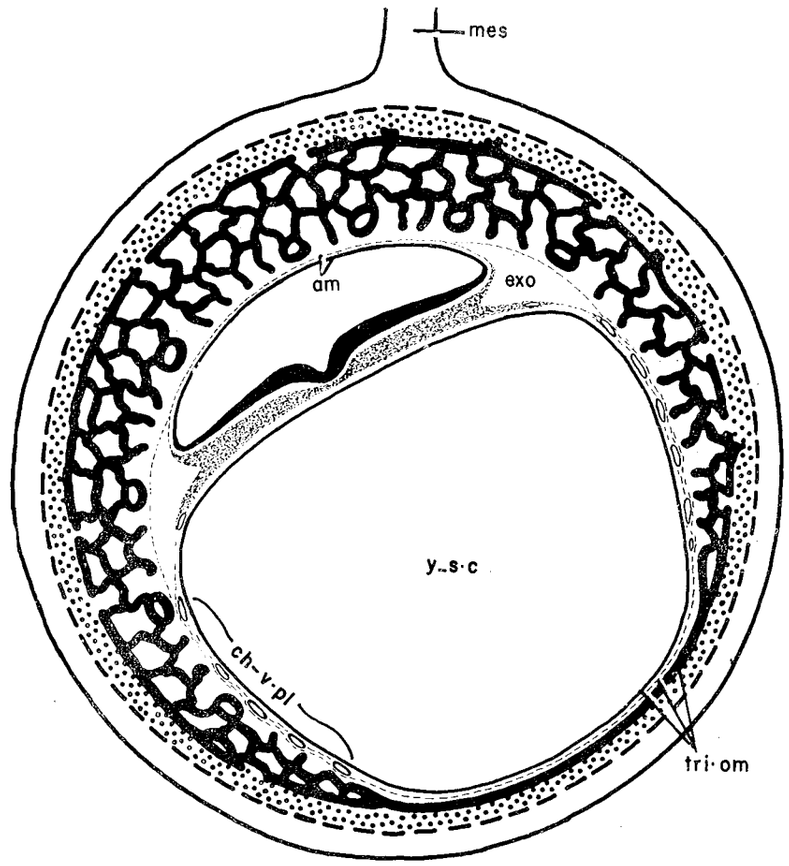


Fig. 15. — Semischematic representation of the arrangement of the foetal membranes of *Hipposideros fulvus fulvus* at the advanced neural groove stage of development of the embryo. *am*, amnion; *ch-v. pl*, *chorio-vitelline placenta*; *exo*, exocoelom; *mes*, mesometrium; *tri. om*, trilaminar omphalopleure; *y-s. c*, yolk-sac cavity.

(Pl. II, fig. 6). The proximal portions of the uterine glands and the cells of the endometrium on the maternal border of the trophoblastic zone have lost their cellular outlines and occur in the form of a cytoplasmic mass in which numerous nuclei appear to be floating freely. Many blood capillaries occur in this region. The deeper regions of the endometrium bordering the myometrium is compact and contains the fundic ends of a few uterine glands in this region.

Stage II : Late implanted bilaminar blastocyst. Four bilaminar blastocysts at a late stage of implantation are available for the present study. The blastocysts and the implantation chamber have expanded considerably as compared to the earlier stage. In all the cases the mesometrial part of the uterine lumen, not occupied by the blastocyst, remains as a narrow slit at the level of implantation. The embryonic mass is oriented antimesometrially with respect to the morphology of the uterus (Pl. II, figs 7 and 8) in three of the specimens studied here, while it is directed between the lateral and the antimesometrial sides of the uterus in the fourth specimen. A small cavity — the primitive amniotic cavity (Pl. II, fig. 9) occurs in the centre of the embryonic mass of three of the blastocysts. The floor of the cavity is slightly curved and is composed of three or four layers of cells, whereas the roof is thinner. In the fourth blastocyst which has a larger amniotic cavity, (Pl. II, fig. 7) the roof of the cavity is thin, and is made up of a row of flat cells, which are in contact with the trophoblastic layer. In all the embryos the primitive amniotic cavity contains some amount of cell detritus.

The endoderm is composed of cuboidal cells containing large vesicular nuclei. In some places the endodermal layer seems to have become peeled away from its original position and lies freely in the blastocyst cavity. This is an artifact caused during the preparation of the stained sections. A thick eosinophilic homogeneous membrane — the Reichert's membrane — which is stained scarlet red in sections stained by PAS procedure (Pl. III, fig. 11), is present between the endoderm and the trophoblast.

The trophoblast can be recognized into a basal layer of cytotrophoblast and a deeper zone of syncytiotrophoblast. The latter has formed a thick mantle on all the sides of the implantation chamber. In many places the basal cytotrophoblastic layer has proliferated and has pushed into the syncytiotrophoblastic zone in the form of solid columns of cells (Pl. III, fig. 10). Numerous maternal capillaries with hypertrophied endothelium run in the syncytiotrophoblastic zone.

The endometrial tissue bordering the syncytiotrophoblastic shell occurs in the form of a symplasma containing numerous nuclei at various stages of disintegration. The distal ends of some of the uterine glands are present near the myometrial border of the endometrium.

4. Trilaminar blastocysts

Two trilaminar blastocysts at approximately the same stage of development (Pl. III, fig. 12) are available for the present study. The implantation chamber has enlarged considerably in comparison with the

earlier stage, the embryonic disc has expanded laterally, and the amniotic cavity has increased in its extent. The embryonic disc is orientated antimesometrially and is composed of several layers of compactly arranged cells. Mesoderm occurs as a sheet of loosely arranged cells below the embryonic disc, and has extended to the extra-embryonic region on all the sides between the endoderm and the trophoblast and around the roof of the amniotic cavity. The endodermal lining of the wall of the large blastocyst cavity — the wolk-sac cavity is composed of cubical cells each with a large centrally places vesicular nucleus. A distinct Reichert's membrane, stained scarlet red by PAS procedure, occurs between the endoderm and the trophoblast.

The placenta at this stage can be recognized into two kinds : — the "trophoblastic placenta" [1] on the dorsal side of the embryonic disc, and the nonvascular yolk-sac placenta in relation to the rest of the blastocyst wall. Histologically the placenta is almost as in the previous stage except for the fact that some of the cytotrophoblastic cords have become hollow near the foetal border of the placenta so that the maternal capillaries invested by the syncytiotrophoblast appear like tubules hanging from the uterine wall (Pl. III, fig. 13).

5. Neural groove stage

Four specimens possessing progressively advanced stages of the embryo in the neural groove stage of development were available for study. In three cases the neural groove was still open, whereas in the fourth it had closed in several places. The embryonic disc lies between the lateral and antimesometrial sides of the uterus in two specimens, towards the lateral side of the uterus in one, and between the lateral and mesometrial sides in one. Whereas the placenta is present on all the sides of the uterus at the level of implantation in the earliest of the neural groove stages, the placenta in the abembryonic region becomes progressively thin as development proceeds, and is altogether abolished by the time the neural groove becomes deep and the exocoelom is formed at the edges of the embryonic disc. Fig. 15 is a semischematic drawing to illustrate the section of the gravid uterus at an advanced neural groove stage in which the embryonic disc is directed midway between the mesometrial and lateral sides of the uterus, and there is a deep neural groove in the centre of the ectodermal plate of the embryo. A thin bilaminar amnion arches over a large amniotic cavity on the dorsal aspect of the embryonic plate. The amnion is in close contact with the chorionic placenta thus forming a chorio-amniotic placenta. Mesoderm occurs as a sheet of loosely arranged cells underneath the ectodermal plate and the early beginnings of somite formation can be noticed in some places. At the edges of the embryonic disc the mesoderm has split into somatic and splanchnic layers incorporating a small exocoelomic space between them. The placenta is in the form of a spherical cup with a thick base on the mesometrial side and thinning margin towards the antimesometrial side. Vitelline vessels have spread on the yolk sac up to the margin of the placental cup converting this part of the placenta into a chorio-vitelline placenta.

There is, however, no marked histological difference in the finer structure of the three types of placenta in the various regions of the gestation sac. Syncytiotrophoblast has penetrated to about three fourths of the thickness of the endometrium, and the cytotrophoblastic villi have entered deep into the syncytiotrophoblastic zone and some of the cytotrophoblastic villi have become branched. The placental tubules (Pl. III, fig. 14) are more distinct than in the previous stage. The maternal capillary within the placental tubule is lined by hypertrophied endothelium and is surrounded by two layers of trophoblast — an inner layer of syncytiotrophoblast with lightly staining sparsely distributed nuclei and an outer layer of cytotrophoblast with darkly stained crowded nuclei. In the region of the chorio-vitelline placenta there are numeruos vitelline capillaries on the foetal surface of the placenta, but they do not enter the placental complex.

DISCUSSION

One of the most outstanding feature which has been revealed by the present study concerns the fact that the orientation of the embryonic mass during early stages and during later stages varies in different specimens and at different stages of development in *Hipposideros fulvus fulvus*. Normally the orientation of the embryonic disc with respect to the morphology of the uterus is constant for a species and often within a family [7] [8]. However, in *Hipposideros fulvus fulvus* during the early stages of development, that is at the early bilaminar blastocyst stages, the embryonic mass is oriented either laterally or antimesometrially or midway between the lateral and antimesometrial sides. However, as the development proceeds there seems to be a progressive change in the orientation of the embryonic disc so that at the neural groove stage of development the disc is mostly between the lateral and mesometrial sides of the uterus. The orientation of the disc at the neural groove stage of development has a considerable impact on the future course of development as the chorio-allantoic placenta is formed on the dorsal side of the embryonic disc since the rest of the uterine wall is occupied by the chorio-vitelline placenta. In *Hipposideros fulvus fulvus* the chorio-allantoic placenta is invariably located on the mesometrial side during later stages of development [5]. Hence, in this animal there is apparently a shift of the embryonic disc towards the mesometrial side irrespective of its earlier location. In this respect this appears to resemble *Megaderma lyra lyra* [3] which is the only other bat where the embryonic disc has a variable orientation during early stages of development, but the definitive placenta is invariably located on the mesometrial side. With the present inadequate knowledge regarding the uterus-blastocyst relationship in the mammals it is not possible to explain this anomalous situation noticed in these two bats.

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DIEL RHYTHMICITY IN THE LOCOMOTORY BEHAVIOUR OF EYE-ELIMINATED BURBOT, *LOTA LOTA* L. (PISCES, GADIDAE)*

BY

ROLF KRONELD

At the same time as the swimming activity of the burbot and its dependency of the light conditions have been studied, a closer description of the channels the light uses in the ruling of the rhythmic behaviour has also been attempted. This experiment indicates that the swimming activity of the burbot is influenced quantitatively by eye elimination. The mechanism of synchronization seems to be of an integrated nature. The fishes stayed night-active throughout the experimental period, during which the burbot according to several investigations made in Messaure (66°42′ N, 20°25′E) usually showed day-active behaviour. This is most probably a consequence of the operation, which eliminates certain seasonally changing visual pigments that correlate with phase shift in the burbot.

In my studies of the biorhythmicity of the burbot [4] [5], which have shown that the phase shift and the rhythmic behaviour in general is closely dependent of the light conditions (light intensity and lenght of light-darkness periods) I have also come to reflect on whether removal of one or more organs from the organism might influence the organism's synchronization to the hypothetical Zeitgeber which induces its rhythmic behaviour.

To test this, the eyes were removed from night-active burbots (age group II, 10–15 cm). This was done in November, in moderated light conditions (dLD). The operation was done according to Eriksson [2]. After the operation, ten days were usually required for the fish to recover before the experiments could start. The locomotory activity was registrated according to Müller and Schreiber [7], with photo-cell registration and an Elmeg counter. After the operation the activity of the fishes was approximately evenly distributed over the day and night.

In January, decade I, two (random choice) of the four operated burbots were placed in normal light conditions (nLD). This was done partly to study the effects of an increase of light intensity on the activity pattern of the fishes, and partly to investigate the possible significance of the eye as a synchronizator in relation to other extraocular factors. The undelying question is of course concerned with the relationship between the cyclical course of the Zeitgeber and the inner circadian rhythm, and also the significance of the Zeitgeber and its strength when the ocular receptors have been removed.

* Economical support to make this work possible has been received from the Delegation of the Swedish Culture Foundation and from the Academy of Finland.

RESULTS

1. Fishes in dLD (< 5 lux). The onset of activity is nearly synchronized to the dusk after the 8th of January, after having been evenly distributed during the day and night after the operation. The light intensity is probably too low for a synchronization or consistence to be induced by external Zeitgeber-signals. Either the reaction is to be explained from the endogenous rhythm of the fishes which appears with variations in the onset of activity or from the synchronization of the fishes through extraocular factors which makes them resume their activity before the operation. The behaviour of the fishes can possibly be the result of a combination of these both alternatives.

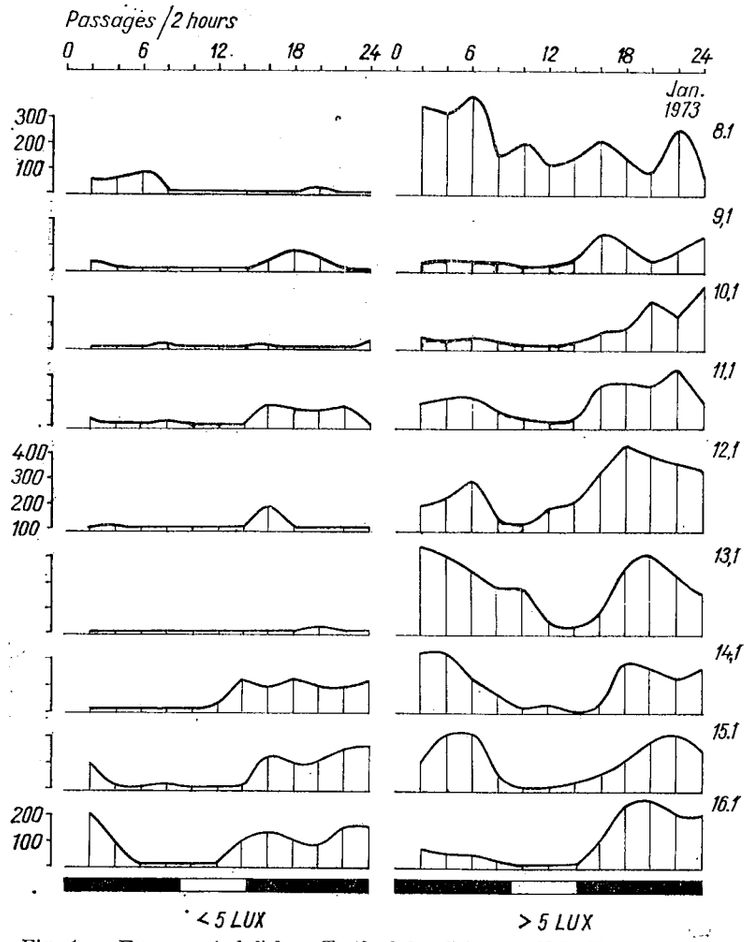

Fig. 1. — Eye-operated fishes. To the left: fishes in dLD. To the right: fishes in nLD. *Ordinate*: passages/2 hours. *Abscisse*: time of the day. 5-lux limit values for sunrise and sunset are marked.

It can of course be said that the locomotory pattern has appeared out of sheer coincidence, but the pattern of activity seems in my opinion too regular to justify such a conclusion.

2. Fishes in nLD (> 5 lux). The activity conditions are unclear on the 8th of January. One day later, when the two eye-eliminated burbots have been placed in normal light conditions (nLD), the onset of activity is synchronized to the dusk, which is clear from figure 1. The quantitative activity is markedly higher than that of the fishes in dLD, and the rhythmic quality of the locomotory activity (night-activity) is clearer.

DISCUSSION

The eye-operated fishes show irregular activity patterns after the operation. They don't get synchronized to day-activity as normal fishes do at this time of the year [6], (Müller 1970). On the contrary it seems like the burbots would adapt to an endogenous periodicity during decade I of January, which at least periodically is synchronized to the light in a seemingly night-active manner. This partial synchronization is characteristic of the burbots that continuously were kept in moderated light conditions (dLD).

The group that was moved into natural light conditions (nLD) on the contrary showed a much quicker and quantitatively greater synchronization to the light. This complete synchronization, however, was in the same direction as that of the former group, namely towards night-activity.

The lack of eyes is probably compensated by other, extraocular receptors. The capacity of comprehending the time signal is made more difficult, but as this experiment shows, it is not made totally impossible in eye-operated fish.

Experiments with fishes, the eyes and pineal organ of which both have been eliminated [3], also show that the organism has got extraocular receptors of light beyond the two mentioned types of receptors. An interesting fact is that the increase in light intensity most directly influences the quantitative activity [1].

In multicellular organisms (for instance, burbots) the synchronization to light is probably of an integrated nature, which means that the removal of one or more organs hardly can touch the "core" of the rhythmic activity, as the "biological" clock is not limited to any isolated part of the whole.

However, Beatty (1969) has shown seasonal changes in the burbot regarding visual pigments with the highest percentage of the retinene$_2$ pigment in the fall and winter (when the burbot has phase shifted from night- to day-activity) and the lowest in spring and early summer (when the burbot is night-active (Fig. 2). Thus the night-activity of eye-eliminated burbots in the winter could be explained partly through the operation, which eliminates these visual pigments.

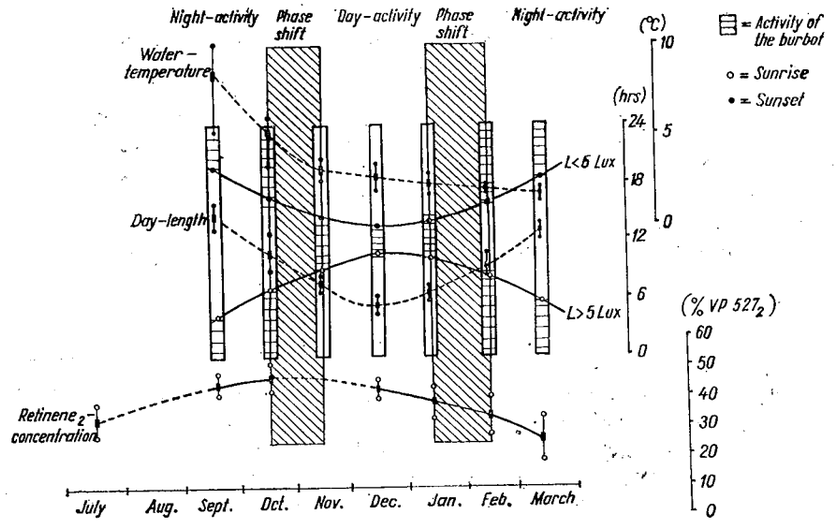

Fig. 2. — Phase shift from night-to-day-activity during the autumn and from day-to-night-activity during the spring in normal burbots, compared to seasonal changes of retinene$_2$-concentration according to Beatty (1969). Water temperature (°C), length of day-light by 5-lux limit values for sunrise and sunset (hours) are marked as means and extreme values during autumn and spring, when my eye-eliminated burbots (Fig. 1) were night-active.

Aeknowledgement. For initiative and material possibilities concerning my performance of the first part of a research projekt on the biorhythmicity of the burbot at the polar circle, I hereby thank prof. Dr. Karl Müller of Messaure biological station. I am also indepted to Dr. Agnes Müller-Haeckel, FK Lars-Ove Eriksson, Dr. Eino Erkinaro and Dr. Jaroslav Figala for interesting contributions of the discussion during my stay in Messaure. For help with drawing, I thank Mrs. Kerstin Bergdahl, while Bo Bergdahl is thanked for technical assistence.

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THYROID INFLUENCES UPON THE THYMUS IN WHITE RATS

BY

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Male white Wistar rats weighing 100 ± 10 g were administered TSH during 3 days (10 IU daily), thyroxine during 7 days (10 μ g daily), or thiouracil during 9 days (25 mg in the first day and 0.1 % in the milk other 8 days) (all doses related to 100 g body weight).

In all treated groups an increase of the thymus weight was noted, as well as an increase of the amounts of total proteins and of DNA. The total nucleic acid content increased in thiouracil treatment only. The RNA content decreased in all treated groups, indicating that the doses used were at the limit between the stimulating and involuting action of the administered substances.

The assumption of a correlation between thyroid and thymus glands is based upon: 1. the common embryological origin of the two glands (from the third and fourth branchial pouch) [11]; 2. the antithyroid action of the thymus [3], [4]; the partial inactivation of the thyreotropic hormone by the thymic tissue and the lymph nodes [7]. Taking into account the role of the thymus in immunobiological reactions [5] and its reactivity against endocrine influences [9], we followed the effect of some thyroid factors on several parameters of the protein and nucleoprotein metabolism, having special importance in the thymus activity [2] [14].

MATERIAL AND METHOD

Experiments were made on male white Wistar rats, weighing 100 ± 10 g. Animals were fed with milk, grains and carrots. They were divided in groups as follows:

1. Controls; these were injected during 10 days with 0.5 ml Tyrode saline daily;

2. Thyroxine treated group; these received daily 10 μg DL-thyroxine (puriss., Fluka) per 100 g body weight, during 7 days;

3. TSH treated group; 10 IU of hormone (Ambinon, Organon) on 100 g body weight were administered during three days;

4. Thiouracil-treated group; the animals were injected with a unique dose of 25 mg/100 g, of 2-thiouracilum puriss (Serva); afterwards, the substance was administereo orally, during 9 days, in a concentration of 0.1 per cent in the milk.

Each group had 10 animals. These were decapitated 24 hours after the last treatment, after a fasting of 18 hours. The thymus was isolated

and immediately weighed on a torsion balance. The following determinations were made:

— total protein content (TP), after the Robinson-Hogben method, modified by Korpáczy [6]; amounts were expressed as milligrams per 100 mg;

— total nucleic acid (TNA), DNA and RNA contents, by the spectrophotometric method of Spirin [10], as modified by Abraham and Pora [1]; results were expressed in mg per g.

The percentage differences between the values obtained in the treated groups and those of the control one were checked for statistical significance using the "t" test of Student.

RESULTS AND DISCUSSIONS

Our data (Table 1) show an increase of the thymus weight as compared to that of the control group : +83% in the thiouracyl group, +37% in TSH treated animals, +23% under the action of thyroxine treatment. It is interesting to note that, in our conditions, tne TSH and thyroxine

Table 1

Influence of TSH, thyroxine and thiouracil upon the contents of proteins and of nucleic acids in the thymus

		CONTROL	TSH	THYROXINE	THIOURACIL
Total proteins	Mean	8.01	13.61	10.79	10.70
	ES±	0.78	0.82	0.78	0.85
	n	8	8	9	10
	±%	—	+69	+34	+33
	p	—	<0.001	<0.01	<0.01
TNA	Mean	31.34	32.09	32.44	37.09
	ES±	1.45	1.06	1.31	2.26
	n	8	9	10	9
	±%	—	+2	+3	+18
	p	—	—	—	<0.05
RNA	Mean	12.37	6.50	7.92	6.14
	ES±	0.67	0.14	1.99	0.21
	n	8	9	10	9
	±%	—	−48	−36	−51
	p	—	<0.001	<0.02	<0.05
DNA	Mean	18.97	25.59	24.52	30.95
	ES±	1.71	1.06	1.26	2.29
	n	8	9	10	9
	±%	—	+34	+29	+63
	p	—	<0.001	<0.01	<0.001
Thymus weight	Mean	142.50	195.70	175.40	261.66
	ES±	13.47	9.81	11.07	17.00
	n	8	10	10	9
	±%	—	+37	+23	+83
	p	—	<0.01	0.10<p<0.05	<0.001

effects are of the same type with those thiouracil, a synthetic antithyroid substance. Milcu et al. reported [7] a thymus hypertrophy in chronical thiouracil treatment, with histological signs of cortical proliferation. In our opinion, the results reported in this paper are in agreement with those of Milcu et al.

Our biochemical data show an increase of TP (+33%, $p < 0.01$), of TNA (+18%, $p < 0.05$), and of DNA (+63%, $p < 0.001$). The question may be put, if in some conditions (depending on the sex of the animals, the dosis, the duration of treatment etc.) there are not an action of the thiouracil on the thymus, independently of its effect upon the thyroid gland. On the other hand, a connection may be supposed between the hyperplasia of the thymus and the inhibition of the fasciculate zone of adrenals in thiouracil treatment, as described by Parhon [8]. This hypothesis is worthy of consideration, as it is known that glucocorticosteroids possess a strong thymolytic action, as well as an effect of enhancing the protein and nucleoprotein catabolism [2] [12] [13] [14].

The stimulating action of STH and thyroxine upon the thymus is to be explained through the data of Cemşa [4]. This author shows, that moderate doses of thyroxine elicit a hyperplasy of the thymus, meanwhile higher doses lead to an involution of the gland. The turning limit of the effects depend on endocrine and metabolic factors, feeding conditions, sex etc.; these dependences may explain the contradictions existing in literature data [3]. In our experiments, the TSH had a stronger stimulating effect that the thyroxine doses we used. In both cases, as well as in that of the thiouracil, we probably reached the maximal limit of thymus stimulation; the decrease of RNA content is an argument for his supposition. Our previous experiments on the thymus involution by hydrocortisone or tumoral stress have shown that the RNA content is the firstly affected parameter [12] [13].

The hyperplasia of the thymus in a light hyperthyroidism may have practical implications. Zondek and Bernhardt [15] assume that the thymus hypertrophy in Basedow's desease plays the role of a contrathyroid mechanism. The complex hormonal influences acting upon the thymus [9] allow also to suppose that the immunobiological capacity of this gland would be also dependent on the equilibrium of internal secretions.

In conclusion, the treatments of white rats weighing 100 g with: thiouracil, 25 mg per 100 g, nine days, 0.1% in the milk; TSH, 10 IU per 100 g daily, three days; thyroxine, 10 μg per 100 g, seven days, lead to an evident thymus hyperplasy. This modification is charaterized by an increase of the weight of the gland, and an increase of the contents of total proteins, DNA, and total nucleic acids (that of the last parameter, in thiouracil treatment only). The RNA content decreases in all treated groups; this shows that the doses used were such, that the turning point was reached between the stimulating and involuting action of the administered substances.

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BOVINE HEPATIC CATALASE SOLUBILIZED WITH SODIUM LAURYLSULPHATE

BY

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The influence of different sodium laurylsulphate concentrations upon the catalase extraction from the bovine hepatic tissue was studied. High detergent concentrations induce the enzyme inactivation. The solubilization of catalase and total proteins was traced electrophoretically. The utilization of laurylsulphate as extraction agent influences the variation of reaction rate with H_2O_2 concentration, with pH, the course of the enzymatic reaction in time, the stability of catalitic activity, as well as the electrophoretic behaviour.

In a previous paper [2] it was shown that the ionic detergent solutions — sodium deoxycholate, sodium laurylsulphate, sodium taurocholate, brij and saponine — used for the solubilization of the catalase from the bovine hepatic tissue extract the greatest quantities of proteins and permit the evidencing of an intense enzymatic activity. It was likewise shown that in the case of the utilization of deoxycholate solutions 0.5% and 3% as catalase extraction agents, the optimum enzyme action parametres are modified, probably, because of the supplementary solubilization of the enzyme bound to cytoplasmatic formations. When sodium deoxycholate 0.5% solution was used as extraction agent, a second maximum was recorded in the curve which renders the reaction rate variation with hydrogen ion concentration, at pH 6.4 while in the zimograms obtained on agar gel a new band in start and the intensification of the electrophoretic band which migrates towards the anode was recorded.

MATERIAL AND METHOD

The enzyme extraction procedure. The bovine hepatic tissue was mortared in the presence of acetone until a powder of white-yellowish colour is obtained. 1 gram acetonic powder was treated with 20 ml water of laurylsulphate solutions, the extraction of proteins being realized under agitation. After one hour, the homogenate is centrifuged at 10,000 r.p.m. for 15 minutes, the supernatant representing the total proteic extract.

Catalase activity determination, was effected according to the colorimetric method described by Sinha [5]. The reaction mixture comprises: 0.5 ml H_2O_2 – solution $1.6 \times \times 10^{-2}$ M; 0.4 ml buffer solution K_2HPO_4/KH_2PO_4, 1×10^{-2} M, pH 7 and 0.1 ml total proteic extract, diluted 1 : 100.

Catalase specific activity was expressed in H_2O_2 (μmoles consumed/mg protein/minute/ 20°C.

Protein concentration was dosed in conformity with Lowry's at al. method [3].

Catalase isoenzymes were separated by electrophoresis in agar gel (1.25%), in a veronal–HCl buffer solution of an ionic strenght of 0.01, four hours migration at 5 mA/plate and 120 V. Visualization of electrophoretically separated proteic fractions was realized with a 1% Amido Schwartz 10 B solution, prepared in 7% acetic acid, while the evidencing of proteic bands with catalase activity was realized in conformity with the method described by Woodbury at al. [8].

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RESULTS

The influence of different sodium laurylsulphate concentrations on the extraction process of the catalase from the acetonic powder of the bovine hepatic tissue, in comparison with water, is given in table 1. At it can be seen, the solubilized protein concentration, expressed in mg/ml, increases from 8.1 to 19.2 when the laurylsulphate concentration varies between 0.25%—2%, for at its higher concentrations, for instance — 3%, 4% and 5% — the protein quantities extracted should drop and then remain stationary. Enzymatic reaction rate, in the case of the utilization of some laurylsulphate concentrations, varying in the domain of 0.25%—2% is constant and higher as value to that recorded in the case of water. At higher concentrations the phenomenom of inactivation appears due to the dissociation of the catalase macromolecule, a fact observed also in the case of other enzymes [4—7].

Table 1

The influence of different concentrations of sodium laurylsulphate on the extraction of bovine hepatic catalase

Laurylsulphate concentration (%)	Protein (mg/ml)	μmoles H_2O_2/ml/min/ 20°C
0	4.8	31,000
0.25	8.1	68,000
0.52	9.8	71,200
0.75	13.6	69,860
1	15.6	71,200
2	19.2	70,400
3	17.8	66,300
4	17.6	46,900
5	18.0	31,700

Following up the effect of protein solubilization from the acetonic powder of the bovine hepatic tissue with water and 0.5%, 3% and 5% laurylsulphate solutions on the spectrum of proteins electrophoretically separated, as well as on zimograms, the appearance of new electrophoretic bands with catalasic activity is recorded in the case of the utilization of a solution 0.5% and 3% laurylsulphate (Fig. 1). The proteic fraction enzymatically active which migrates towards the anode is more intense in the case of the use of the detergent in a concentration of 0.5% than in the case of water and of the 3% solution, disppearing completly when the 5% laurylsulfate is used. The cathodic band is very slightly visualized in the case of a 5% solution of detergent. The spectrum of proteins, in all the four cases, differs both quantitatively as well as qualitatively.

The course of the enzymatic reaction during the five minutes in the case of the utilization as extraction medium — water and 0.5%, 3% and 5% laurylsulphate solutions is shown in figure 2. In the first case, the 80 μmoles H_2O_2 introduced initially into the reaction mixture are consumed in the course of five minutes, the rate growing directly proportionally only during the first reaction minute. When the extraction medium is the laurylsulphate solution 0.5% and 3% the entire quantity of substrate is consumed in 3 and respectively 4 minutes. The curve which renders the course of enzymatic reaction, in the case of the utilization of a 5% detergent concentration it has a particular aspect as we have to do with two reaction orders.

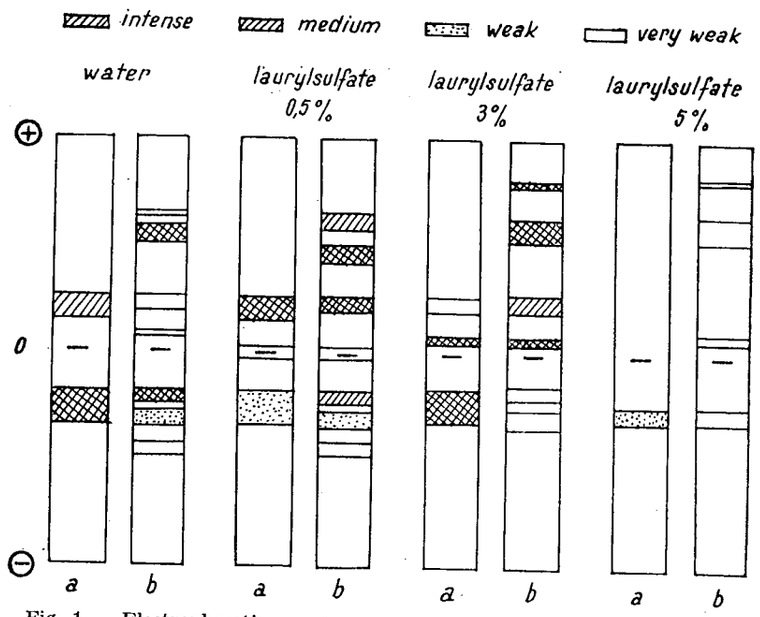

Fig. 1. — Electrophoretic spectrum of active enzymatic fractions and total proteins extracted with water, laurylsulphate, solution 0.5%, solution 3% and solution 5%. *a*, sketches of catalase molecular formes; *b*, sketches of proteic fractions electrophoretically separated.

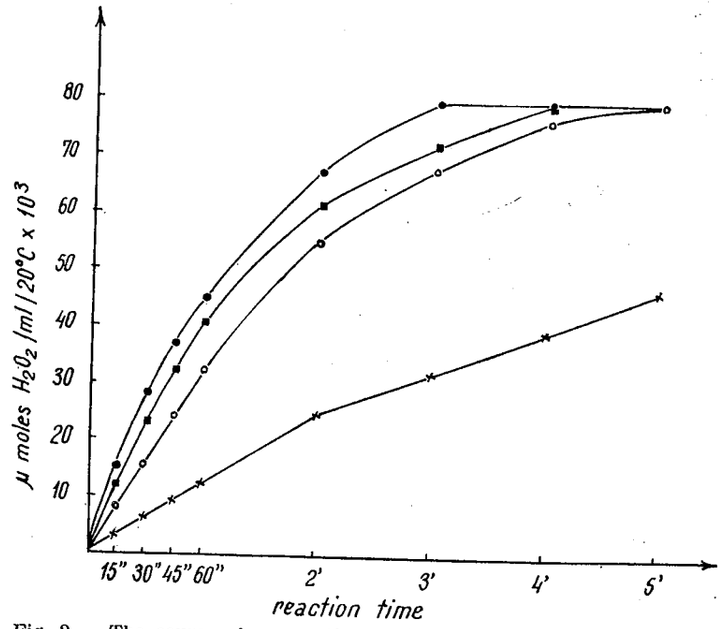

Fig. 2. — The course of enzymatic reaction in time when the extraction medium of catalase is: —o—o— water; —●—●— laurylsulphate, solution 0.5%; —■—■— laurylsulphate, solution 3%; —×—×— laurylsulphate, solution 5%.

The influence of the present H_2O_2 concentration in the reaction mixture on the enzymatic preparations obtained by the extraction with different solutions is presented in figure 3. It is found that in the case of the utilization of the three concentrations of laurylsulphate as extrac-

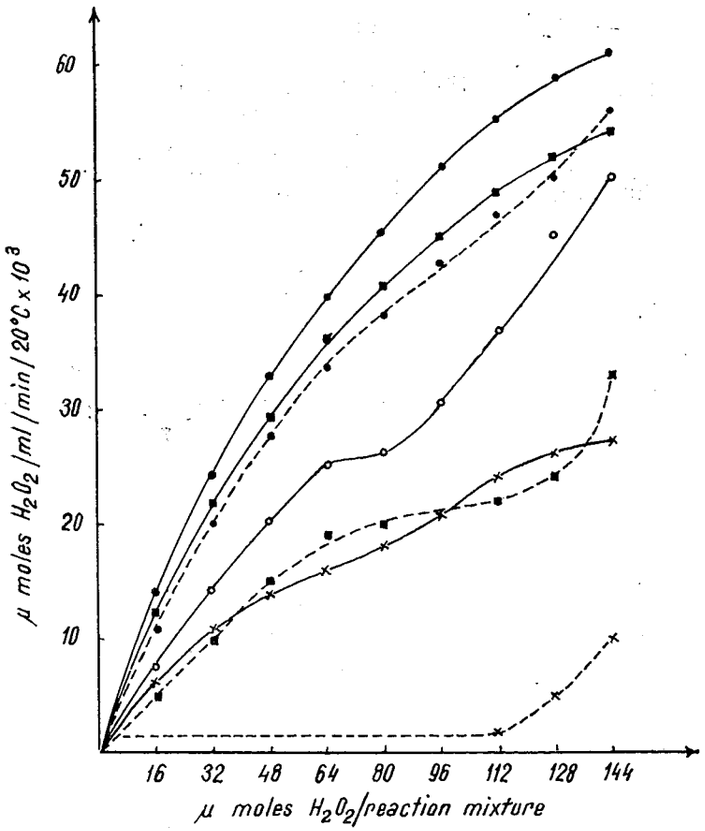

Fig. 3. — Influence of substrate concentration on catalized reaction rate of the enzyme extracted with: —o—o— water; — ● — ● — laurylsulphate 0.5% solution; — ■ — ■ — laurylsulphate 3% solution, and — × — × — laurylsulphate 5% solution.

tion agent, the aspect of curves which render the reaction rate variation with the substrate concentration is modified when the determinations are effected after 24 hours from the preparation of the total proteic extracts. In the case of the use of the 0.5% laurylsulphate solution for the extraction of catalase from the acetonic powder of bovine hepatic tissue, the variation of the rate with the concentration in H_2O_2 follows the trajectory of an hyperbole; the second day from the preparation of the extract presents lower values to those previously dosed and a somewhat sigmoicity in the domain of 128—144 μmoles substrate. The inactivation

in the course of 24 hours of keeping the proteic extract at 7°C is more marked in the case of the utilization of the 3% solution, at the same time the sigmoicity phenomenon presented by the curve recorded the second day of the solubilization experiment being more accentuated. The hyperbolic variation of the enzymatic reaction rate determined in the presence of various substrate concentrations disappears by the increase of the quantity of laurylsulphate present at 5% in the extraction medium. By keeping at 7°C, for 24 hours, the enzyme from the proteic extract realised with a 5% solution of laurylsulphate becomes active only in the

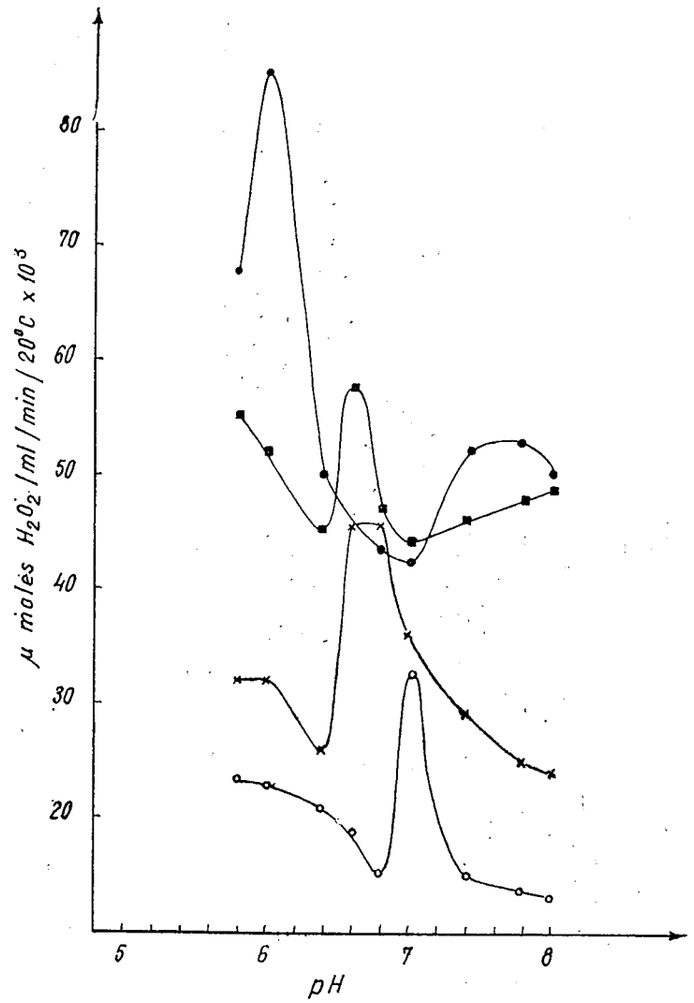

Fig. 4. — Optimum pH variation of the catalase depending on the extraction medium utilized: —o—o— water; — ● — ● — laurylsulphate 0.5% solution; — ■ — ■ — laurylsulphate 3% solution, — × — × — laurylsulphate 5% solution.

presence of great concentrations of H_2O_2, 120—144 μmoles. It is observed that the differences between catalytic activities recorded for the preparations obtained by the utilization of various extraction agents are more marked in large substrate concentrations.

The utilization of laurylsulphate solutions of 0.5%, 3% and 5% for the extraction of bovine hepatic tissue catalase, has as a result different variations of catalysis rate depending on the pH reaction mixture (Fig. 4). In the case of the utilization of water for the extraction of catalase it is recorded that catalytic activity is maximum at pH 7. When reaction was realized with the three laurylsulphate concentrations, the catalase presents an optimum activity at more acid pH values. A phenomenon worthy to be noticed is the appearance of two maxima of catalytic activity in the case of the performing of the experiment of the solubilization with a 0.5% solution of laurylsulphate.

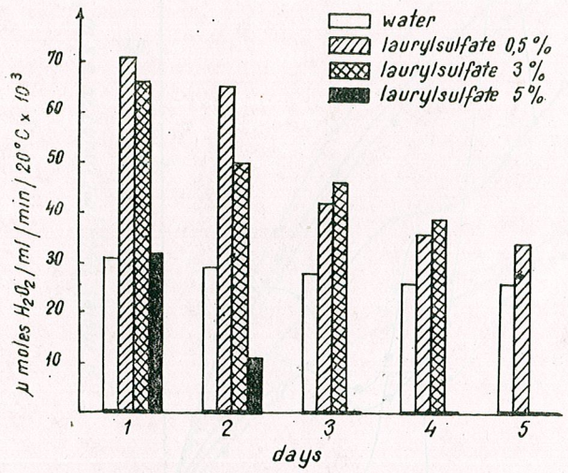

Fig. 5.—Activity stability of some catalase preparations achieved by the utilization of three extraction media, in the course of five days' keeping at 7°C.

The denaturating effect of this anionic detergent was traced by determining the enzymatic activity, periodically, for five days from the preparation of total proteic extract (Fig. 5). The enzymatic preparation realized with laurylsulphate 5% solution is completely inactivated by its being kept for two days, while the one obtained with the 3% solution for four days. At the same time, the enzymatic preparations in which the total proteins were extracted by means of water are much stabler.

DISCUSSIONS

The laurylsulphate solutions of various concentrations have a solubilizating action both upon total proteins from the acetonic powder of the bovine hepatic tissue, as well as upon the catalase. Protein concentration in the extracts realized by the use of 0,25%—2% detergent solutions is all the greater as the concentration in laurylsulphate increases, but the enzymatic activity of preparations, though clearly higher to the

one obtained in the case of the use of water as extraction agent, does not increase proportionally with total protein concentration, which evinces a non-selective solubilization. By the increase of laurylsulphate concentration in extraction medium to 3%, 4% and 5%, the solubilization rate of total proteins decreases and then becomes stationary. This fact is accounted for, probably, by the formation of detergent micelles which involves also protein molecules. The marked decrease of catalase activity, in the case of protein extracts prepared with a 4% and 5% laurylsulphate solution may be due to the cooperation of three phenomena: — the involving of catalase molecules in the formation of micellar process — to an inhibition effect by the redistribution of electrical charges at the level of active catalytic site, or — to the scission action of the quaternary structure of catalase, which is formed of 18 polypeptidic chains with molecular weights of 14,000 in subunits which possess a fraction of the native molecule activity [1]. The fact that the laurylsulphate modifies the distribution of electric charges at the level of active catalytic site is evinced by the modification of optimum pH values at which the different catalase preparations act. The greatest pH optimum displacement is recorded in the case of the utilization of 0.5% laurylsulphate solution in the quality of extraction agent. This fact to which the existence of a second maximum of activity (pH 7.8) is also be added suggest that at small detergent concentrations, in conditions in which the critical concentration of micelles formation has not been reached, the electric interactions catalase-laurylsulphate are stronger. The increase of detergent concentration leads to the appearance of micelles which may include or adsorb also some protein molecules, leading to the decrease of the field of interaction between electric charges of the laurylsulphate and those of the enzyme which remain free and permit that the value of parametres taken into study would tend towards the characteristic one of native catalase.

The inclusion of catalase into the laurylsulphate micelles at concentrations higher than 3% is evinced also by the data concerning the course of enzymatic reaction in the course of five minutes. By catalase solubilization with a 0.5% solution of laurylsulphate, the 80 μmoles of H_2O_2 introduced into the reaction medium are completely consumed in only three minutes, as against the five minutes necessary to the preparation obtained by extraction with water. At the concentration in detergent of 3% the same quantity of substrate is descomposed in four minutes, though the total protein concentration (17.8 mg/ml) is higher to the two foregoing solubilization cases. At a 5% concentration in laurylsulphate predominates the dividing action of the catalase molecule into subunits. The quantitative determination data agree with the results obtained by electrophoretic separations, which evidence the formation of some proteic fractions with larger migration rates instead of the intermediary ones.

The data presented permit our supposing the following sequence of events concerning the catalase-laurylsulphate interactions. At a 0.5% detergent concentration, the laurylsulphate induces a rearangement of electric charges at the level of active catalytic site, manifested by an activation. The formation of laurylsulphate micelles, supposed to be initiated at concentrations of 2%—3%, involves also the catalase molecules, partially including them and becoming inaccessible to the substrate.

It is probable, that at these concentrations begins also the partial dissociation of the polymere catalase molecule, a phenomenon which becomes predominant in the case of the 5% laurylsulphate solution. All these phenomena are accentuated in time, in the case of keeping the total protein extracts.

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L'ÉVOLUTION DES MODIFICATIONS CYTOGÉNÉTIQUES DANS LES CELLULES DE LA MOELLE OSSEUSE ET DE LA RATE CHEZ LE RAT BLANC INTOXIQUÉ PAR LE BENZÈNE

PAR

FL. TEODORESCU, Al. CĂLUGĂR et R. DUDA

A group of white rats has been subject to a chronic intoxication with benzene vapors. The authors tried to observe the eventual reshufflings in time, the new aspects which could be found in karyotype and the variations of the mytotic index in medullary cells and in spleen after a 4 months treatment interruption. In the karyotype of the investigated cells after the above mentioned period, stable chromosomic anomalies were recognized; the mytotic index did not reach normal values.

Des observations cliniques directes ou des études expérimentales ont maintes fois confirmé, en dehors des autres aspects pathologiques, la capacité cytotoxique des hydrocarbures au noyau benzénique. Chez les ouvriers exposés à long terme, par leur profession, aux vapeurs de benzène, Pollini et Collombi [11], Tough et Court Brown [12], Forni [3], Pollini et collab. [10], Forni et collab. [4], ont réussi à mettre en évidence des altérations structurales et numériques dans le caryotype des cellules médullaires et des lymphocytes du sang périphérique. Dans la majorité des cas, l'état pathologique évoluait vers les états leucémiques, souvent de type myéloblastique, même après un longue période de latence.

Nos résultats expérimentaux sur la capacité mutagène du benzène ont été communiqués dans un travail antérieur [2]. Les éventuelles modifications à la longue et les nouveaux aspects qui pourraient être trouvés dans le caryotype des cellules après l'arrêt du traitement, ainsi que la variation de l'index mitotique, ont constitué les prémisses de ce travail.

MATÉRIEL ET MÉTHODE

Ce travail a été effectué sur un lot de 10 rats exposés chaque jour, pendant 6 heures, aux vapeurs de benzène dans un espace fermé; la concentration moyenne a été de 13 mg/mc air. Après 4 mois d'intoxication on a effectué des observations cytogénétiques chez la moitié du lot (5 rats, lot A), en utilisant des cellules en division de la moelle osseuse et de la rate [2]. Le reste des animaux (lot B) a été sacrifié après un autre intervalle de 4 mois et on a effectué les mêmes analyses, dans des conditions identiques. Parallèlement on a étudié un lot témoin.

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Tableau I

Différents types d' anomalies (exprimés en %)

MOELLE OSS.	total cell. comptées	apparemment normales	avec des anomalies	lacunes	cassures	fragments	anneaux	délétions	translocations	assoc. chromos.	déficience de spiralisation	chromosomes pycnotiques	aneuploïdies	polyploïdies
lot intoxiqué (A)	500	55,8	44,2	20,6	12,6	15,6	0	0,8	0,4	0	3,4	0,4	3,6	0
lot après 4 mois (B)	310	68,4	31,6	11,9	8,4	5,8	0,3	2,5	0,3	1,0	0	0	4,5	0,3
lot témoin	272	72,0	28,0	16,5	8,0	5,5	0	0	0	0	0,5	0	1,8	0
RATE														
lot intoxiqué (A)	115	67,0	33,0	10,4	4,7	3,1	0	2,6	2,7	5,2	0,9	0	3,5	0
lot après 4 mois (B)	56	68,1	31,9	10,8	3,9	3,8	0	3,6	1,8	7,2	0	0	10,7	0
lot témoin	64	81,2	18,8	12,5	3,1	1,6	0	0	0	0	0	0	3,1	0

RÉSULTATS

Après 4 mois d'interruption de l'intoxication sont intervenues des modifications évidentes (tableau 1). Le rapport entre le pourcentage des cellules avec des anomalies et les cellules apparemment normales s'est modifié en faveur de la seconde catégorie ; celle-ci est sensiblement rapprochée de la valeur caractéristique du lot témoin par la diminution du pourcentage des anomalies considérées instables (cassures, fragments) et non spécifiques (déficience de spiralisation, chromosomes pycnotiques). Parmi les anomalies qui se sont maintenues aux mêmes valeurs ou même ont augmenté, on peut remarquer les translocations, les délétions chromosomiques, les aneuploïdies. Le fait que seulement une partie du total des réarrangements (ceux qui comprennent une quantité majeure de matériel chromosomique) peut être dépistée par observation directe, permet la supposition qu'il existe en réalité un chiffre supérieur pour cette catégorie. La différence entre la proportion des métaphases avec des anomalies des deux lots (A et B) est significative (le test « t ». Cette affirmation est valable aussi quand on recherche la modalité de représentation des différentes anomalies (le test χ^2). Au contraire, la différence entre le lot B et le lot témoin n'est pas significative, à l'exception de la distribution des différents types d'anomalies.

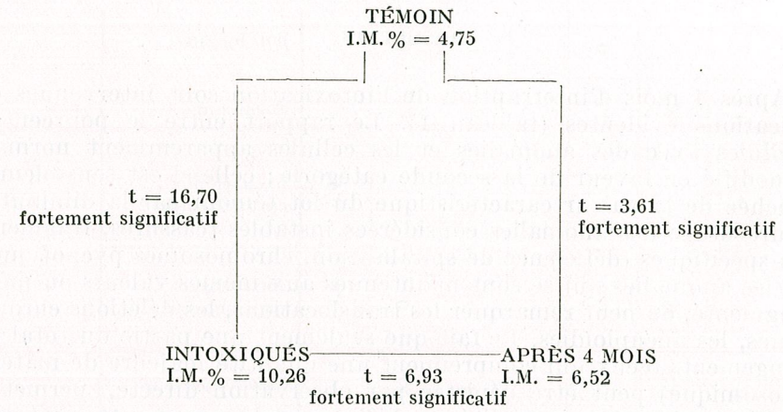
Dans la rate, on ne remarque pas de différences évidentes, calculées en pour-cent, entre les deux lots soumis à l'intoxication (tableau 1). L'importance accrue des anomalies chromosomiques stables peut constituer une explication de la presque constance des valeurs globales, par comparaison à la situation rencontrée pour les cellules médullaires.

Les valeurs qui expriment l'index mitotique chez les 3 lots sont sensiblement différentes. Le nombre des cellules médullaires en division est plus grand chez le lot A (I.M. = 10,26 %) par rapport au témoin (I.M. = 4,75 %), la différence étant significative statistiquement. Après avoir interrompu 4 mois l'exposition, l'I.M. baisse considérablement. Il enregistre une valeur moyenne (I.M. = 6,52 %), la différence étant fortement significative par rapport aux deux lots (tableau 2).

Tableau 2

Modifications de l'index mitotique dans les cellules médullaires

	Intoxiqués au benzène	Après 4 mois	Témoin
total cell. comptées	7500	4500	4500
moyenne I.M. %	10,26	6,52	4,75
erreur de la moyenne	±0,35	±0,37	±0,32



DISCUSSION ET CONCLUSIONS

Le retour des animaux intoxiqués aux conditions normales a déterminé ultérieurement l'installation d'un certain équilibre dans le caryotype. Nous avons déjà remarqué que la baisse de la proportion des anomalies instables, bien représentées chez le lot A (où les conditions d'intoxication ont eu un caractère permanent), a fait diminuer le chiffre global des anomalies observées chez le lot B. Cette situation pourrait expliquer d'une manière satisfaisante la situation dans laquelle la différence entre la distribution des types d'anomalies, non significative entre les lots A et témoin, devient significative quand on compare le lot B (chez lequel un nouveau tableau, caractéristique, s'est installé) et les deux autres lots. Le fait que chez les animaux sacrifiés 4 mois après l'interruption de l'exposition les délétions chromosomiques, les translocations, les aneuploïdies sont représentées par des valeurs comprises entre des limites égales (ou plus larges) à celles trouvées chez les animaux du lot A, confirme le caractère remanent, dans certains cas, de ces anomalies reconnues comme initiatrices des lignées cellulaires anormales (fig. 1, 2, 3, 4).

Les aneuploïdies observées sont assez importantes parmi les anomalies caractéristiques des deux lots (tableau 1). Elles sont orientées surtout vers le déficit de chromosomes (hypodiploïdie).Le pourcentage plus élevé de aneuploïdies peut être considéré comme une des conséquences de l'intoxication [3] [6] [7]. On ne peut pas exclure du calcul les cas dans lesquels le déficit en chromosomes dans une métaphase pourrait être dû aussi aux artefacts d'ordre technique.

Le principal effet nocif du benzène réside dans l'action toxique directe sur les cellules primordiales. Les valeurs supérieures par lesquelles on exprime l'I.M. chez le lot A démontre une hématopoïèse particulièrement active. Quoique étant après une période de régime normal, l'I.M. apparaît diminué par rapport au lot testé antérieurement, mais reste éloigné toutefois des limites normales, ce qui démontre une influence profondément irritative et traînante de l'agent toxique. Grâce aux cellules qui restent porteuses d'une tare génétique, la tendance de perpé-

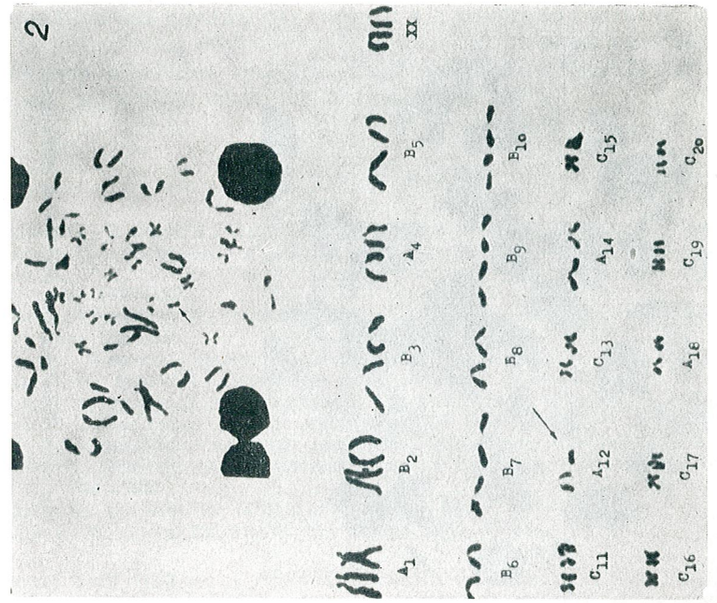


Fig. 2. — Chromosome « minute ».

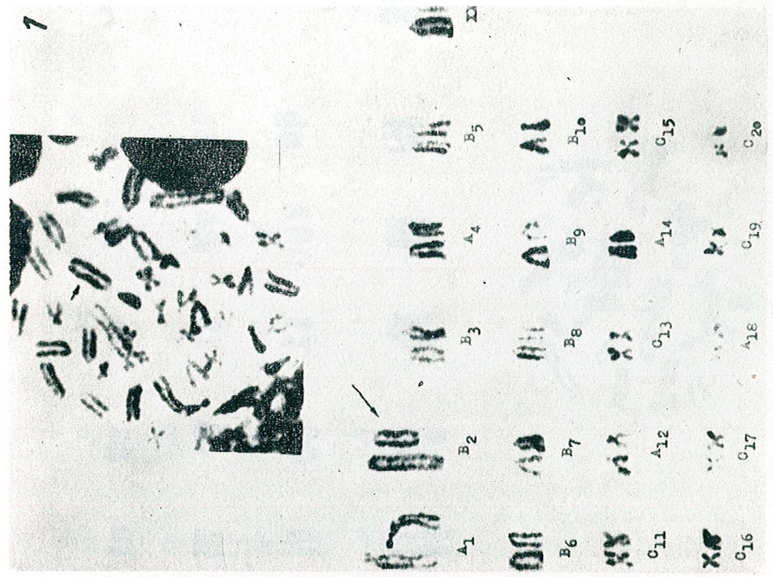


Fig. 1. — Délétion chromosomique.

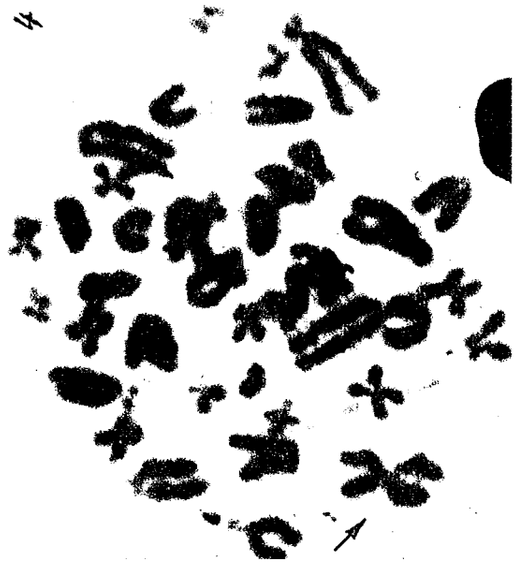


Fig. 4. — Translocation par fusion centrique.

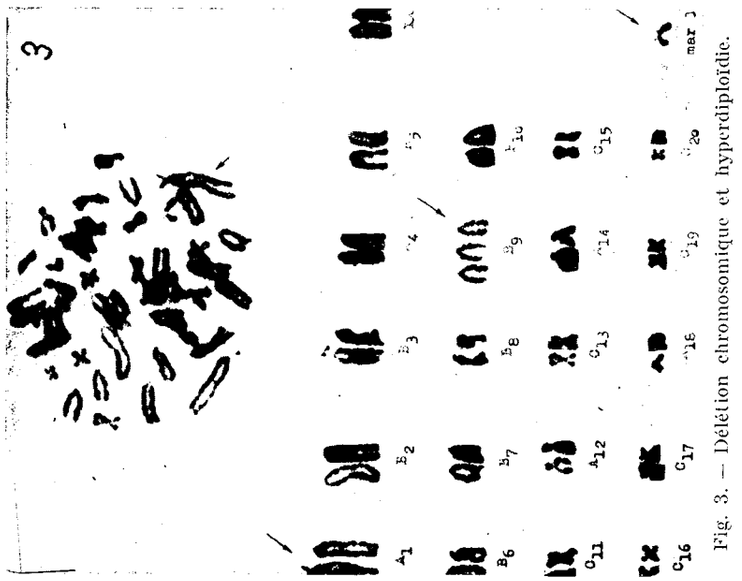


Fig. 3. — Délétion chromosomique et hyperdiploïdie.

tuation des lignées anormales est évidente. Cette exacerbation du potentiel de division des cellules sous l'influence persistante du benzène, à côté des anomalies des noyaux par rapport à la désorganisation de la prophase ou de la métaphase, déplace un équilibre initialement normal vers différents aspects morbides, qui sont d'ailleurs décrits dans la pathologie humaine ou démontrés chez les animaux [5] [9].

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