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LES CRITÈRES DE L'ESPÈCE. LE CRITÈRE MIXIOLOGIQUE

PAR

E. V. NICULESCU

The work points out that the mixiological criterion is not valid in all cases, such as distinct genitalia species which still cross among themselves. It is valid only as "species criterion" to differentiate species from sub-species ; as "interspecific criterion" aimed at delimiting and identifying several species of a genus it cannot be employed either, because all the species appear isolated from the reproductive viewpoint. The author refutes the thesis supported by a number of zoologists whereby the criterion of reproductive isolation is the only scientific one, upholding that it is neither the unique nor the best one.

Le critère mixiologique de l'espèce est le critère biologique (mieux physiologique) de l'isolement reproductif. Ce critère se trouve à la base du concept biologique de l'espèce. La plupart des adeptes de ce concept admettent que dans la délimitation des espèces l'isolement reproductif est un critère important. Mais il y a aussi des zoologistes qui exagèrent l'importance de ce critère et, ignorant d'autres critères, accordent à ce dernier une valeur absolue. Ainsi, on affirme que le critère biologique de l'espèce est le seul critère vraiment objectif et scientifique.

Nous ne pouvons être d'accord avec cette affirmation pour les raisons suivantes :

1. Les espèces ne sont pas seulement reproductivement isolées, mais aussi morphologiquement, écologiquement, éthologiquement et génétiquement.

2. Comme corollaire du point précedent, il résulte qu'il y a aussi d'autres critères que celui mixiologique, critères, eux aussi, scientifiques.

3. Le critère mixiologique non seulement n'est pas le seul critère de l'espèce, mais il est non plus le meilleur.

Des lignes précedentes il résulte que l'espèce ne peut être correctement et complètement définie selon un seul critère.

L'espèce a une structure où est inscrite son évolution même. La structure (les genitalia chez les insectes) et la fonction (l'isolement reproductif) constituent un tout unitaire, morpho-physiologique, qui ne peut être dissocié. Considérer seulement la fonction et ignorer la structure signifie avoir une conception unilatérale qui ne peut nous aider à nous faire une idée claire sur l'espèce.

Une espèce nouvelle apparaît à la suite d'une triade de phénomènes : 1. isolement morphologique, 2. physiologique (isolement reproductif), 3. génétique ; tous ces trois phénomènes définissent l'espèce (5). C'est pourquoi nous n'acceptons pas l'espèce biologique telle qu'elle est présentée par les adeptes du concept biologique c'est-à-dire définie seulement biologiquement. Même Mayr, le promoteur du concept biologique de

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l'espèce, dit (3) que « l'isolement reproductif, les différences écologiques et la *distinction morphologique* (notre soulignement) sont les trois propriétés les plus caractéristiques de l'espèce ». Par conséquent, selon Mayr aussi, il y a d'autres critères de l'espèce et parmi ceux-ci figure le critère morphologique donc l'espèce ne se définit pas seulement sous l'aspect biologique. Toujours Mayr dit, dans le même travail (3), que « L'espèce biologique est habituellement aussi une espèce morphologique (à l'exception de quelques espèces jumelles) ». Certains zoologistes repoussent aussi cette affirmation car ils considèrent que « nous ne pouvons affirmer que deux formes voisines sont devenues espèces distinctes que si l'on constate l'isolement reproductif dans la nature ». C'est cette même opinion unilatérale que nous réfutons catégoriquement puisqu'elle ne tient pas compte des autres isolements mentionnés ci-dessus. Deuxièmement elle ne correspond pas à la réalité puisque l'isolement reproductif n'est pas un phénomène absolu, général. On sait que dans la nature il y a un grand nombre de bonnes espèces qui s'intercroisent. Donc, elles ne sont pas isolées reproductivement. Ainsi le phénomène d'interstérilité n'est pas général. Pour accorder au critère mixiologique la plus grande valeur, on affirme que le critère morphologique est déficient, et que la délimitation et l'identification des espèces selon les caractères morphologiques est subjective et arbitraire. Si nous nous basons, dans les opérations taxonomiques uniquement sur le graphisme et le coloris, les palpes ou la formule chromosomique, les délimitations et les identifications peuvent naturellement être très souvent subjectives et arbitraires. Mais aujourd'hui l'entomologiste utilise avant tout l'armure génitale qui, si elle est examinée et interprétée selon nos recommandations (4), nous fournit d'excellents caractères spécifiques et génériques tout à fait objectifs. Mais les zoologistes qui méconnaissent la valeur de ce « phénomène morphologique » qu'est l'armure génitale, considèrent que l'étude exagérée des genitalia a une teinte typologique et les entomologistes qui font de telles études sont «typologistes» !

Certainement, le phénomène de l'isolement reproductif est un phénomène réel et il caractérise l'espèce — à quelques exceptions près. Puisque les exceptions sont beaucoup moins nombreuses que les « cas conformes », nous devons accepter cette notion et admettre que l'espèce doit comprendre dans sa définition aussi ce critère.

Nous ne rejetons pas le critère mixiologique comme les adeptes du concept biologique rejettent le critère morphologique. Nous l'acceptons, et lui accordons « la place » qui lui revient, mais non pas comme « le seul critère vraiment objectif scientifique ».

Ainsi, si nous nous référons au critère d'espèce (5) c'est-à-dire au critère qui nous aide à délimiter l'espèce de la sous-espèce, ce critère peut être, dans la plupart des cas, utilisé avec succès puisque les espèces sont interstériles et les sous-espèces interfertiles. Mais, dans certains cas, ce critère est déficient. Ainsi, par exemple, dans une vaste région faunistique on constate qu'à la périphérie de l'aire de distribution géographique certaines formes d'une espèce sont tellement différenciées dans l'habitus qu'il est difficile de dire si ces formes sont des sous-espèces ou espèces vicariantes. Le critère mixiologique ne peut être utilisé puisque les formes extrêmes sont séparées par des barrières ou par une discontinuité spatiale et par conséquent elles ne se rencontrent pas et nous ne pouvons savoir

si elles sont interfertiles ou interstériles. Voici l'une des déficiences du critère mixiologique. Le critère morphologique des genitalia (chez les Insectes) resoud simplement et rapidement ce problème, vu que, chez les espèces, les genitalia sont distincts, et identiques, chez les sous-espèces.

Enfin, si nous nous réferons au critère interspécifique (5), c'est-à-dire au critère utilisé dans la délimitation des espèces entre elles pour les identifier, le critère mixiologique est complètement inutilisable puisque les espèces considérées (supposons trois espèces d'un même genre) sont, toutes, isolées reproductivement.

Une autre déficience du critère mixiologique existe aussi dans la définition des espèces jumelles. Voici l'une des définitions présentées : « Les espèces jointives sont des espèces extrêmement voisines entre elles, ne différant que par des caractères morphologiques insignifiants et même souvent sans différences morphologiques apparentes, mais se distinguant alors biologiquement ; elles présentent cependant le caractère essentiel des „bonnes espèces" qui est l'isolement reproductif » (2). Dans toutes les définitions apparaît le phénomène de l'isolement reproductif. C'est là que réside le paradoxe : comment constater l'isolement reproductif chez les espèces jumelles qui ne se distinguent pas morphologiquement ? L'isolement reproductif peut se constater, chez les espèces sympatriques distinctes par l'habitus, en observant les formes intermédiaires. S'il y a des formes intermédiaires, cela signifie qu'elles sont interfertiles, donc elles ne sont pas isolées reproductivement ; si les formes intermédiaires manquent, les espèces dont il s'agit sont interstériles, donc isolées reproductivement. Mais comment constater des formes intermédiaires quand les deux espèces parentales sont identiques dans l'habitus ? Aussi comment constater des formes intermédiaires chez les espèces jumelles allopatriques ? Et, toutefois, tous les zoologistes qui ont écrit sur les espèces jumelles (dans le sens positif) ont affirmé qu'elles sont isolées reproductivement, mais personne n'a prouvé cette chose.

Enfin, affirmons encore que l'existence des espèces jumelles dans la nature a été considérée par les supporters du concept biologique comme une bonne preuve de la déficience du critère morphologique puisque ces espèces ne se distinguent pas morphologiquement, mais sont toutefois de bonnes espèces étant isolées reproductivement. Si le concept biologique est soutenu par de tels « arguments » comme l'existence des espèces jumelles — pure spéculation scientifique, selon G. Warnecke (6) — alors la faiblesse de ce concept est évidente et d'autres commentaires sont inutiles.

Le critère mixiologique n'est pas «souverain », comme pensent certains zoologistes. Mayr (3) parle des populations dont la spéciation n'est pas complètement terminée, par exemple les espèces où « la différenciation morphologique a eu lieu sans acquisition de l'isolement reproductif ». Ces cas sont assez nombreux chez les Lépidoptères parmi les Papilionidae, Pieridae, Hesperiidae (Pyrgus malvoides) et surtout parmi les Sphingidae. Les espèces en cause ont les genitalia distincts mais elles s'intercroisent, n'étant pas isolées reproductivement. Ainsi Mayr, présentant le phénomène de la différenciation morphologique sans acquisition de l'isolement reproductif, reconnaît la déficience du critère mixiologique et, peut-être sans le vouloir, la valeur du critère morphologique. Des

lignes ci-dessus il résulte d'une part que le critère morphologique est vraiment un critère de l'espèce et, d'autre part, que le critère mixiologique peut être, parfois, déficient quand l'isolement reproductif n'existe pas.

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THE GENUS *PUNTIOPLITES* SMITH (PISCES, CYPRINIDAE)

BY

P. M. BĂNĂRESCU

Puntioplites is a valid genus, only distantly related to *Puntius;* by the disposition of the striae on the scales, it is closer to the generic group *Tor*. It includes, besides *P. proctozysron*, the Indonesian "*Puntius*" *bulu*, *waandersi* and *lawak*, this one based perhaps on an anomalous specimen of *bulu*. *Puntius nini* is a synonym of *Puntioplites waandersi*.

The generic name *Puntioplites* Smith, 1929 was proposed for the species *Puntius proctozysron* Bleeker, 1865; *Adamacypris* Fowler, 1934 with the same generotype, is an objective synonym. According to Smith [3], *Puntioplites* differs from *Puntius* in having the last simple anal ray spinified and serrated; Smith mentions that in a few *Puntius* species the ray is spinified, too, but smooth; he implicitly retains these species (all Indonesian) within *Puntius*. Weber & De Beaufort (4) give a comparative description of the four *Puntius* with anal spine.

The comparison of *P. proctozysron* with the four nominal species having a smooth anal spine, convinced me that they are closely related and must be ascribed to the same genus, *Puntioplites*, that differs from *Puntius* not only in having the anal ray spinified (a character found in no true *Puntius)*, but also in other characters : 1. Gill rakers more numerous (25—42 as against less than 10) and longer than in *Puntius*. 2. Scales with parallel striae (Fig. 1), as in *Tor* and *Labeo* (but fewer than in these genera); in *Puntius* the striae are divergent. 3. Large size is rarely if ever reached by *Puntius*.

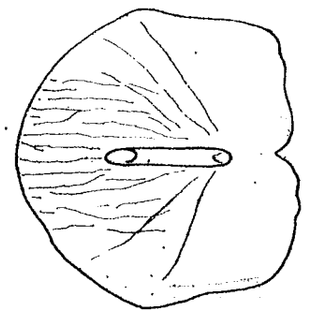

Fig. 1. — *Puntioplites bulu*, lateral line scale.

Other characters shared by all *Puntioplites*, but also by some *Puntius* are : body very deep; lateral line complete, with 35—38 scales; dorsal spine strong and denticulated; 16, 18 or 20 circum-peduncular scales; no barbels; back below dorsal fin compressed and narrowed; base of dorsal and anal covered by scales; pharyngeal teeth in three rows, rather pointed, with reduced grinding surfaces; intestine short; peritoneum silvery.

A special remark deserves the disposition of the striae on the scales; this character is considered important in delimiting the main subdivisions (here considered tribes) of Cyprininae s. lato: in Torini (Labeonini) the striae are parallel, in Barbini and Schizothoracini numerous and divergent,

in Cyprinini and Puntiini few and divergent (Karaman, 2). *Puntioplites* is, in the general habitus, similar to *Puntius*, but has an anal spine, like the Cyprinini; hence it could be considered intermediate between the two tribes. But the disposition of the striae is more similar to that in Torini. This fact proves that *Puntioplites* is not closely related to *Puntius*, their common characters being partially due to convergence (general similarity) or to synplesiomorphy (pointed teeth, thin and simple lips).

The specimens this study is based on belong to the following collections: British Museum (BMNH); Inst. Ştiinţe Biologice Bucureşti (ISBB), Muséum National d'Histoire Naturelle, Paris (MNHN), Rijksmuseum van Natuurlijke Historie, Leiden (RMNH), Zoologish Museum Amsterdam (ZMA)

SYSTEMATIC ACCOUNT

Genus ***Puntioplites*** Smith, 1929. (Fig. 2)

1. *Puntioplites proctozysron* (Bleeker, 1865). Fig. 2.
Synonyms: *Puntius smithi* Hora, 1923; *Puntius falcifer* Smith, 1929.

Specimens examined: syntypes of *Puntius proct.*: MNHN 1830, three specs., 76.5, 67.0 and 63.0 mm standard length; the largest is here declared lectotype (Fig. 2); MNHN 1868, two specs., 64.0 and 54.5 mm (all five from Thailanda, with no indication of river): MNHN 9 636 and 9 635, Laos, four specs., 151.0—168.5 mm; MNHN A 1114, southern part of Vietnam, one spec., 134 mm; MNHN 63—47, Menam R., one spec., 81.0 mm.

D III 8; A III 5; L. lat. 35 $\frac{9-10}{5-6}$ 36; Sp. br. 25—30; D. phar. 5.4.3—4.3.2—2.3.4; Circumpeduncular scales 20 ($= 2 \times (4^1/_2 + 1 + 4^1/_2)$[1].

Anal spine strongly denticulated. In % of standard length: depth 45.0—51.0; caudal peduncle length 15.7—19.8; least depth 13.7—16.6; predorsal distance 56.5—63.0; preanal 70.0—76.0; preventral 46.0—51.0; distance from pectoral to pelvic insertion 18.3—24.0; from pelvic to anal 23.8—28.8 (always longer than the preceeding); pectoral length 21.6—26.0; pelvic length 23.8—27.6; head 26.8—30.2; snout 5.2—7.4; eye diameter 7.2—11.4; snout 19.1—25.4% of head; eye 25.2—33.6% of head and 64.0—100.0% of interorbital width.

Widely distributed throughout the basins of Mekong and Menam rivers in Thailand and adjacent countries and in the coastwise rivers of the eastern slope of continental Thailanda of the Malakka Peninsula; absent from Salwin and the other rivers of the eastern slope.

2. *Puntius bulu* (Bleeker, 1851). Figs 3, 4, 5.

Specimens examined: MNHN 3838, Sumatera, 160 mm (Fig. 3), registered as holotype, actually not even paratype, since no specimen from Bleeker's collection is in Paris (Dr. M. Boeseman, in litt., 14 July, 1977); paratypes RMNH 2913, Bandjermaesin, Kalimantan, two specs.,

[1] This formula indicates that there are $4^1/_2$ scales above the lateral line, one on the lateral line and $4^1/_2$ below it.

77.0 and 77.5 mm; RMNH 8055, Indonesia (no locality), two specs. 110.0 and 75.5 mm (the third spec. of the series is a *P. waandersi);* ZMA 115152, Indonesia (no locality), 55 specimens, 104—167 mm (initially labelled *Puntius waandersi-bulu-nini)* (Fig. 4, 5); ISBB 2958, Deramakor R., northern Kalimantan, 1 spec., 161.0 mm; ZMA 115.194, West-Koetei, Kalimantan, 3 specs., 118—191 mm, determined by De Beaufort as *P. waandersi.*

D III 8; A III 5; L. lat. (35) 36 $\frac{7^1/_2-9}{4^1/_2-5^1/_2}$ 37 (38); Sp. br. 27—42; circum-peduncular scales usually 16 ($= 2 \times (3^1/_2 + 1 + 3^1/_2)$, more rarely 18 ($= 2 \times (4^1/_2 + 1 + 3^1/_2)$.

Dorsal spine strongly denticulated, anal spine smooth. In % of standard length: depth 37.8—48.0; caudal peduncle 16.4—20.4; least depth 13.3—15.5; predorsal distance 54.5—64.0; preanal 71.0—78.0; preventral 47.5—54.5; P—V distance 18.9—24.4; V—A distance 21.5—26.6; pectoral length 20.8—25.4; pelvic length 22.3—24.5; head 27.8—34.4; snout 6.5—10.1; eye 7.0—10.7; snout 24.5—33.5% of head; eye 24.4—33.5% of head and 60—96% of interorbital. The wide ranges of variation of body depth, snout length and eye diameter depend above all on body size.

Also the number of circumpeduncular scales is variable: 16 (e.g. $3^1/_2$ above lateral line) in most specimens, including the paratypes, 18 ($4^1/_2$ above the lateral line) in others. This is an individual variation, with reduced significance, since one can see that one scale above the lateral lines is splitted in some specimens, while in others there are $3^1/_2$ scales in one row and $4^1/_2$ in the neighbouring row.

More significant is the variation of the number of gill rakers (Sp. br.). 40 of the 55 specimens ZMA 115152 have 16 circumpeduncular scales and among them 15 have 27—34 gill rakers ($M = 31.5 \pm 0.47$) and the 25 others have 36—42 ($M = 39.6 \pm 0.64$). e.g. the variation is discontinuous, no specimen having 35 gill rakers. Considering all 40 specimens together, one obtains $M = 37.62 \pm 0.54$, the probable fluctuation of the mean being higher than in each of the two groups separately. In the 14 specimens of the same series with 18 circumpeduncular scales, there are 36—42 gill rakers ($M = 39.0$), e.g. the same values as in the second group of specimens with 16 scales.

This sharply discontinuous variability probably is the expression of a genetically determined polymorphism. It is also possible that *P. bulu* in its present acception actually includes two sibling species; the problem can be solved through field investigations.

The following number of gill rakers was found in other specimens: 27 and 29 in the paratypes, 33 in MNHN 3838, 32 and 33 in RMNH 8055, 38 in ISBB 2958, 36, 38 and 40 in ZMA 115194. Inger & Chin [1] found in 15 North Borneo specimens 27—40 gill rakers, $M = 35.8$, i.e., a range of variation including both groups of the series ZMA 115152.

If it is proved that *P. bulu* includes two species, the name will remains valid for the species with Sp. br. 27—34.

The range of *P. bulu* extends over the eastern slope of Sumatera and both slopes of Kalimantan.

3. *Puntioplitea waandersi* (Bleeker, 1859). Figs 6, 7.

Synonyms : *Barbus proctozysron* (non-Bleeker) — Volz, 1904 (Sumatera) ; *Puntius nini* Weber & de Beaufort, 1916 ; *P. lawak* (non Bleeker) — Weber & de Beaufort, 1916 (partim : Djambi, Sumatera).

Specimens examined : syntypes of *P. nini*, ZMA 111505, 4 specs., 167—210 mm, the largest (Fig. 7) is declared lectotype ; it became ZMA 115181. ; RMNH 8055, Indonesia, 1 spec., 112 mm, in a series of *P. bulu;* ZMA 114961, Djambi, Sumatera, 7 specs., 54—121 mm, determined *P. lawak* by Weber & de Beaufort ; ZMA 115194, Palembang, 2 specs., 128 and 148 mm.

D III 7—8 ; A III 5 ; L. lat. 36 $\frac{9^1/_2-10}{5-6}$ 37 ; Sp. br. (30—) 32—35 : (36) ; Circum-ped. scales $20(2 \times (4^1/_2 + 1 + 4^1/_2)$.

In % of standard length : depth 37.4—51.0 ; caudal peduncle 17.1—20.7 ; least depth 13.0—15.7 ; V—A distance 18.8—25.2 ; head 29.0—33.0 ; snout 6.5—8.9 ; eye 8.1—11.0 ; other values as in *P. bulu.*

This species differs from the preceding in having 20 circumpeduncular scales. The difference is sharp, it seems improbable that it represents intraspecific variation. Also the number of scales above and below the lateral line is different.

Weber & de Beaufort [4] described *P. nini* as a distinct species, with 6 branched anal rays. Actually its four syntypes have but five rays, like *bulu;* being large specimens, the last ray is slightly more deeply forked than in other specimens. There is no other difference between these two nominal species which actually are synonymous.

4. *Puntioplites lawak* (Bleeker, 1855).

This species is known only after the type specimen in the British Museum, not seen by me. It is similar to the two preceding ones, but has 7 branched anal rays ; this character, mentioned in the literature [4] was confirmed by Mr. G. Howes who examined the specimen *(in litt.*, 23.XI.1977). There are 16 circumpeduncular scales. The specimen may be an anomalous *P. bulu.*

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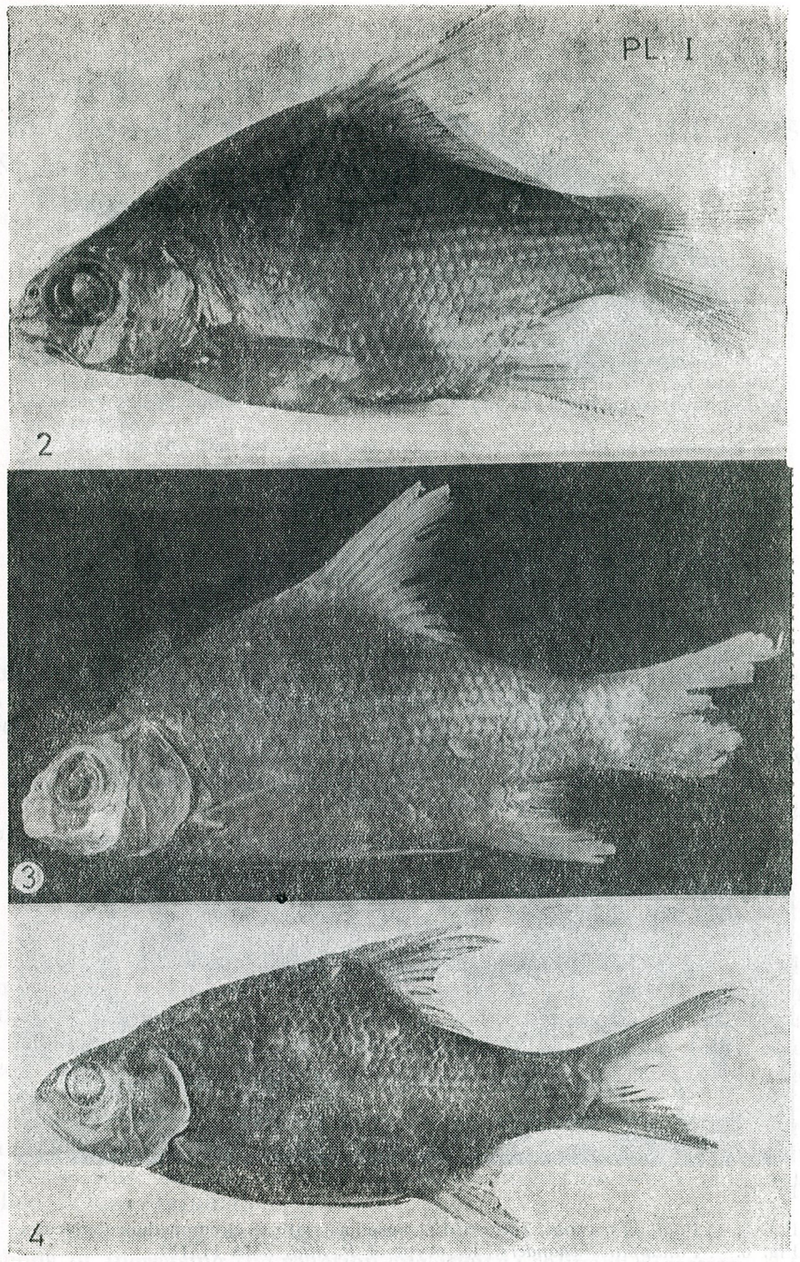
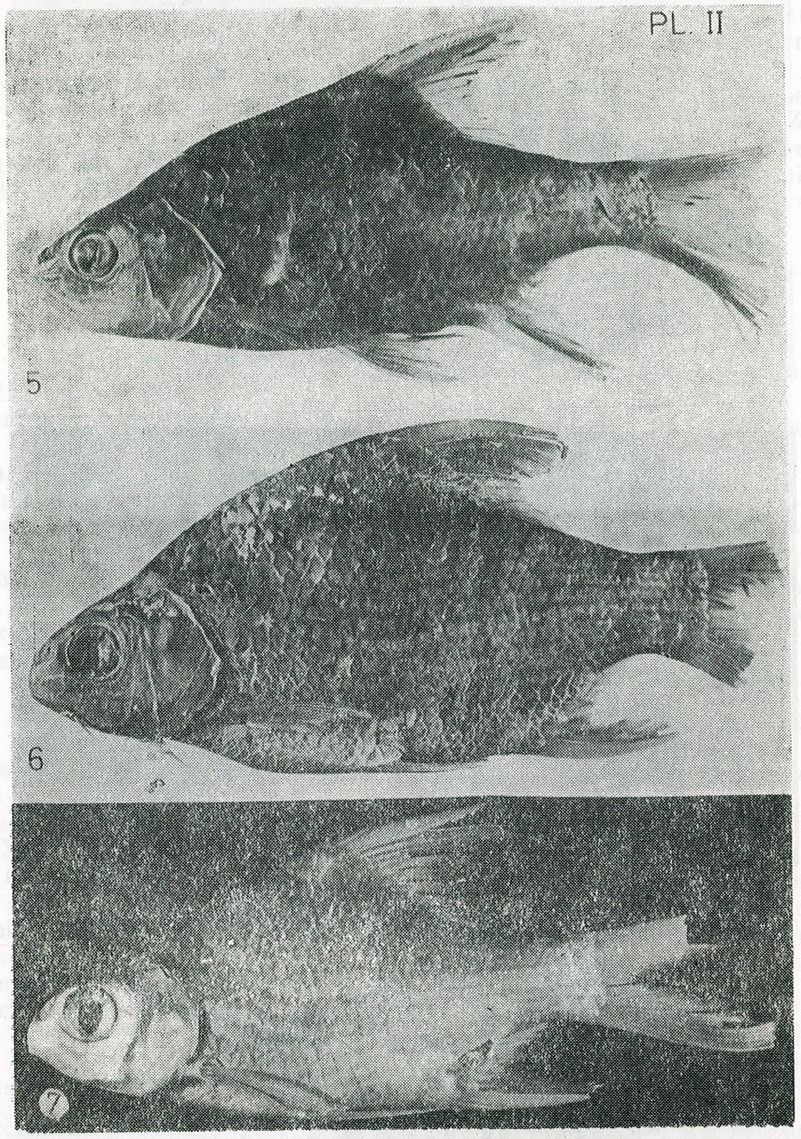


Pl. 1.

Fig. 2. — *Puntioplites proctozysron*, lectotype, MNHN 1830.
Fig. 3. — *Puntioplites bulu*, MNHN 3838.
Fig. 4. — *Puntioplites bulu*, ZMA 115152, specimen with 16 circumpeduncular scales.



Pl. II.

Fig. 5. — *Puntioplites bulu*, ZMA 115152, specimen with 18 circumpeduncular scales.
Fig. 6. — *Puntioplites waandersi*, lectotype of *P. nini*. ZMA 115181.
Fig. 7. — *Puntioplites waandersi*, ZMA 114961.

MORPHOHISTOCHEMICAL STUDY OF PAROTID, SUBMAXILLARY AND RETROLINGUAL GLANDS IN *CITELLUS CITELLUS* L. (RODENTIA, SCIURIDAE)

BY

LUCIA DUMITRESCU

The structural characteristics and histochemical properties of the parotid, submaxillary and retrolingual glands of *Citellus citellus* L. were studied.
It has been established by histochemical investigation that the parotid and submaxillary glands belong to the seromucous type and the retrolingual gland to the mucous type.
The presence and the nature of acid mucosubstances (AMS) revealed that for the parotid and submaxillary glands two cellular types can be established whereas for the retrolingual gland only one type.
According to the simultaneous presence of AMS and neutral mucosubstances (NMS), cells secreting both AMS and NMS, as well as cells secreting AMS only were identified in the submaxillary gland, while cells secreting both AMS and NMS were identified in parotid and retrolingual glands.

Many years ago, Ionescu-Varo and Homeiu [4] investigating the submaxillary gland of *Citellus citellus*, have established that in its serous cells the zimogen granules are formed by mucopolysaccharides bound to cell proteins.

Recently, we reported [2] the histology and histochemistry of the small sublingual gland of *C. citellus*.

In the present paper, the parotid and retrolingual glands of *C. citellus* are described for the first time from a morphohistochemical point of view.

MATERIAL AND TECHNICS

Tissues were fixed in Bouin-Hollande, Carnoy and Gendre fluids prior to paraffin embedding and sectioning at 7 μ. For the preliminary histological examination, the sections were hemalaun-erythrosin, azan and mucicarmine stained. In order to identify the nucleic acids, neutral mucosubstances (NMS), acid mucosubstances (AMS) and $-NH_2$ radicals, the following tests were used :

— for the nucleic acids : Feulgen procedure and methyl green pyronine (MGP) staining. The substrate specificity of MGP staining procedure was tested by the enzymatic digestion with ribonuclease applied before staining ;

— for neutral mucosubstances (NMS) : PAS reaction [8] with methods of increasing the reaction specificity : salivary digestion + PAS, acetylation + saponification + PAS [9] ;

— for acid mucosubstances (AMS) : alcian blue (AB) at pH 1.0 [7]; AB at pH 2.5 [11] with methods of increasing the reaction specificity : methylation + AB pH 2.5 [17], methylation — saponification + AB pH 2.5 [18], mild acid hydrolysis + AB pH 2.5 [14] AB pH 1.0 + alcian yellow pH 2.5 (AB + AY) — Ravetto's method modified by Sovari and Sovari [16];

— for neutral and acid mucosubstances : AB pH 2.5 — PAS [12];

— for glycogen : PAS method with and without previous salivary extraction (1 h at 37°C); carmine Best stain was also used

— for $-NH_2$ groups : bromphenol blue stain (BPB) [10].

RESULTS

Parotid gland. Morphologically, the glandular acini belong to the serous type and according to their histochemical properties to the seromucous type.

In acinous cells was noticed a granular secretion with an intense or moderate positive to PAS reaction, unaffected by the salivary digestion, but which was obviated by acetylation and restored by saponification, thus indicating the carbohydrate nature of the positive PAS material. The cells were slightly of moderately alcianophile with AB at pH 1.0 and pH 2.5 and some cells were negative.

Following AB + AY staining, in some acini all the cells were negative. In others, some cells were slightly or negligibly AB positive, while others presented alcianophilia for both dyes, staining in green or blue green (Fig. 1). With AB pH 2.5 — PAS sequence, some cells stain red, while others stain uniformly purple-blue. The cellular cytoplasm is moderately BPB positive.

The intercalated ducts, short structural segments, are sparse visualized in histological sections. Their cells are negligibly PAS positive, and the intensity of BPB reaction is like that remarked at the acini level.

The intralobular ducts, with an epithelium composed by high cells delimiting a wide lumen, are well represented. The basal striations are low and the cytoplasm is non-reactive to methods of the AMS identification, but presents a negligible PAS positive reaction. The BPB reaction is strong compared to that of acini cells and intercalated ducts cells. By PAS reaction, carmine Best and amylase digestion, the presence of glycogen in the cytoplasm of the duct cells was revealed.

Submaxillary gland. The light microscopic observations reveal a considerable development of the intralobular ducts which sometimes prevail on the acini. Morphologically, glandular acini belong to the serous type and according to the nature of secreted mucosubstances, to the seromucous type. In clearly delimited glandular cells, one or two round-shaped and clearly outlined nuclei were observed. In these nuclei, one or three nucleoli were distinguished. The fine granular chromatin appears slightly Feulgen positive. A granular pyroninophilic material, uniformly distributed into the cytoplasm was revealed by MGP. In the basal cytoplasm, the ergastoplasmic lamellae were also revealed by the same method. The brown colour of the cytoplasm evidenced after the extraction with

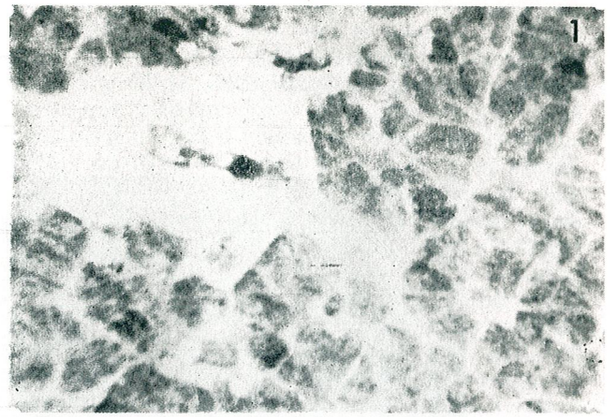


Fig. 1. — Section through the parotid gland of *Citellus citellus*. The glandular cell s present a different affinity to AB and AY in the AB+AY sequence. Note the negative reaction in the cells of intralobular duct. 1104×.

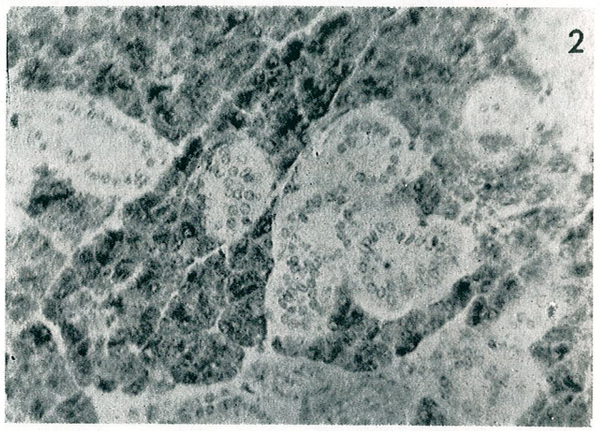


Fig. 2. — Section through the submaxillary gland of *Citellus citellus*. Glandular cells appear not uniformly alcianophile with AB or AG in the AB + AY sequence. 460×.

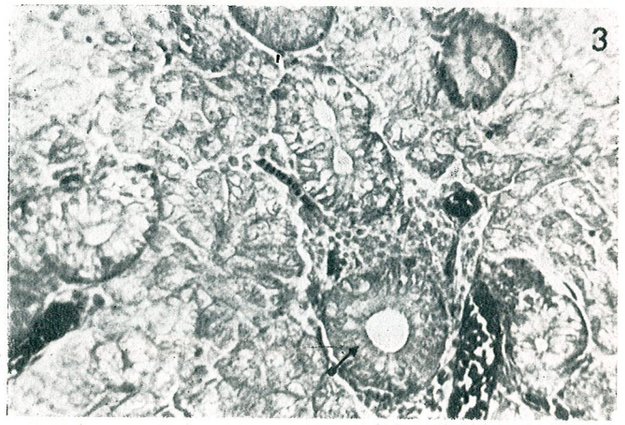


Fig. 3. — Section through submaxillary gland of *Citel lus citellus*, stained with BPB. Note an intensely positive reaction in the epithelial cells of the proximal segment of intralobular duct (arrows) 920×.

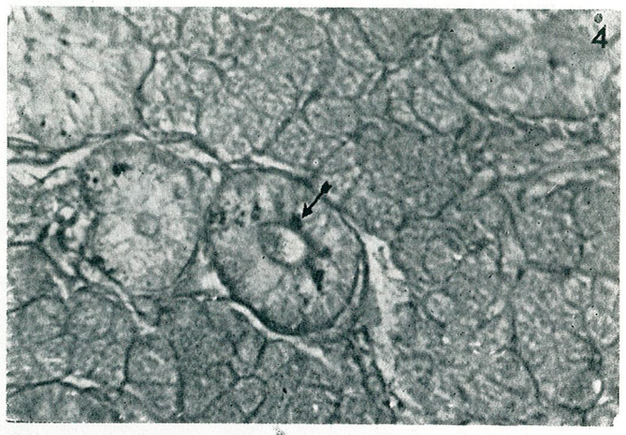


Fig. 4. — Section through submaxillary gland of *Citellus citellus*. Glandular cells present an affinity to PAS reaction. Notice the presence of particles of glycogen (arrow) in the striated duct. 1104 x.

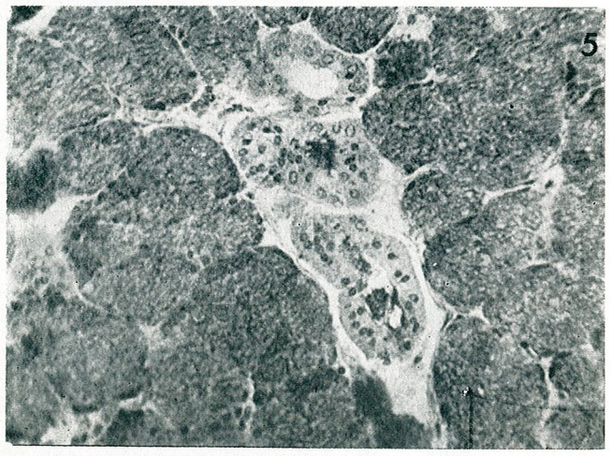


Fig. 5. — Section through retrolingual gland of *Citellus citellus*. Glandular cells present an affinity to AB and AY, being uniformly stained in AB + AG sequence. 460 x.

ribonuclease followed by MGP, suggests the presence of a mucopolysaccharidic material. The fine granular material secreted is moderately PAS positive and its carbohydrated nature is well demonstrated by increasing technics of PAS reaction specificity. With BPB tests, the granules appear moderately blue stained due to the presence of $-NH_2$ groups. All cells were alcianophile with AB staining at pH 1, some of them being negligibly positive and others slightly positive. The cells were slightly or moderately alcianophile with AB at pH 2.5. Concerning the affinity of cells to AB and AY, in the AB + AY sequence (Fig. 2), two cellular types were established : 1) cells with affinity both to AB and AY ; several of them exhibiting a predominant affinity to the AB, homogeneous staining in green-blue, while other cells showing a special affinity to AY and being homogeneously green stained ; 2) cells with affinity to AY only, yellow stained. The cells of the first type secrete carboxylated and sulfated AMS, while those of the second type, carboxylated mucosubstances.

By methylation for 5 hours at 60°C, AB or AY alcianophilia was removed, while by saponification, the affinity was restored only for AG, thus revealing the presence of carboxyl groups and removing of sulfate groups. By using the AB pH 2.5—PAS method we noticed cells that secreted only AMS (moderately AB positive) as well as cells that secreted both AMS and NMS (moderately PAS and AB positive, predominantly PAS or AB positive). This histochemical polymorphism was observed only in histological sections performed through the glands fixed in Bouin-Hollande, while those fixed in Carnoy or Gendre fluids presented only a moderate PAS positive reaction. In all cells, the presence of a granular material, moderately BPB positive, was revealed.

The intercalated ducts are well represented, being narrow and often ramified. The cytoplasm of their cells was slightly PAS positive and intensely BPB positive, unstaining with cationic dyes.

The long and sinuous intralobular ducts are lined by high epithelial cells. Generally, in the structure of intralobular ducts there are two well-distinguished segments : the proximal and distal segment, which can be distinguished clearly after fixing the tissue in Bouin-Hollande. The proximal segment of the intralobular duct system is lined by tall columnar cells which contain a large number of discrete secretory granules strongly BPB positive (Fig. 3) and moderately PAS positive. Cell boundaries are not distinct. The proximal segment may be considered homologous to the granular tubules, generally described in the rat submaxillary gland. The basal striation clearly appears in the basal cytoplasm. The cells of the distal segment of the intralobular ducts are more clearly delimited. Generally, the apical cytoplasm does not present any affinity to BPB and to PAS, but in some cells it showed a slight moderate affinity due to the small number of secretory granules.

The intralobular ducts cells of the submaxillary gland of the *C. citellus* are characterized by the presence of a large quantity of glycogen (Fig. 4), which was demonstrated both by the PAS reaction and carmine Best staining.

Retrolingual gland. The retrolingual gland is an entirely mucous gland. The acini are large (Fig. 5) and the cytoplasm of the glandular cells is slightly eosinophilic, presenting an affinity both for mucicarmine and

aniline blue. The PAS reaction is intensely magenta, the positive PAS material having a granular aspect. This reaction was unaltered by the treatment with the salivary amylase, indicating the absence of glycogen. It was abolished by acetylation and restored by saponification, suggesting the presence of carbohydrates.

By usual histochemical methods for the AMS identification it could be demonstrated that all the glandular cells secrete sulfated and carboxylated AMS. Thus, after AB pH 0.5 and AB pH 1.0, the cytoplasm was slightly positive and after AB pH 2.5 it was moderately or intensely positive.

The mild methylation removed alcianophilia after AB pH 2.5 and saponification partially restored it, demonstrating the presence of carboxymucines. All cells appear intensely green stained with AB+AY suggesting the presence of sulfate and carboxyle groups (Fig. 5). We noticed the specific reaction for sulfate groups (AB pH 1.0 and AB pH 1.0 in the AB + AY sequence) were influenced by the fixator used. Thus, after fixation in Bouin-Hollande fluid, reactions were positive; after fixation in Carnoy and Gendre they were negative.

The cytoplasm of glandular cells appears slightly blue stained with BPB and uniformly violet stained with AB pH 2.5 + PAS.

Generally, the intercalated ducts are sparse visualized in the histological sections. In their epithelial cells, the round-shaped nucleus occupies a large area, and the cytoplasm contains the PAS and BPB positive material and is unreactive to methods used for the AMS testing.

In the intralobular ducts, the epithelial cells are low as compared to those of the submaxillary glands. They delimit a wide lumen and their cytoplasm contains $-NH_2$ radicals which are BPB reactive. The presence of NMS in the cytoplasm of these cells was revealed by PAS reaction.

DISCUSSION AND CONCLUSIONS

According to the classification proposed by Shackleford and Klapper [15], we may include the parotid gland of *C. citellus* into the seromucous type because its glandular cells secrete both NMS and a small quantity of AMS.

In rodents, the presence of a sialomucine AMS type was histochemically revealed only in the Hamster parotid gland [3, 13, 19] and in the Rat parotid gland [3]. Aureli et al. [1] and Junqueira and Toledo [6] demonstrated biochemically the presence of the sialomucins in the parotid of some Rodents, however they could not be rendered evident histochemically. This fact, according to Mundho's standpoint [13], may be due to the masking of the carboxyle group by the basic proteins.

The present findings related to the AMS presence in the parotid gland of *C. citellus* show a marked histochemical polymorphism. Thus, besides the cells which do not secrete AMS, we did find both the cells secreting carboxylated and sulfated AMS and cells secreting sulfated AMS only.

From the morphohistochemical examination of the submaxillary gland it may be seen that this gland belongs to the seromucous type.

According to Ionescu Varo and Homeiu's observations [4], we conclude that glandular cells of the submaxillary gland secrete NMS. Our findings reveal that beside NMS these cells secrete a considerable quantity of AMS. The intensity of reactions of AMS decelation was increased as against that remarked at the level of the parotid glandular cells.

Related to the nature of secreted AMS, we ascertained two cellular types: 1) cells secreting both carboxylated and sulfated AMS and 2) cells secreting carboxylated mucosubstances only.

After the simultaneous presence of NMS and AMS, two cell types of the submaxillary gland were established: 1) cells secreting both AMS and NMS and 2) cells secreting AMS only.

Concerning the intralobular ducts of the submaxillary gland, in contrast with those of other rodents (unpublished data), in *C. citellus* we distinguished both their evident development and the large amount of the glycogenic material.

The retrolingual gland of *C. citellus* is a purely mucous gland. The glandular cells uniformly secrete carboxylated and sulfated AMS in contrast to those of mouse and rat retrolingual gland, secreting exclusively carboxylated AMS [19].

Josza and Szederkenyi [5] using various fixators, excluding Gendre and Bouin-Hollande fluids (used in our present study), have remarked the loss of a certain quantity of mucopolysaccharides during fixation.

Regarding the fixation of AMS secreted by the glandular cells, we suggest the use of Bouin-Hollande fluid because after fixation in the Carnoy and Gendre fluids we remarked the loss of the AMS.

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COMPARATIVE STUDY OF ^{85}SR AND ^{90}SR CONCENTRATION AND ELIMINATION IN *CARASSIUS AURATUS GIBELIO*

BY

I. CHIOSILĂ and E. REVIU

This paper presents several experiments concerning the concentration in *Carassius auratus gibelio* of ^{85}Sr and ^{90}Sr from contaminated waters with 1,200 pCi/ml. Concentration of the two radionuclides in fish, after a month's contamination, is almost identical, the concentration factors amounting to 10. The elimination of ^{85}Sr and ^{90}Sr from fish bodies takes place with the same T_b (136 days), while T_e takes about 44 days for ^{85}Sr and 136 days for ^{90}Sr.

The utilization of nuclear techniques and technologies in economy and social fields requires the construction of nuclear installation and apparatus contributing, by their wastes, to the radioactivity increase in the biosphere. In order to protect the environment it is necessary to know exactly all the radioactive contaminating sources of the biotope, migration of the radioactive isotopes in the abiotic environment as well as along the terrestrial and aquatic trophic chains, and finally, the ways of contamination and exposure of the human body [1], [2], [3], [4], [5], [7].

As a final link in several aquatic trophic chains and, at the same time, as useful production of fresh water basins, fish represent an important transfer way of radionuclides from the aquatic environment to man.

Strontium is dangerous for animal organisms and especially for man by its radioactive isotopes appearing in the uranium fission process, and by its uptake in bones, constitutes an irradiation source with noxious effects at the level of the endosteal tissue and hematogenous marrow.

Among strontium radioactive isotopes, ^{90}Sr with T_r (radioactive half-life) of 28.1 years and ^{89}Sr with T_r of 51 days are the most dangerous, both being β^- radiation emitters with efficiency of 4.79 and 5.77 per cent in fission process. ^{90}Sr is more dangerous for human organism by its T_e (effective half-life) of about eight years [8] as well as by its daughter product ^{90}Y with T_r of 64 hours and hard β^- radiation emitter (2.27 MeV). The other strontium radioactive isotopes resulted from the fission process ^{91}Sr, ^{92}Sr, ^{93}Sr and ^{94}Sr, even if they have a similar occurrence rate, are not very dangerous owing to their low T_r : between $3.94 \cdot 10^4$ and $0.81 \cdot 10^2$ sec.

In our paper we present a series of experiments concerning ^{85}Sr and ^{90}Sr concentration and elimination by the goldfish (*Carassius auratus gibelio*).

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The experiments were performed in aquaria of 30 l where we had introduced 5—7 fish of 30—40 g each. Before contamination, the fish were kept about seven days under laboratory conditions for acclimation. We administered uncontaminated food made up of *Tubifex* sp., having in view radionuclide concentration through the branchiae but also through the tegument.

Water was initially contaminated with 1,200 pCi/ml of $^{90}SrCl_2$ and $^{85}SrCl_2$, respectively (Amersham — England).

The activity of the two radionuclides in fish was counted in the whole body system on a Philips analyser with NaI(Tl) crystal of 5.0. 4.5 cm with an efficiency of 2.4 per cent for ^{85}Sr where we counted γ radiation of 0.515 Mev and of 1.02 per cent for ^{90}Sr, a radionuclide evidenced by 'bremsstrahlung', radiation which is given by its and ^{90}Y β^-. A plastic fish-like phantom filled with 40 ml initial radioactive solution was used for counting the efficacity.

After fifty-two days (for ^{85}Sr) and seventy days (for ^{90}Sr) fish were drawn out of the contaminated water and introduced into aquaria with clean water changed every two-three days.

The counting values of the activities obtained for the two radionuclides (Fig. 1) in the first twenty days are different. The activity in

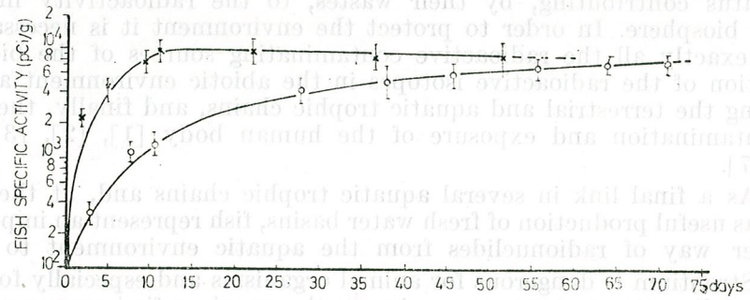

Fig. 1.—Dynamics of concentration of ^{85}Sr (× — ×) and ^{90}Sr (O—O) by *Carassius auratus gibelio*.

fish with ^{85}Sr increases suddenly, reaching values of 9.830 ± 501 pCi/g with a concentration factor of 8.60 ± 0.44 in the first contamination period, in opposition to the activity of ^{90}Sr which increases slowly thus, in the first ten days it hardly reaches 1.228 ± 145 pCi/g, with a concentration factor of only 1.33 ± 0.14.

The ratio between the activities of ^{85}Sr and ^{90}Sr in fish decreases from about 6.5 in the first days to 3.9 between the 10th and the 20th day, and below 2 after a month from water contamination. The activity of ^{85}Sr in fish reaches the concentration plateau in ten days after contamination, while that of ^{90}Sr tends to a plateau only thirty days after. Theoretically, however, such a big difference in the two radionuclides uptake in fish should not occur in the first days. This is explained by the fact that by counting ^{90}Sr in fish in the 'whole body' system for the first twenty days, there occurs a discrepancy that may be revealed by measuring a lower amount of ^{90}Y (the radioisotope occurring by disintegration of ^{90}Sr). Good counting is performed only after a fortnight when a balance between ^{90}Y and ^{90}Sr is reached. Also, while ^{90}Sr is an osteotropic radionuclide, ^{90}Y is fixed in soft organs possessing a high metabolism with rapid elimination [1], [3], [8].

At the end of the experiments, the results indicated similar concentrations of the two radionuclides in fish. The activity of fish in water contaminated with ^{85}Sr decreases slowly after forty days (owing to the disintegration of the radionuclide), while fish activity in water with ^{90}Sr increases slowly. The concentration factors for ^{90}Sr in fish reach some higher values by the end of the experiment, between 10.48 and 12.51.

The study of ^{85}Sr and ^{90}Sr elimination in fish revealed lower values* for T_e of ^{85}Sr obtained about 44 days, while T_e of ^{90}Sr is 134 days (Fig. 2). After counting T_b** (biological half-life) we found out that this time was about 136 days (136.19 for ^{85}Sr and 136.49 for ^{90}Sr), both in fish contaminated with ^{85}Sr and ^{90}Sr. We noticed that the elimination of the two strontium isotopes is not affected by atomic mass. These results demonstrate that ^{85}Sr and ^{90}Sr are identically metabolized.

In conclusion, strontium radioactive isotopes with a relatively short life — ^{85}Sr and ^{89}Sr — for water unique contaminations, are taken

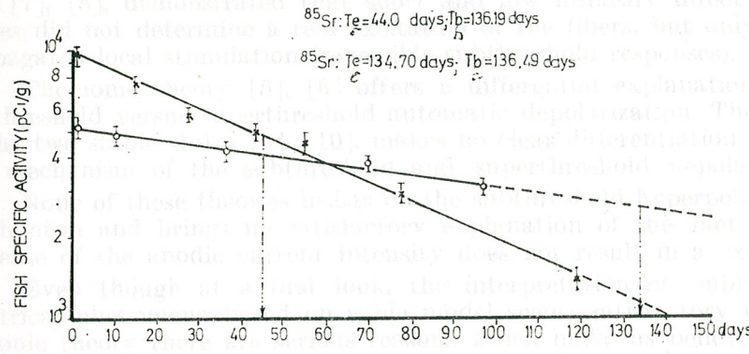

Fig. 2.—Dynamics of elimination of ^{85}Sr (× — ×) and ^{90}Sr (O—O) by *Carassius auratus gibelio*.

up in fish reaching the highest values during a month; after that, these isotopes decrease owing to the fact that disintegration exceeds their concentration. ^{90}Sr, having a very high T_r, is uptaken continuously by fish like the other strontium isotopes, but its activity tends to a plateau only after two months from contamination where it remains while the isotope is in water. In case fish are removed to clean waters, ^{85}Sr and ^{89}Sr disappear completely from fish body in about 450 days, while the isotope with long life (^{90}Sr) is found in fish for about 1,340 days.

* T_e calculated both graphically and from the formula:

$$T_e = \frac{t_2 - t_1}{\log\Lambda_1 - \log\Lambda_2}$$

** $$T_b = \frac{T_e \cdot T_r}{T_r - T_e}$$

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CABLE MODEL OF THE EXCITABLE FIBER AND THE "2-M.S.I." CONCEPT

BY

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According to the "2-M.S.I." concept, the subthreshold electrical phenomena are the result of the interaction between ionic currents (imposed by the applied external current) and the spontaneous ionic diffusions through the membrane layers rather than capacitive transmission phenomena. These are best explained on the basis of "positive inside and negative outside" polarity of the resting membrane and of the distribution of the electric field applied locally and transversally across the membrane. They are a result of a deionization or superionization of the intramembranous compartment, taking place with no real change in the membrane permeability.

The investigations regarding the electrical excitation of cell membrane [4], [7], [8], demonstrated that short and low intensity direct current pulses did not determine a real excitation of the fibers, but only a non-propagated local stimulation (reversible subthreshold responses).

The ionic theory [5], [6] offers a differential explanation of the subthreshold versus superthreshold automatic depolarization. The theory of the two stable states [9], [10], makes no clear differentiation between the mechanism of the subthreshold and superthreshold depolarization.

None of these theories insists on the subthreshold hyperpolarization mechanism and brings no satisfactory explanation of the fact that an increase of the anodic current intensity does not result in a excitation.

Even though at a first look, the interpretation of subthreshold electrical phenomena based on cable model seems satisfactory in terms of ionic theory there are serious reasons which make us believe that it is completely inadequate.

In the membrane, the phenomena appearing at the application of a subthreshold stimulus is local and stationary. After its installation it is not propagated along the fiber membrane, since there is no real excitation to be conducted along the fiber as in an electrical cable.

The applied stimulatory current effectively passes through the membrane. Hence, there is no transmission as in the cable. Moreover, closing the circuit in the portion of biological preparation is achieved by ionic currents which have, as charge carriers, cations and anions moving simultaneously in both directions rather than by a simple unidirectional electronic current. In terms of the polarity of the applied stimulus and also of how the membrane separates spontaneously the positive from negative ions, an increase, respectively a decrease in the membrane electrical charge takes place.

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Besides, the signal attenuation with distance in the cable is achieved during its longitudinal movement, whereas in the membrane, during a transversal passage of the current, the attenuation with distance is local.

However, the ionists' demonstration finally revealed the local phenomena taking place in the membrane when a current passes through it, which actually is no more than abandoning the cable model itself. We should make the general remark that in the case of the considered biological system (the membrane and the adjacent phases) one does not deal (at the application of a subthreshold stimulus) with an electrical signal conducted along the extracellular fluid (as through a series of R_e resistors in the cable) which could have a transmission effect conducted in parallel and simultaneously along the intracellular fluid (series of R_i resistors).

Our explanation regarding the effects of subthreshold electrical stimuli on the resting charge of the membrane is based on the fact that these are local stationary effects, which appear at the application of a low intensity short lasting direct current through the membrane. The membrane potential variation in time and space depends upon the interaction of the ionic currents imposed in the system by the external current with the spontaneous ion movements through the membrane placed in a transversal electric field. This is not a result of the attenuation of a capacitively transmitted signal, but a consequence of the conditions in which the installation of the applied electric field takes place in time and space, as well as of the interactions determined in the system by it.

In respect to the intimate mechanisms of the subthreshold electrical phenomena one should take into account, as previously pointed out [2], that a cathodic current depolarizes the membrane provided that its internal surface had a positive and the external one a negative charge. For such a polarity of the membrane, an anodic current brings about the hyperpolarization of the membrane.

The biological membrane, according to the "2-M.S.I." concept, having two independent layers with different ion exchange properties (the external layer predominantly cationic, and the internal layer predominantly anionic), subthreshold depolarization is accompanied by a deionization of the intramembraneous compartment whereas hyperpolarization by its increment.

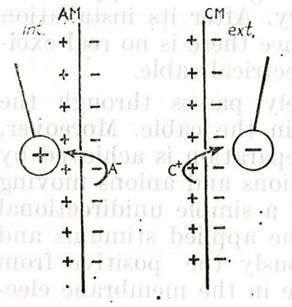

Fig. 1. — Subthreshold depolarization of the membrane in a cathodic current.

Thus, subthreshold depolarization is characterized by a decrease of the potential difference across each layer, and at the same time, as a result of the intramembranous compartment deionization, a pronounced increase of the concentration gradients through layers occurs (Fig. 1). It should be emphasized that, due to the very reduced volume of the intramembranous compartment, removing of a relatively reduced number of ions from it is sufficient to produce a very pronounced decrease in the electrolite concentration.

As long as the applied cathodic current is able to oppose the increased gradients of concentration through layers regardless of the produced discharge of the layers, the depolarization (which remains for this reason subthreshold) attains an equilibrium point with definite value, function of the intensity of the applied stimulus. This corresponds to the depolarization plateau for this particular stimulus intensity. At the interruption of stimulus, the normal diffusions through layers are re-established, and the concentration gradients as well as the electrical charge return in short time to their resting values.

When the applied cathodic current has a higher intensity than the threshold value, determining a more pronounced electrical charge of layers (thus, of the factor normally slowing down the diffusion) and producing at the same time increments in the concentration gradients through the layers which are no longer under their control, a disruption of the equilibrium between the action of the electrical factor and that of the chemical factor (gradient of concentration) takes place; the ions removed from the intramembranous compartment in the adjacent phases (the cations from the extracellular phase and the anions from the intracellular one) are again strongly pushed by the concentration gradients towards the intramembranous compartment. It is clear, that in these conditions, the first ions entering "massively" in the intramembranous compartment will be those with greater mobility, that is, the cations from the extracellular phase (e.g. Na^+ ions).

The anions taken previously in the intracellular phase will return with greater difficulty to their usual places. Thus, Na^+ ions which penetrated between the membrane layers will find the internal layer relatively labilized by the absence of anions and will be able to exert their "structure breaking" action (phase transition) on it, starting the automatic display of the spike [1].

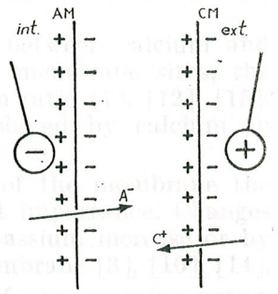

Fig. 2. — Subthreshold hyperpolarization of the membrane in an anodic current.

An anodic stimulus leading to a hyperpolarization of the membrane and also to the superionization of the intramembranous compartment (Fig. 2) will produce by these specific actions a condition which is opposing to the start of phase transition and consequently to an excitation. By superionization the layers become simultaneously superstabilized (the internal layer due to anions, the external one to cations) and thus, they will become more "resistent" to the action of some stimuli. It clearly appears why the anodic stimuli are unable to produce a propagated excitation.

The ions involved in the subthreshold phenomena are Na^+ for the external layer and A^- (diffusible) for the internal one. K^+ ions seem to play no essential role in these phenomena.

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PROCAINE EFFECT ON MEMBRANE DEPOLARIZATION IN CALCIUM-FREE MEDIUM

BY

I. NEACȘU and ȘT. AGRIGOROAEI

Investigations were carried out on membranes of frog sartorius muscle fibres in calcium-free Ringer at pH = 7.2 and 6.0.
As a consequence of the modification of the $K^+ : Ca^{2+}$ ratio in favour of K, a membrane depolarization takes place, better expressed at alkaline pH than at acid pH. The phenomenon is based on a phase transition of the laminar phospholipidic micellae within the external membrane layer to globular micellae.
The cationic form of procaine (2.5mM) reduces less the amplitude of depolarization at pH = 7.2 and completely at pH = 6.0. An acid pH increases the capacity of replacement of Ca^{2+} from the external membrane layer by the anesthetic.

There are numerous investigations regarding the effect of variation of external calcium concentration upon the membrane potential [10], [14], as well as the interaction of local anesthetics with the calcium whithin the membrane [10], [14], [18]. Nevertheless some aspects such as those regarding the localization of the effects, their mechanisms, time course, pH influence etc., are still unclarified.

On the other hand, it is well known that between calcium and procaine there exists a competition for the same membrane sites, the occupation of which depends on their concentration ratio [7], [12], [15]. Thus procaine may partially substitute the role played by calcium in the membrane [4], [9].

It is known that for the electrical charge of the membrane the $K^+ : Ca^{2+}$ ratio in the external medium is of great importance. Changes in this ratio in favour of potassium, either by potassium increase or by calcium decrease, lead to a depolarization of the membrane [3], [10], [14].

In a previous paper [5], the interference of the procaine action with that of increased external potassium has been followed up. In order to extend our understanding of the mechanisms of the phenomenon, in the present paper the mode of action of procaine in external calcium-free medium is being investigated.

MATERIALS AND METHODS

Experiments were performed on membranes of frog *(Rana ridibunda)* sartorius muscle fibres by the method of intracellular glass microelectrodes. Each experiment was performed on five muscles at room temperature. Ringer solutions buffered with bicarbonate at pH = 7.2 and phosphate at pH = 6.0 were used. Ringer solutions without Ca^{2+} were obtained

by equimolar replacement of this ion by Na^+. The procaine solutions 2.5 mM) were prepared by adding the anesthetic to the Ringer.

RESULTS

The resting potential recorded in normal conditions had a value between 94.11 and 94.74 mV (SE about 0.50 mV) (Figs. 1 and 2 : RP).

The effect of the lack of calcium on the membrane potential has been followed up at pH = 7.2 and pH = 6.0. Replacing normal Ringer (pH = 7.2) by Ca^{2+}-free Ringer a depolarization can be observed, which after 20 minutes reaches an amplitude of 8.09 mV, a lower value than that reported by other investigators [10]. By replacing the fibres back into normal calcium Ringer (NR) the potential is recovered completely in about 20 minutes (Fig. 1, trace A).

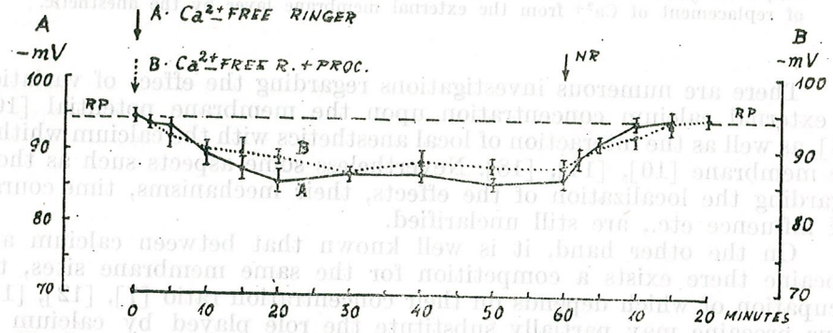

Fig. 1. — The membrane depolarization in a Ca^{2+}-free Ringer solution (A) and the effect of 2.5 mM procaine on this depolarization (B), at pH = 7.2.

At pH = 6.0 a depolarization was registered too, which takes place quicker and which has an amplitude of only 2.89 mV. This is easily reversed by replacing the fibres in normal Ringer. (Fig. 2, trace A).

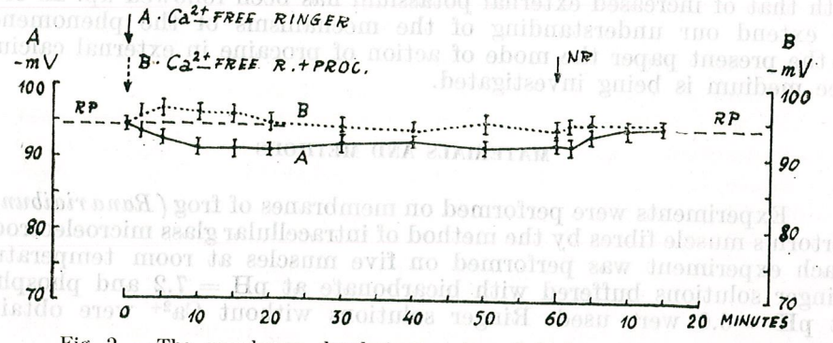

Fig. 2. — The membrane depolarization in a Ca^{2+}-free Ringer solution (A) and the effect of 2.5 mM procaine on this depolarization (B), at pH = 6.0.

In other experiments the effect of procaine (2.5 mM) on the membrane potential in Ca^{2+}-free medium at alkaline and acid pH has been investigated.

At pH = 7.2, in Ca^{2+}-free Ringer containing 2.5 mM procaine a slow starting depolarization takes place, reaching an amplitude of 6.17 mV. Replacing the fibres into normal Ringer the potential recovers to the resting value in about 15 minutes (Fig. 1, trace B).

It can be seen that in the presence of the anesthetic the amplitude of depolarization is lower (6.17 mV compared with 8.09 mV) and the onset of the phenomenon is slower (30 minutes compared with 20 minutes) as well as the repolarization in normal Ringer.

At pH = 6.0 the membrane potential in Ca^{2+}-free Ringer containing 2.5 mM procaine shows but slight modifications during the entire period of observation (60 minutes) : a slight initial hyperpolarization (1.09 mV) and a very little after-depolarization (0.43 mV) (Fig. 2, trace B).

It can be observed that the depolarizating effect due to the lack of calcium is completely overcome at acid pH.

DISCUSSION

The increase of the $K^+ : Ca^{2+}$ ratio in the external medium, either by an increase in K^+, or by a decrease in Ca^{2+}, leads to membrane depolarization [3], [10], [14] but between the two ways of depolarization there are significant differences.

One, brought about by high external potassium shows a rapid initial, and slow subsequent phase, the amplitude of the phenomenon increasing much with the K^+ concentration. Recovery of the potential by bringing back the fibres into normal Ringer is much slower than the depolarization.

Membrane depolarization, even at total reduction of calcium, starts much slower and the depolarization amplitude is much more reduced. But the potential recovery in normal Ringer is much faster than after depolarization by high potassium.

In order to explain our experimental data we took into consideration the membrane structure and properties as suggested by the concept "2-M.S.I." [1], [2]. We admitted that the K^+-Ca^{2+} antagonism is based on the phospholipid membrane structures, a view supported by other data, too [11], [19].

Thus, depolarization caused by a lowering of external calcium, as well as caused by a potassium increase, may be explained through the phase transition of the laminar phospholipid micellae whithin the external membrane layer to globular micellae (by the replacement of Ca^{2+} ions, which are involved in the normal structure, by K^+ ions which are in excess). The difference between the two kinds of depolarization would result in the fact that, in each of these cases, phospholipid micellae of different sensibility against the implicated ions (K^+ respectively Ca^{2+}) and different in number are involved [1]. The data presented in this paper bring new proof in support of our hypothesis.

In the experiments carried out at pH = 6.0 we utilized a phosphate buffer, condition in which the external pH change does not implicate a

modification of the intracellular one [8], [17], so that the effect of this pH is localized only at the level of the external membrane layer. The influence of the acid pH on the start and recovery in the case of depolarization in the absence of external Ca^{2+} may be explained by the same action on the membrane structure as in the case of depolarization by high K^+ [3]. The decrease in the depolarization amplitude would result in the fact that it starts with the phospholipid micellae of reduced K^+ sensibility and small number.

As shown already, the interaction of local anesthetics with Ca^{2+} has been studied by many investigators, the depolarization reduction induced in the absence of Ca^{2+} by procaine being also mentioned in the literature [13], [14], [16]. Our data bring new evidence in this regard.

In previous experiments [4] we found that procaine (2.5 mM) in normal Ringer induced a hyperpolarization, better expressed at pH = 7.2 and less obvious at pH = 6.0, simulating partially the role of calcium. If, in the case of procaine action in the absence of Ca^{2+}, the two effects appeared as in the case of a separate occurrence we would expect stronger depolarization reduction. But, in these conditions there appear two different micellar areas on which procaine exerts its action : globular micellae existing in the normal resting structure, where it induces a hyperpolarization, and laminar micellae, affected by the absence of Ca^{2+}, in which case it replaces partially this ion. This distribution of the agent leads to reduced hyperpolarization, but at the same time, the depolarization amplitude caused by the lack of Ca^{2+} is lower.

In acid medium procaine leads to a total reduction of membrane depolarization caused by the absence of Ca^{2+}. It is known that such a pH causes certain membrane structure modifications [3], under which conditions procaine is almost totally in the cationic form [6], and the depolarization amplitude caused by lack of Ca^{2+} is smaller, too. In acid medium procaine capacity to substitute for Ca^{2+} increases much, the action of K^+ being thus inhibited, although the $K^+ : Ca^{2+}$ ratio is changed in favour of potassium. This effect is enhanced by the pronounced increase in the concentration of the cationic form of the anesthetic. Thus it may be stated that the substituting effect of procaine in respect to Ca^{2+} is well put into evidence in the absence from the medium of this ion, being much enhanced at an acid pH.

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THE MEMBRANE POTENTIAL OF DDT-TREATED MUSCLE FIBRES IN K^+-FREE MEDIUM

BY

V. CRĂCIUN and ȘT. AGRIGOROAEI

The experiments were performed on single muscle fibres of the frog by using the intracellular glass microelectrode technique. DDT reduces and delays the membrane hyperpolarization in K^+-free medium, an action depending on the pH. The authors consider that in the membrane hyperpolarization in K^+-free Ringer, the basic process is a phase transition from globular to laminar micellae, through Ca^{++}-binding and that DDT reduces the action of Ca^{++} on globular micellae.

It is known that the basis of DDT actions on the excitable membranes is a prolongation of the sodium conductance increase and a suppression of the potassium conductance increase [6], [10], [11], [14] resulting in an augmentation of the negative after potential and subsequent repetitive discharges [5], [12]. Taking into consideration the effects of DDT on the Na^+- and K^+-conductances during the action potential, some authors admitted that the site of the DDT action is on the inner surface of the membrane [6], [9], [10], [11].

Regarding the molecular mechanisms of these actions, some authors hypothetically admitted a DDT interaction with the membrane proteins [7], [8], [13], but more recent studies demonstrated the possibility of DDT binding to the membrane phospholipids and forming charge transfer compounds [16]. In these studies they also show that a DDT binding to the membrane proteins is uncertain.

In a previous paper concerning the DDT effects on the membrane potential in high K^+-Ringer [3] we have shown that the DDT action upon excitable membranes is caused by the interactions of the insecticide with the phospholipids of the external membrane layer. In order to further test this idea, we followed in the present experiments the DDT effects on the membrane potential in a K^+-free Ringer.

It is well known that a diminution of the external K^+ concentration by equimolar substitution for Na^+ results in a hyperpolarization of the striated muscle fibre membrane [1], [15]. In another paper [2] we have shown that this type of hyperpolarization, as well as other modifications of the resting potential induced by changes in the external ionic composition, are the result of some complex phenomena located at the level of the external membrane layer.

Assuming that the membrane phospholipids play a basic part in bioelectrical and permeability phenomena, we correlated the ionic exchanger properties of the external membrane layer with the supermolecular organization of the active membrane phospholipids, especially phospha-

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tidyl-ethanolamine (PE). The predominant cationic properties of the external membrane layer in the resting state are due especially to the laminar organization of the PE micellae, which bind Ca^{++} in their structure. But this layer has also, in the resting state, the anionic properties less pronounced than the cationic ones. These anionic properties are related to the existence, beside the numerous laminar micellae, of a limited number of globular PE micellae which bind K^+ in their structure.

The normal ratio between the two types of micellar structures is determined by the normal ratio $K^+ : Ca^{++}$ in the extracellular solution; these ions represent in fact the structuring and respectivelly destructuring ions, for laminar organization, on the one hand and for globular organization on the other. The membrane hyperpolarization obtained by lowering the external K^+ concentration is due to the modification of the normal ratio $K^+ : Ca^{++}$, to the Ca^{++} advantage. In these conditions a phase transition takes place in the external membrane layer from globular to laminar micellae; the direct consequences of this phase transition are the increase of the packing degree of the layer and the extension of their cationic properties. Of course, this results in an increase in their electric charge, that is in the membrane hyperpolarization on the whole.

In our experiments, performed on frog striated muscle fibres by using the intracellular glass microelectrode technique, at a total replacement of external K^+ by Na^+ and at a pH = 7.2 (carbonate buffer), we obtained an average membrane hyperpolarization of 21.70 mV (Fig. 1). Returning the fibres to normal Ringer, the membrane repolarization took place, but more suddenly than the hyperpolarization.

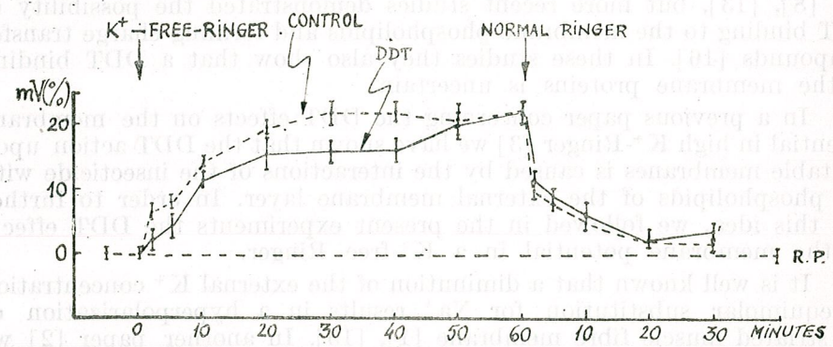

Fig. 1. — The effect of DDT on membrane potential (per cent) in K^+-free medium at pH = 7.2. R.P., initial resting potential.

In the presence of DDT (5.10^{-4} M) (the DDT-containing Ringer was prepared as shown in a previous paper [3]) the hyperpolarization in K^+-free solution began more slowly and without the rapid initial step. The hyperpolarization reached after 20 minutes a plateau of 14.90 mV amplitude. Subsequently the hyperpolarization becomes faster again, as if the insecticide had ceased its action. The repolarization taking place by return to normal Ringer was not significantly influenced by DDT.

At a pH = 6 (phosphate buffer), the membrane hyperpolarization in K^+-free Ringer showed some specific features (Fig. 2): after a faster start than at normal pH, the phenomenon took place generally more slowly and finally reached a lower plateau, of 20.66 mV amplitude. Returning to normal Ringer, the membrane repolarization was very sharp initially, then it proceeded at a very slow rate.

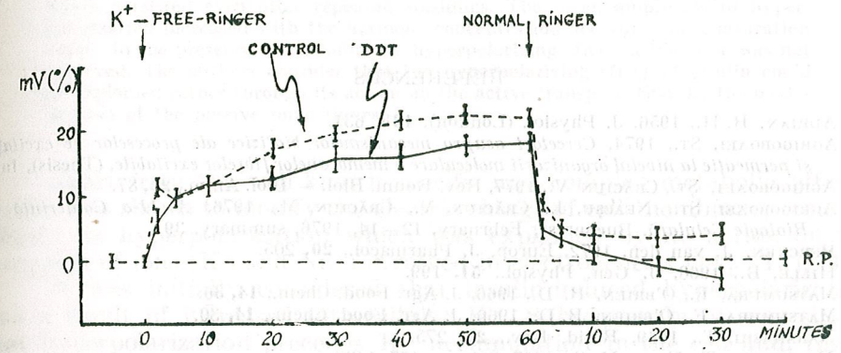

Fig. 2. — The effect of DDT on membrane potential (per cent) in K^+-free medium at pH = 6. R.P., initial resting potential.

In the presence of DDT (pH = 6), the initial sharp phase of hyperpolarization was not observed and the amplitude increased slowly till the end of the observation period, when it was lower than in the absence of the insecticide. After washing with normal Ringer (pH = 6) the membrane repolarization was very rapid, reaching the resting level in 15 minutes and being followed by depolarization (2.90 mV).

On the basis of the above data we can conclude that DDT generally reduces and delays the membrane hyperpolarization in K^+-free solution, depending on pH. In order to explain this effect of DDT we must consider that the membrane hyperpolarization in K^+-free Ringer takes place in conditions resembling those of the membrane repolarization after a K^+-induced depolarization [4]. The basic process is also a phase transition from globular to laminar micellae through external Ca^{++}-binding. The effect of DDT on the membrane hyperpolarization can thus be also attributed to their ability to reduce the action of Ca^{++} on the globular micellae [3].

Regarding the effect of DDT at an acid pH, we must remark that, because of their tendency to produce a relatively more accentuated depolarization in the laminar regions of the external membrane layer [3], the actual hyperpolarization in these conditions would be considered as having a higher amplitude than the recorded one. It would be partially masked by the depolarizing effect that overlaps the hyperpolarizing effect. In support of this idea seems to come the fact that, by returning to normal Ringer, the membrane potential becomes lower than the initial resting potential. We also observed that, because of the effect of an acid

pH on the packing degree of globular membrane areas, the ability of DDT to prevent the phase transition from globular to laminar organization by the binding of Ca^{++} is less pronounced at a pH = 6 than at a pH = 7.2.

Therefore, the results of the above experiments confirm our previous conclusion [3] that the DDT effect on excitable membranes relies on some interactions of the insecticide with the phospholipids of the external membrane layer.

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ON THE BIOELECTRICAL EFFECTS OF INSULIN

BY

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The experiments were performed on single frog muscle fibres by using the intracellular microelectrode technique. Insulin caused a membrane hyperpolarization which persisted even after repeated washings. The mean amplitude of hyperpolarization increased with the hormone concentration according to a saturation curve. In the presence of ouabain, the hyperpolarizing effect of hormone was not observed. The authors consider that the hyperpolarizing effect of insulin could be explained rather through its action on the active transport, than by the modification of the passive ionic fluxes.

Apart from the well-known specific and complex metabolic effects, insulin was shown to modify the electric charge of cell membrane, conducting to its hyperpolarization which was explained by its action on the transport of ions (K^+ and Na^+) involved in electrogenesis [14], [15], [16].

It was initially considered that insulin-induced hyperpolarization was a result of increased intracellular K^+. Subsequently, it was shown that hyperpolarization precedes K^+ accumulation in the cell and, therefore, it is the cause and not the effect of K^+ increased concentration in the cell [14]. However, a few years later, the same author observed that this phenomenon was much more complex, implying Cl^- transport and especially the active Na^+ efflux [16], [17]. A more recent work [11] brought conclusive proofs that the primary action of insulin was exerted on the active Na^+ transport, whereas the increase of intracellular K^+ was explained by a decrease of intracellular fluid without affecting the net K^+ influx.

However, up to the present, there is no agreement upon the connection between the insulin-induced hyperpolarization and the modification of ionic fluxes through the membrane. Since membrane hyperpolarization could be induced by different mechanisms, it seemed worthwhile to establish whether there are enough proofs favouring the hypothesis of a membrane permeability change caused by insulin, or if the transport through the membrane is influenced in some other way. To this end, the insulin-induced variations of the membrane potential in different experimental conditions were recorded.

MATERIAL AND METHODS

Our experiments were performed on single sartorius muscle fibres of the frog, by using the intracellular glass microelectrode technique, and an electronic Tönnies device for amplification and recording. A Ringer bicarbonate buffer (pH = 7.2) was used throughout the experiments. Insulin solutions (0.01, 0.03, 0.05, 0.08 and 0.1 I.U./ml) were prepared by adding corresponding amounts of hormone (Biofarm, 40 I.U./ml) to Ringer solution. The ouabain solution (Nutritional Biochemicals corp., Cleveland, Ohio) was prepared by adding $5 \cdot 10^{-6}$M glycozide to Ringer,

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solution. The experiments were carried out at room temperature (22—25°C), in summer, on groups of 4—5 frog sartorii. The statistical significance of the observed changes for each experimental series was evaluated by Student's test.

RESULTS

In the first series of experiments we followed the variation of the membrane potential at different concentrations of insulin (0.01, 0.03, 0.05, 0.08 and 0.1 I.U./ml). As shown in Fig. 1, the hyperpolarizing effect of

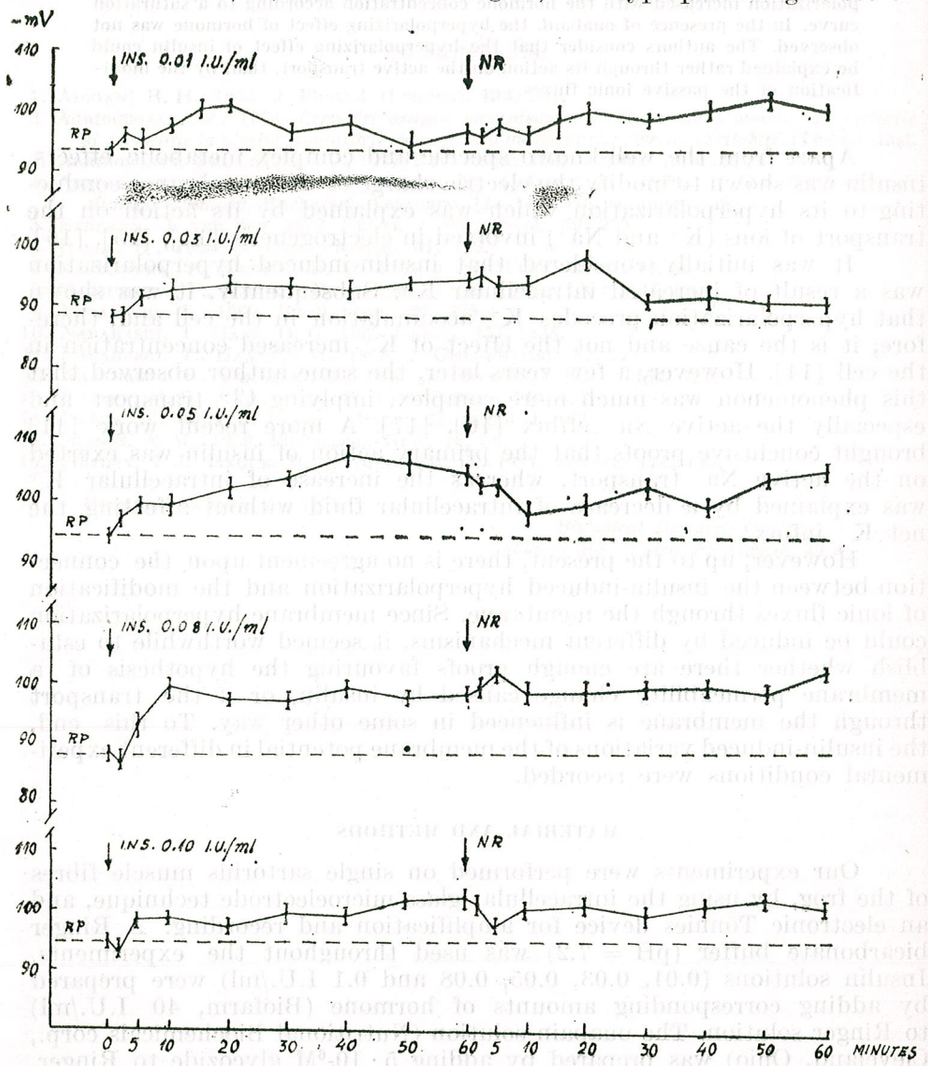

Fig. 1. — Effects of different concentrations of insulin on the membrane potential. R.P. — initial resting potential; N.R. — normal Ringer.

the hormone is present throughout the experiment (1 hour) at all five concentrations tested. By returning the fibres to normal Ringer, the membrane potential did not return to its initial level even after repeated washings, during 60 min., suggesting that the hormone firmly bind to the membrane receptor.

It should be pointed out that the mean amplitude of hyperpolarization increased with the hormone concentration, according to a saturation curve (plateau between 0.05 and 0.08 I.U./ml). At an insulin concentration of 0.1 I.U./ml the amplitude of the hyperpolarization suddenly decreased (Fig. 2).

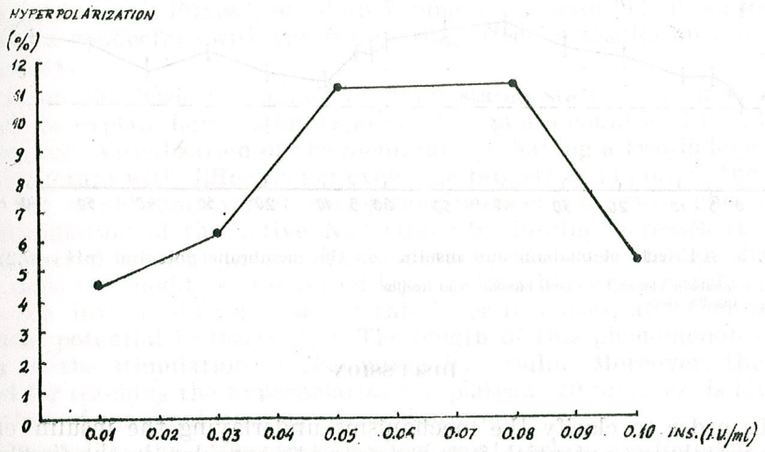

Fig. 2. — Variation of the insulin effect (%) on the resting potential of the striated muscle fibres, depending on the hormone concentration (pH = 7.2).

In another paper [2] we already admitted that insulin acts at the level of the (Na^+—K^+)-pump mechanisms. In order to further test this idea, in the second series of experiments we followed the effect of ouabain, a generally accepted selective inhibitor of the (Na^+—K^+)-pump, on the membrane potential in the presence of insulin. As shown in Fig. 3a, the ouabain ($5 \cdot 10^{-6}$ M) initially caused a slight and transient membrane depolarization (5.58 mV), then the membrane potential oscillated around the control value. In the presence of both insulin (0.05 U.I./ml) and ouabain, the hyperpolarizing effect of the hormone was no longer observed, in these conditions the (Na^+—K^+)-pump being inhibited by the glycoside. But the initial depolarizing effect of ouabain was also reduced by the hormone (Fig. 3 b). We must remember that insulin alone, at the same concentration, caused an average membrane hyperpolarization of 10 mV (Fig. 3 c).

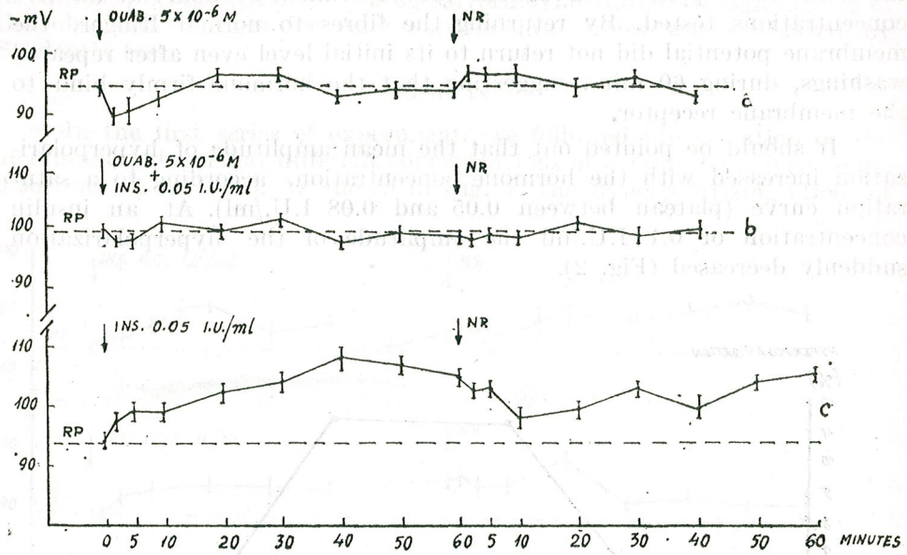

Fig. 3. — Effects of ouabain and insulin on the membrane potential (pH = 7.2):

a) — ouabain alone; b) — both ouabain and insulin;
c) — insulin alone.

DISCUSSION

In order to clarify the mechanisms underlaying the insulin effects on the membrane potential, it is necessary to consider, in the first place, the interactions of the hormone with the membrane constituents. Edelman et al. [6] reported that the striated muscle fibre membranes were able to bind the insulin, both electrovalently and covalently, and that the permeability changes caused by the hormone were initiated by the inter-charge reactions (thiol-disulfide) between S-S group of the hormone and —SH groups of membrane proteins. However, Green et al. [7] demonstrated that the structural proteins of the membrane are lacking sulfhydryl and disulfide groups.

More recent works [4], [5] did not exclude the possibility of an insulin-membrane protein interaction (by a different mechanism) and pointed out its dependence on the state of membrane phospholipids.

On the other hand, Perry et al. [13], using artificial phospholipid membranes, demonstrated the possibility of an interaction between insulin and some phospholipids (phosphatidic acid, phosphatidyl-choline) and the absence of such interactions with phosphatidyl-ethanolamine, sphingomyelin, cholesterol etc. The phospholipid-insulin complex could favour the bimolecular leaflet to turn into a micellar structure which would explain (according to these authors) the changes in permeability related to the physiological and biochemical effects of the hormone.

Other workers [3], [10] made valuable contributions to the role of membrane phospholipids; however, the experiments on model systems do not seem to supply real information on the interaction between insulin and the membrane hydrophobe zone, since the hormone does not interact with the basic functional phospholipids (phosphatidyl-ethanolamine and sphingomyelin) to form complexes (globular micellae). Therefore, some other mechanisms than the phase transitions should be admitted, since a reaction between insulin and phosphatidyl-choline (an essentially static phospholipid) would lead to a disorganization of the membrane structure by decoupling the lipids from the protein network.

Since all these data do not support the idea of a direct modification of the membrane permeability by insulin (by a phase transition), it is evident that the hyperpolarizing effect of insulin could be explained rather through its action on the active transport than by the modification of the passive ionic fluxes. Considering some recent data [11] it is necessary to make a connection with the stimulating effect of insulin on the active Na^+ efflux.

If on the basis of "ionic" or "two stable states" theories it was difficult to explain how a stimulation of Na^+-pump could lead to a hyperpolarization. Visualization of the membrane as having a two-independent-layer structure with different ion exchange properties [1] offers the possibility for establishing direct correlation between the two phenomena. The stimulation of the active Na^+ efflux by insulin increases the Na^+-concentration gradient across the membrane, and even though the hormone does not modify the external layer permeability (its micellar structure), the inward diffusion across this layer increases, and therefore its electrical potential increases, too. The length of this phenomenon corresponds to the stimulation of Na^+-pump by insulin. Moreover, the time needed for reaching the hyperpolarization plateau (20 minutes) is identical with that of maximal binding of the hormone.

Regarding the concentration-effect relationship, we found that the hyperpolarization increased with the hormone concentration up to 0.08 I.U./ml (that is insulin can bind to the membrane in higher amounts than in normal physiological conditions) but hormone concentrations over the saturation value (0.1 I.U./ml) resulted in some side effects, disturbing the normal ones.

Some authors already reported a depolarizing effect of the ouabain [8], [9]. The specific inhibition of (Na^+—K^+)-pump by ouabain supplied new data with respect to a possible relationship between insulin-stimulated active transport and membrane potential. Our experimental data furnish new evidence that the bioelectrical effect of insulin is based on its interaction with the molecular mechanisms of the active ionic transport.

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MEMBRANE POTENTIAL AND THE EXTERNAL POTASSIUM CONCENTRATION

BY

GABRIELA AGRIGOROAEI and ŞT. AGRIGOROAEI

The paper shows the inconsistence of the arguments brought by the ionic theory in favour of the excitable fiber membrane behaviour as an electrode.
Neither the experimental values of the membrane resting potential, nor the linear relationship between the membrane potential and the K^+ external concentration logarithm in the depolarization experiments are real proofs in this respect.
A similar linear relationship was also found for other agents producing membrane depolarization.

The ionic theory considers that the excitable fiber membrane behaves in resting state as a K^+ electrode [6] and that the resting potential value measured experimentally is in good agreement with the value calculated on the basis of the Nernst equation which takes into consideration the external and internal concentrations of K^+:

$$E_m = \frac{RT}{F} \ln \frac{[K^+]_e}{[K^+]_i} \qquad (1)$$

In a previous paper [2] we have shown that, despite appearances, the use of this equation is not justified, since it is applicable only in the case of a membrane potential of the Donnan type. Or, the ionists themselves abandoned the idea of such an equilibrium for the biological membrane. The experimental values also refute this idea, being misinterpreted by the ionists and unjustifiably related to the Nernst equation given above.

Evidently, this equation can be considered as valid in expressing the potential of a K^+ concentration cell. But, if one finds out that the value obtained by calculation is in good agreement with the experimentally recorded one, it is obvious that the membrane potential must be admitted as being null in resting state, since the calculated value represented only the electromotive force (e.m.f.) of the K^+ concentration cell, without any revelance on the part played by the membrane potential in it.

The 'small difference' (— 5 mV) between the experimentally recorded value (— 90 mV) of the 'membrane potential' of the striated muscle fiber [1], [8], [9], [10], and the calculated value (— 95 mV) based on the Nernst equation using K^+ external and internal concentrations is only apparently small and insignificant.

All the ionists' reasoning in this respect are strongly influenced by the fact that membrane and phases properties are viewed in a too simplified light. Taking into account the K^+ and Na^+ distributions, it

appeared clearly enough that there was no justification to consider the system intracellular fluid-membrane-extracellular fluid and the recording electrodes only as a K^+ concentration cell.

However, if one agreed to consider the e.m.f. of this concentration cell only as a function of K^+ concentrations and since the theoretical equation (1) yielded, in these conditions, only the potential difference between the K^+ cell electrodes, it resulted that :

$$E_{exp} = E_{conc} \pm E_{mem} \qquad (2)$$

(+ or — function of the membrane potential polarity).

Since the concentration cell electrodes are reversible with respect to Cl^- anion, the negative pole of the cell is the intracellular electrode. On the other hand, it has been admitted that the charge of the internal surface of resting membrane is negative.

Since the potential of the concentration cell has the same polarity with the membrane potential it follows that one has to admit the equation (2) in the form :

$$E_{exp} = E_{conc} + E_{mem}$$

Considering the polarity of each of these potentials and the known values, it follows that :

$$-90 \text{ mV} = -95 \text{ mV} - E_{mem}$$

All the terms of equation (2) having the same sign, it becomes irrational by replacing E_{conc} for the potential value of the K^+ cell obtained by the the Nernst equation.

Therefore, there are no serious arguments in favour of the intracellular fluid-membrane-extracellular fluid system as forming together with the recording electrodes only a K^+ concentration cell with semi-cells separated by a membrane. It is also obvious that the membrane in resting state cannot be considered as having a potential based only on the ratio between $[K^+]_e$ and $[K^+]_i$.

But the most conclusive data taken into consideration by the ionists in favour of membrane behaviour as an K^+ electrode were those obtained in the depolarization experiments of the membrane by high external potassium [1], [5], [7], [11].

In these conditions there was a linear relationship between the 'membrane potential' values determined experimentally and the logarithm of external K^+ concentrations.

Because the experimental linear curve was superimposed (some authors claimed it ran parallel to) upon the theoretical one, derived from the Nernst equation for the same values of the K^+ external concentration, this was interpreted as a confirmation of these equations validity for membrane potential calculation [1], [4], [8]. So, it was thought that this indicated that the membrane potential was dependent only upon the ratio $[K^+]_e : [K^+]_i$.

With regard to these results of depolarization experiments with high external K^+, several remarks should be made.

A perfect agreement between the calculated values and those experimentally recorded can only demonstrate that for the entire K^+ external concentration range for which the linear relationship is valid, the membrane potential strictly speaking must be admitted as being zero, if one admitted an equilibrium of the Donnan type.

The experimentally recorded potential values at different K^+ concentrations do not represent the membrane potential values but the potential differences of the entirely complex systems as mentioned above, in these conditions. Thus it is not obvious that the membrane potential has a linear variation with the logarithm of K^+ external concentration.

In fact, there is not a perfect agreement between the calculated values and those experimentally recorded, which is very important, just like the case of resting potential, in demonstrating that the Nernst equation is inapplicable even for the concentration cell potential calculation of the considered system. The actual concentration cell potential is not determined only by K^+ concentrations.

We also think worth pointing out that the depolarized membrane by high external K^+, as several authors have already shown [12], has properties distorted from those present in normal resting state. The membrane in a non-excitable state (the impulse spreading is blocked), has a much higher ionic conductance, while in a stationary electrical state (increased ionic conductance) it is not accompanied by a continuous reduction of the concentration gradients. So, it is difficult to admit that the membrane behaviour in non-excitable state could serve as an argument in favor of its similar behaviour in normal resting state (as a "K^+ electrode").

It is also known that a linear relationship has been found and in the case of a depolarization by Rb^+ high external concentrations, although this ion is not present in the intracellular medium.

We have found a similar linear variation and in the case of membrane depolarization by TBA/3/, which does not penetrate in the fiber and acts only at the external surface of membrane. In these cases it is not possible to apply the Nernst equation (1) to the membrane potential calculation.

Finally, we consider of great importance to point out that Nerst relation true for the electrode is :

$$E_{el} = E^0_{el} + \frac{RT}{F} \ln a_{sol}$$

where E^0_{el} is the standard potential of the electrode and a_{sol} is the activity of the ion in the solution.

This relation of Nernst for electrode cannot be confounded with the relation of the same author of the concentration cell potential.

The membrane potential is much more complexly determined than by the ratio of external to internal K^+ concentration, and the depolarization by external K^+ increase is the result of profound changes in the membrane structure and properties.

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ON THE BIOELECTRICAL ACTIVITY OF THE LATERAL MUSCLES IN SHARK

BY

C. WITTENBERGER and C. GALERA-GARCÍA

Biopotentials were extracellulary recorded from the lateral musculature of *Scyliorhinus canicula*, as typical spikes generated both spontaneously and on electrical stimulation, either *in situ* or *in vitro*. No spikes were recorded from isolated pure red muscle pieces. It is likely that all the spike activity recorded from the trunk musculature of the fish was generated in the white muscle only, and not in the red one.

Only a few investigations exist on the bioelectrical phenomena in the trunk musculature of fish. Clear-cut differences were observed between the two lateral muscles, the white (LWM) and the red (LRM) one. The mean value of the membrane potential is about 55 mV in the LRM and about 90 mV in the LWM [1]. Correlations were found between the electrical activity of the two lateral muscles and the types of movements in living fish : the LWM gives bursts of potentials only when the fish performs vigorous movements of the tail or when its swimming speed exceeds a given value ; potentials form the LRM are recorded during slow movements [2], [3], [4]. The LWM exhibits typical spikes on electrical stimulation of the motor nerve [1] ; as far as we know, no similar data are as yet available concerning the LRM. With direct electrical stimulation *in situ*, the electrical activity of the same general shape is recorded from both muscles [6].

The above quoted data led their authors to the conclusion that the lateral white muscle of the fish performs the rapid swimming movements, the slow ones being carried out by the red muscle. This conclusion seemed to us as being insufficiently founded, given also the results of one of us concerning the metabolism of both muscles (see [7]). We tried, therefore, to obtain further data on the bioelectrical activity of the LWM and LRM.

MATERIAL AND METHODS

Experiments were carried out on *Scyliorhinus canicula* (60—70 cm long). Platinum wire electrodes were used for both stimulation and recording. In the *in vitro* experiments, small pieces were cut off from the lateral musculature and placed in a small vessel filled with selachian Ringer solution. The electrodes (ca. 0.1 mm diameter) were inserted microscopically in the same bundle of a single myomere. *In situ* recordings were also made (in beheaded fishes), stimulation being performed by a pair of electrodes inserted either in the same muscle, several centimetres from the recording point, or in the spinal cord of the tail region.

Rectangular impulses were used throughout, and biopotentials recorded on a Tektronix RM 565 type oscilloscope, with a Grass Instruments C4L kymograph camera.

RESULTS

Experiments on isolated muscles. The electrical behaviour of isolated muscle pieces was different according to fibre composition : all white, all red, or both kind of fibres. Spontaneous potentials were recorded in white muscle pieces, or in those including both white and red fibres. These were spikes of 50—200μV and 2—5 ms, at irregular intervals (Fig. 1). No spike in pure red muscle preparations (8 experiments) was ever obtained. The presence of several white fibres in the isolated piece was, however, sufficient for the generation of spikes, which were then recorded irrespective of the nature of fibres in which the recording electrodes had been inserted.

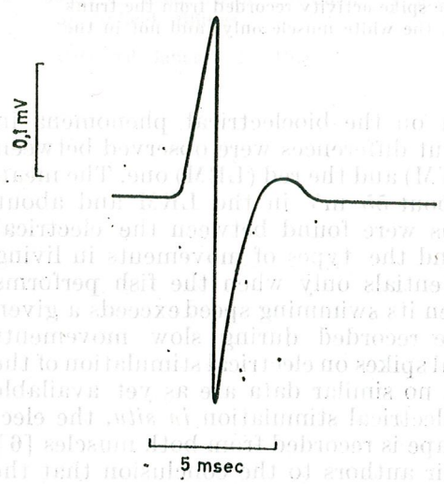

Fig. 1. — Spontaneous spike from isolated white muscle tissue.

With the aim of proving that we were dealing with real biopotentials, the isolated muscle piece was vigorously stimulated for several minutes, until no fibre exhibited any contraction as seen microscopically. The spontaneous spikes disappeared. They appeared again after several minutes, at first with low amplitude and frequency, gradually regaining the initial values (Fig. 2).

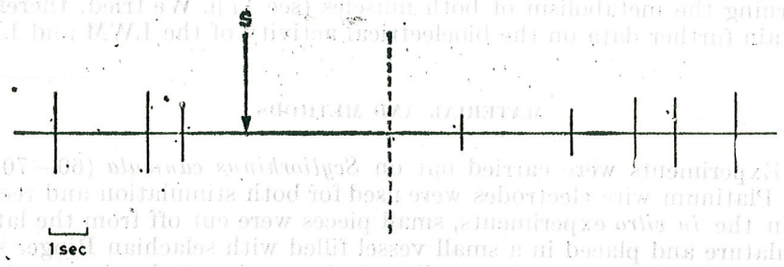

Fig. 2. — Spontaneous spikes from an isolated white plus red muscle piece. S : stimulation for 3 min, with impulses of 1 V and 5 ms, at a frequency of 10 Hz. Vertical dotted line : 3 min interruption of the recording.

In the experiments with electrical stimulation, we did not succeed in eliminating the very large stimulus artefact. So, it was not possible to follow the development of elicited biopotentials on the records. On some records from LWM clear, small spikes are visible on the ascending side of the negative stimulus curve (Fig. 3). Such elicited potentials were never recorded in fibre bundles of LRM (12 experiments). Nor did the fibres of LRM present any microscopically observable mechanical response to stimulation.

Experiments on muscles in situ. Spontaneous action potentials were recorded in both LWM and LRM. The amplitude was 50—100 μV when recorded in the former, and 20—40 μV in the latter. Duration was 3—4 ms. Spontaneous as well as mechanically elicited movements of the tail were clearly correlated with an intense electrical activity of the muscles. The amplitude of the spikes reached in this case, as well as under electrical stimulation, was 200—300μV. No difference was observed in respect to the muscle from which recording was obtained or to the nature of stimulation (direct muscular or indirect medullary).

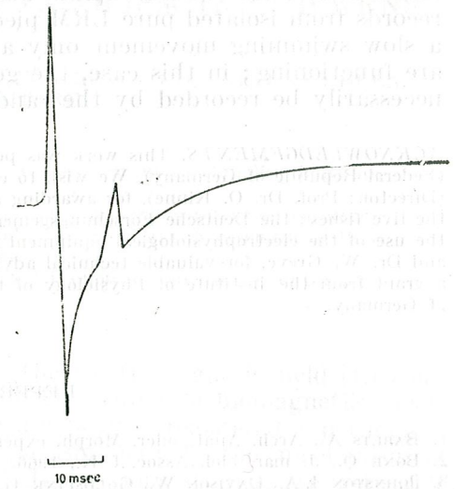

Fig. 3. — Electrically elicited spike superimposed on the stimulus artefact.

DISCUSSION

It is obvious from the above data that the electrical behaviour of LRM is different from that of LWM. Freshly isolated from the organism and placed in an appropriate saline, the LRM reacts neither by mechanical contraction nor by action potentials to electrical stimulation. One cannot suppose that the LRM tissue would die immediately after isolation, since by incubation in a Warburg apparatus it exhibited oxygen consumption for several hours (see [7]). One may suppose that either the LRM fibres are not excitable by low frequency or unique impulses, or a depolarization of the sarcolemma occurs but does not elicit a propagated spike. The second hypothesis is supported also by data in the literature related to teleost fishes [1], [5].

Our recordings were made with extracellular macroelectrodes. Results reported in the literature on the electrical activity of LRM were obtained in the same manner [2], [4]. Only Takeuchi [5] used intracellular microelectrodes, and recorded small spikes from red fibres in a teleost fish ; but these were fibres of another muscle, which may not have the special peculiarities of the LRM [6], [7]. Thus, we have no certain information about the precise origin of the potentials recorded. The current interpretation of these results, as specified in the introduction of this paper, is, therefore, doubtful. Only if we could be sure that LWM is electrically silent during the slow movement, as assumed by some authors

[2],)[3], [4], we might maintain the hypothesis of the division of labour according to swimming speed. But two points must be kept in mind in this respect : 1) all electrical activity recorded might well be originating in the white fibres, as macroelectrodes cannot give sufficiently precise information ; this would explain also the complete absence of spikes on records from isolated pure LRM pieces ; 2) it is very likely that during a slow swimming movement only a few fibres of the very large LWM are functioning ; in this case, the generated action potentials would not necessarily be recorded by the randomly inserted electrodes.

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THE EFFECT OF THE ELECTROMAGNETIC FIELD ON THE CAROTENO-LIPID METABOLISM IN CHICKENS

BY

P. ROTINBERG, MATILDA JITARIU, ECATERINA DUCA, ELENA CHERA and P. JITARIU

The action of the electromagnetic field on the caroteno-lipid metabolism in 24 hrs-hatched chickens was studied.
The quantitative and qualitative modifications in total carotenoids, carotenoid fractions, total lipids, free fatty acids indicated the active involvement of these compounds in the animal cell metabolism.
A possible pattern for a direct action of the EF upon the functioning of the caroteno-lipid system is discussed.

Cell reactivity to the action of the electromagnetic field (EF) has been revealed by many investigations. The reality of biomagnetic effects was confirmed by the multitude and diversity of registered experimental data. The major problem of biomagnetism, namely the mechanism of action of the EF is still unexplained. In previous studies, we were able to show an active participation of the carotenoid pigments in the general cell metabolism [8], [9], [14]. These biological active substances have the possibility to form structural and functional complexes, with lipids and proteins, through noncovalent links. Consequently, the proteo-lipid-carotenoidic system, characterized by a specific spatial configuration and a functional lability, plays an active role in the cell metabolism. Thus, the hypothesis stating a lack of carotenoid involvement in the animal cell metabolism has been infirmed [12].

Based on the known structural and functional lability of the caroteno-lipid complex, we tried to investigate its dynamics under the action of the EF. This working hypothesis was put forward as an experimental model in order to reveal a possible direct mechanism of action of this energy form in the animal cell. This study was undertaken bearing in mind the energetic and morphogenetic importance of these substances within the cell.

MATERIAL AND METHODS

The experiments were performed on White Rock chickens hatched from EF treated or untreated eggs. The biological embryonic material was exposed to the action of a pulsatory rhythmic EF of 300 Oe, 100 Hz, 2.8 A generated by a magnetodiaflux device according to the following protocol :

— the first treated group (1 TG) exposed to the EF action three consecutive times for 10 minutes each on the 3rd, 6th, and 9th day of incubation ;

— the second treated group (2 TG) exposed to the EF action three consecutive preincubation times, twice for 10 minutes and once for 3 minutes respectively, as well as a postincubation treatment of 3 minutes on the 3rd day;

— control group (CG) handled in the same manner but without being irradiated.

The electromagnetic treatment was applied during the first stages of embryonic development, when the biological material was characterized by an increased sensitivity to the action of external factors. This increased sensitivity is due to the functioning of only the intracellular selfregulating mechanisms.

The external EF interaction with the biological microfields and the effects of this interaction on the reactivity of the caroteno-lipid complex was studied by the investigation of total carotenoid and carotenoid fractions [4], total lipids [17] and free fatty acids [2].

The determinations were carried out on liver tissue, harvested immediately after sacrification by decapitation of the experimental 24 hrs-hatched animals, during which interval they were kept on water without food.

The experimental data were statistically analyzed by the "t" Student test [15].

RESULTS

1. *The EF effect on the caroteno-lipid complex.* The mean values of the total carotenoids, total lipids and free fatty acids of the three experimental groups are shown in Fig. 1. A significant increase in liver carotenoid concentrations can be seen in the EF-treated groups. At the same time, in the treated groups a significant decrease of total lipids as compared to the control values is evident. The same type of modifications is shown by the liver free fatty acid concentration.

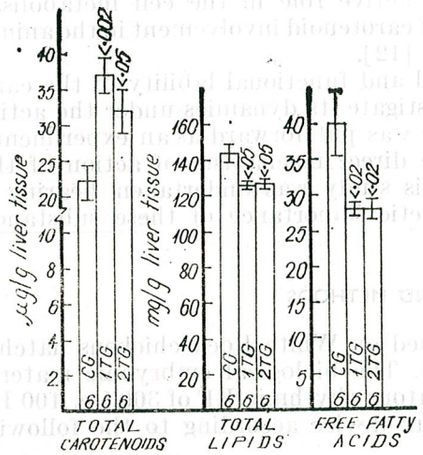

Fig. 1. — The effect of electromagnetic field on the liver total carotenoids, liver total lipids, and liver free fatty acids, in 24 hrs-hatched chickens. 1 TG = the first treated group; 2 TG = the second treated group; CG = control group. Figures in columns represent the number of samples and significance. Values are M ± SE.

2. *The quantitative and qualitative effect of EF on the liver carotenoid fractions.* The qualitative and quantitative modifications of the liver carotenoid fractions, are presented in Fig. 2. Thus, it can be observed the existence of a first fraction in the EF treated groups. This fraction is missing in the control group but appears subsequently in the ontogenetic development (unpublished data).

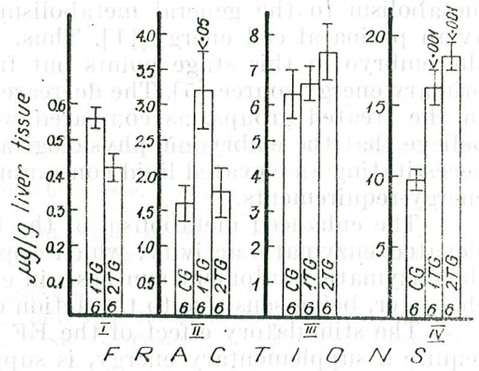

Fig. 2. — Quantitative and qualitative modifications of the liver carotenoid fractions to the electromagnetic field action.
Legend as in Fig. 1.

Also, significantly increased values of the second and fourth carotenoid fractions were registered in the EF-treated groups. The last fraction includes the most oxidized pigmentary forms, therefore the most reactive and of greatest functional significance.

DISCUSSIONS

Our data confirm the realness of the electromagnetic field action on cell reactivity, thus demonstrating the sensitivity of the living systems to its action.

The quantitative increase of the total carotenoids and the presence of new pigmentary forms in the EF-treated groups, as compared with the control group, supports the possibility of animal cells to synthetize these macromolecules; the hypothesis claiming a lack of carotenoid synthesis and of their metabolic significance in the animal cells has also been infirmed by our previous data [8], [9], [14].

The present results clearly point out that the liver carotenoid pigment synthesis is enhanced by the EF. This intensified synthesis, is probably, determined in terms of the functional implication of these chemical constituents in the cell metabolism stimulated by EF.

Further evidence for the action of EF on the carotenoid metabolism is provided by the qualitative and quantitative modifications of the carotenoid fractions in this experimental condition. In this respect, the existence of the new pigmentary forms and the increased values of these fractions should be remembered.

Considering that the levels of the determined indices are an expression of the dynamics of prenatal metabolism, the stimulatory effect on

EFFECT OF STRESS INDUCED BY POPULATION DENSITY INCREASE UPON REPRODUCTION ABILITY IN *MICROTUS ARVALIS* L. FEMALES

BY

ANCA PETRESCU-RAIANU, LILIANA BABEŞ, M. HAMAR and A. TUŢĂ

The effect of overpopulation stress upon the reproductive ability in *Microtus arvalis* females was investigated. The stressor was demonstrated to inhibit ovulation in a high proportion of adult females (90 p.c.) as indicated by the histological and histochemical picture of the adrenal gland.

Studies concerned with population density in rodent species revealed the occurrence of cyclic variations consisting in step-by-step density increase, up to overpopulation levels, followed by natural abrupt decrease. Christian [4] was the first to suggest a disturbance in the function of the pituitary-adrenal axis, as well as the stress determined by increased social pressure, to account for the above cyclic density declines. Recent studies confirmed the stressing effect of an increase in social contacts frequency, in natural or laboratory conditions [1], [3], [5], [6], [13], [16], [19]. In spite of the great number of stressing factors able to induce neuro-endocrine disturbances, a few indications exist in the literature for their ability to affect reproductive function. So far, psycho-social stress [3], [14], [21], surgical stress [2], [15], [22], hospitalization [18] and alimentary restriction [11] were demonstrated to induce such disturbances.

Though psycho-social stress was shown to induce reproductive function disturbances, neither the component of the hypothalamo-hypophyseal-gonadal axis upon which the stressing factor acts, nor the mechanism of action of the latter are known.

Based upon the observed decrease in gestation occurrence among the females of a very high density population of *Microtus arvalis* (90 p.c. of adult females were not pregnant), the problem arose of the influence of an increase in social interaction frequency upon reproductive system.

MATERIAL AND METHODS

Population density estimations were performed for a *Microtus arvalis* population during the 1970—1975 period in a pasture in Braşov county, by recording the number of the individuals captured in cylinders placed on a 1,000 m² area for 6 days monthly. Materials for the histo-physiological study of the adrenal cortex and reproductive system were collected in the following periods: June 1971, October 1971, June 1972, July 1973, April 1974 and October 1974. For capturing the animals,

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traps killing instantaneously the animal were used, avoiding any stress due to captivity. The adrenal glands and reproductive organs were removed immediately after capture and the following parameters were taken into account :

a) relative weight of the adrenal glands, as determined with a torsion balance and expressed versus body weight (after removal of the digestive tract from cardia to anal orifice in order to avoid the errors due to the different quantity of food) ;

b) histological structure of the adrenal cortex and ovary, studied on paraffin sections stained with haemalun-eosin and Heidenhain's azan stain ;

c) total lipid content as revealed by Sudan black B staining, using glands fixed in Baker's formol-calcium and sectioned with a freezing microtome ;

d) free and esterified cholesterol, as demonstrated by examination in polarized light of sections obtained with a freezing microtome and treated with digitonin, using glands fixed in Baker's formol-calcium ;

e) ascorbic acid, as demonstrated by silver nitrate reduction, according to Sosa [12].

RESULTS

Graph 1 reveals the fact that periods of maximal density (1970, 1974) are followed by years of decreasing population density, which display normal average density. (Fig. 1).

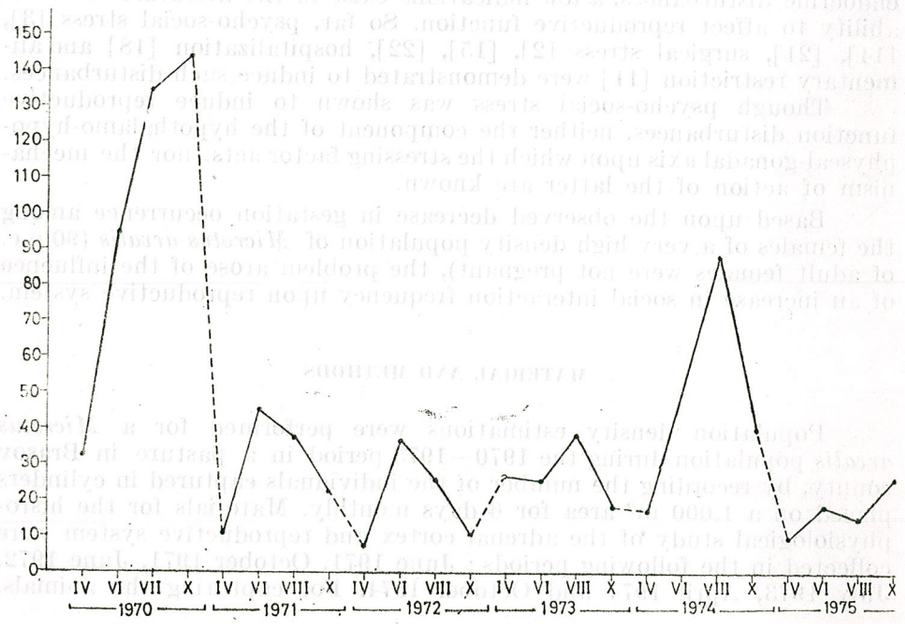


Fig. 1. — Population densities of *Microtus arvalis* (1970—1975).

I. *Morpho-functional state of the adrenal cortex.* Relative weight of the adrenal cortex in *Microtus arvalis* females during the June 1971—April 1974 period displayed fluctuations within the limits of seasonal cyclic variations. However, in October 1974, the relative weight of the adrenal gland displayed a much higher value, as compared to that recorded for the period June 1971—April 1974 (Fig. 2).

Histological structure of the adrenal gland in females captured during the reproduction seasons between June 1971 and April 1974 displayed a rather characteristic activity, as against the seasonal cyclic variations related to the sexual cycle. In spite of fascicular and reticular zones hypertrophy and the occurrence of a great number of compact cells, the changes did not reasonably allow to diagnosticate a stress. The considerable amount of sudanophilic lipids and ascorbic acid displayed by the adrenal cortex in animals captured between June 1971 and April 1974 supports the above affirmation. The adrenal cortex collected in October 1974, a high density period of the population, appears, however, quite different. The adrenal cortex hypertrophy becomes even more apparent, leading to the frequent occurrence of giant nuclei (Figs 3, 4) which sometimes contain big nucleoli of vesicular appearance (Fig. 4). The reticular zone displays a dramatic structure alteration, due to the occurrence of a great number of cells in different degeneration stages, with highly acidophilic cytoplasm and strong basophilic, angular, pycnotic nuclei. In certain females, the degenerative process was even more drastic, leading to the destruction of cell cords and the occurrence of lysis zones (Fig. 5).

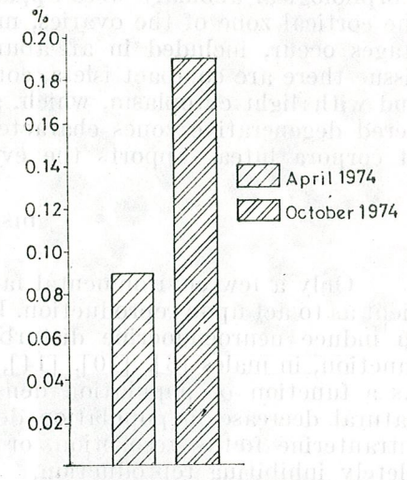


Fig. 2. — Relative weight of adrenal glands in *Microtus arvalis* females.

The enhancement of the adrenal cortex activity in females of the high density population is also confirmed by the frequent occurrence of subcapsulary nodules and accessory adrenal glands (Fig. 6).

The lipid content in the adrenal gland was much lower in females captured in October 1974 (Figs 7, 8). The same is true of cholesterol and ascorbic acid contents (Fig. 10).

Therefore, one might reasonably conclude that the adrenal gland in females captured at the moment when the population density reached maximum values, displays a certain exhaustion state, due to their maximal activity enhancement.

II. *Morpho-functional state of the reproductive system.* It should be noted that the reproduction season in *Microtus arvalis* extends under normal conditions up to October, most of the females captured during this month between 1971 and 1973 being pregnant.

In April 1974, when the population density did not reach such a value as to become a stressing factor as confirmed by the state of the adrenal gland, ovaries displayed follicles in different development stages, from primordial follicles up to mature ones, as well as a poor stromal tissue and the occurrence of well-developed corpora lutea. A macroscopic approach revealed hypertrophied uteri, while the number of embryos (6—10) was easy to count along the uterine horns.

In 90 p.c. of the adult females living in a population with a high density level in autumn 1974, though neither weight regression, nor morphological anomaly were apparent, ovaries lacked corpora lutea. In the cortical zone of the ovaries, numerous follicles in different evolution stages occur, included in an abundant stromal tissue. In the stromal tissue there are compact islets, consisting in cells of globular appearance and with light cytoplasm, which, according to certain authors are considered degenerative zones characteristic of the oestrus [7]. The absence of corpora lutea supports the evidence that ovulation did not occur.

DISCUSSION

Only a few environmental factors were demonstrated to be so efficient as to act upon reproduction. However, overpopulation stress appears to induce neuroendocrine disturbances acting upon the reproductive function, in males [3], [10], [14], [16], [17], as well as in females [21]. As a function of population density level, the stressors might induce natural decrease in population density by affecting lactation, inducing intrauterine fœtus resorption, or even, in case of severe stimuli, completely inhibiting reproduction.

In the population we investigated, a great number of the sexually mature females captured in October 1974 were stressed by overpopulation. The histologic appearance of the ovary for any of these females was typical of persistent oestrus, with many follicles reaching maturity and no corpus luteum. What was blocked by stress consisted in the release of mature follicles, i.e., ovulation, with subsequent failure of fertilization even when mating occurred.

An examination of our results reveals that very high values of population density coincided with hyperactivity of the adrenal cortex, associated with inhibition of ovulation, which indicates that the high population level was a factor of maximal intensity. In order to explain the ovulation inhibition mechanism, several hypotheses might be taken into account:

1) Inhibition of hypophyseal LH production, resulting in turn from an inhibition of LH—RF production in the hypothalamus. The cyclic LH—RF production, characteristic of females is known to assume, unlike continuous secretion occurring in males, certain connections between the nuclei of the hypophysiotropic area and other hypothalamic nuclei (preoptic, anterior and lateral hypothalamic regions and premammillary one), as well as zones remote from the hypothalamus (amygdala, septum, hypocampus, epithalamic-epiphyseal region, reticular mesencephalic substance) [9]. The last ones receive, in their turn, impulses resulting from the action of environmental stimuli, whose incidence increases with

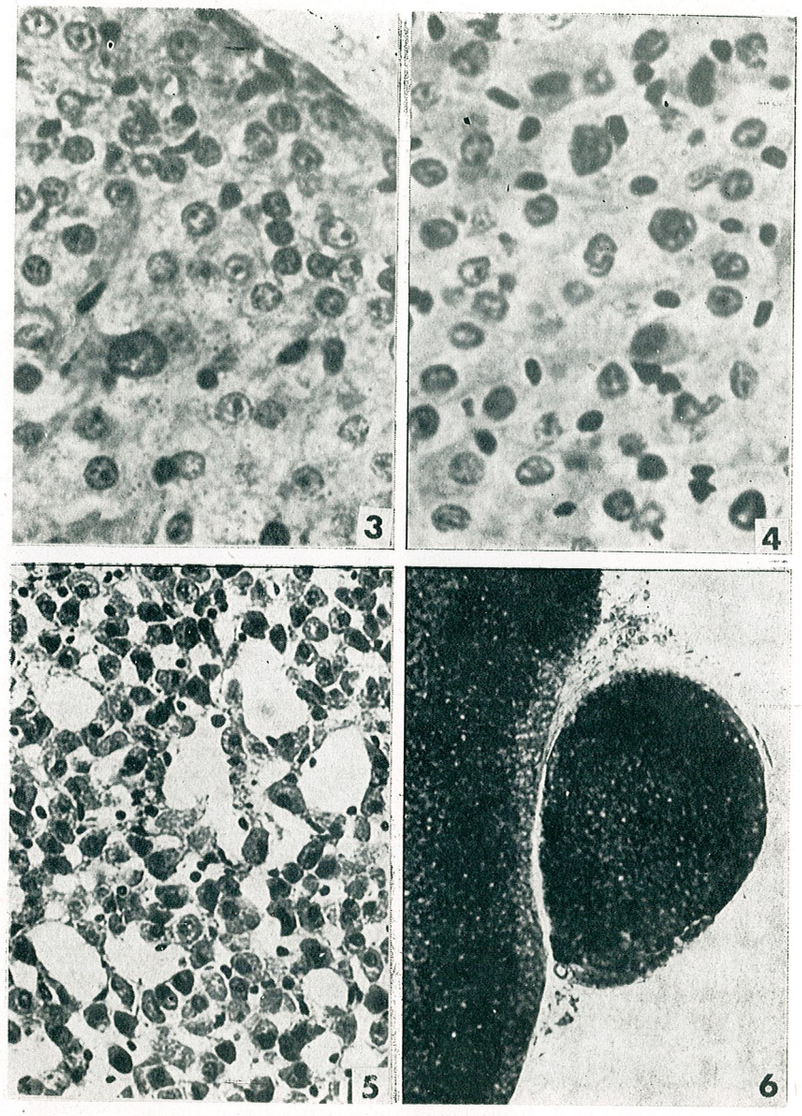
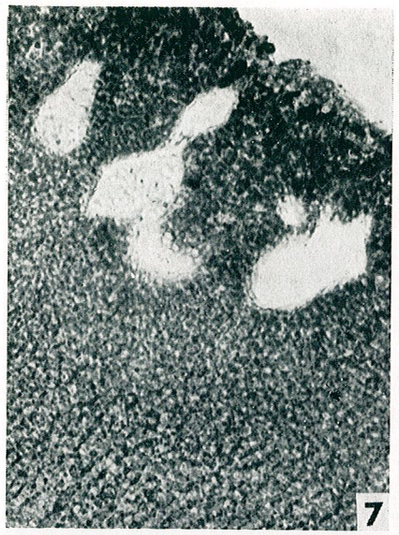
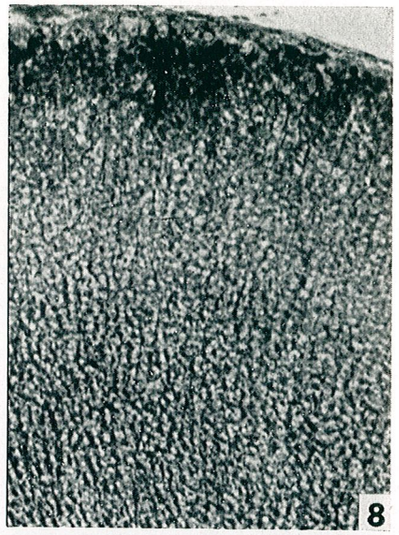
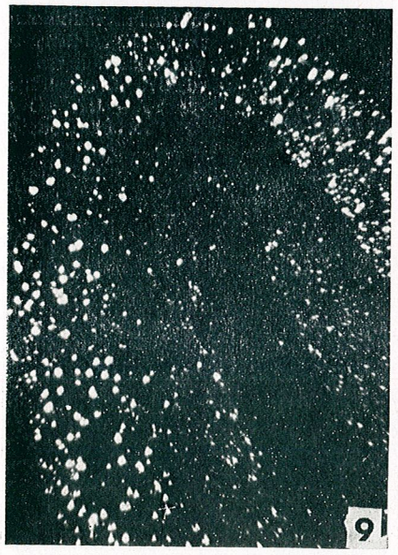


Fig. 3. — Hypertrophied nuclei and a giant nucleus in the fascicular zone of the adrenals.
Fig. 4. — Pycnotic and giant nuclei with vesicular nucleoli in the reticular zone of the adrenals.
Fig. 5. — Degenerative changes in the reticular zone.
Fig. 6. — Accessory adrenal gland.







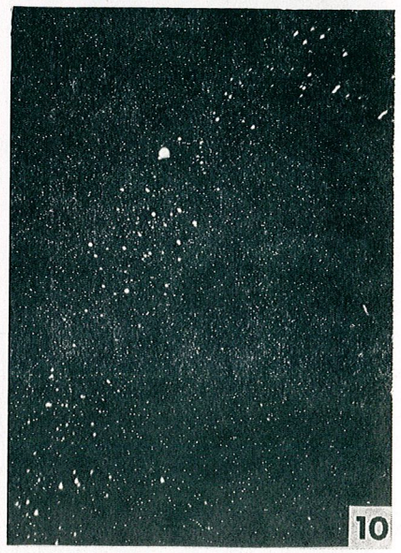


Fig. 7. — Depletion of lipids and degenerative changes in the fascicular zone.
Fig. 8. — Depletion of lipids in the adrenal cortex.
Fig. 9. — Cholesterol in the adrenal cortex of females in a period of normal population density level.
Fig. 10. — Depletion of adrenal cholesterol in females in a period of very high population density level.

social interaction in high density populations, which was demonstrated to be a stressing factor. Overstraining of the above extra-hypothalamic formations might impose an alteration of the function of the hypothalamic nuclei connected with them and involved in LH production and release.

2) The deficit of plasma LH might result from the failure of adenohypophyseal cells to produce this hormone, as they are involved in the ACTH overproduction induced by stress [22].

3) As adrenal cortex is an important source of progesterone, under ACTH control and not under the control of gonadotropins like ovarian progesterone [8], [20], high levels of ACTH, characteristic of stress might induce in the adrenal gland the synthesis and release of considerable amounts of progesterone known to inhibit ovulation.

4) A lowering of target organ sensitivity to gonadotropins might be also taken into account, according to Aono et al. [2], who reported a lowering of testes sensitivity to gonadotropins during surgical stress and subsequent to it.

5) Finally, the functional state of the reproductive ability of males in the population has to be further investigated.

Thus, the conclusion might be drawn that the numerical decrease of populations, following overpopulation periods, originates in a natality lowering due to female infertility by ovulation inhibition, in addition to mortality increase associated with the adaptative syndrome. For the population investigated by us, the above conclusion was confirmed by the density decline recorded in 1975.

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IDENTIFICATION OF SUBFRACTIONS WITHIN POLYSOMES DERIVED FROM RIBONUCLEO-PROTEIN PARTICLES

BY

R. BRANDSCH, P. ROTINBERG, CSÖNGE BRANDSCH and SMARANDA KELEMEN

Free polysomes obtained from postmitochondrial supernatants of rat tumor and rat liver tissue by ultracentrifugation on sucrose step-gradients were dissociated into ribosomes and ribonucleo-protein particles with the aid of EDTA (ethylen-diamine-tetraacetic acid) or puromycin. Chromatography of the ribonucleo-protein particles on microcrystalline cellulose revealed the existence of several subfractions. Analysis of the protein moiety of different subfractions on polyacryl-amide gels showed different migration patterns. Sedimentation of sucrose gradients of the RNA extracted from subfractions of ribonucleo-protein particles revealed differences in their sedimentation behavior, too.

In eucaryotic cells the primary transcription product as well as the messenger RNA (mRNA) molecules are found in the form of a complex with proteins, the so-called ribonucleo-protein particles (RNP). The role played by these RNA associated proteins is not clear and represents a subject of intense investigation. At least one of these proteins seems to be specifically linked to the poly (A)-sequence, characteristic of the 3′ end of most m/RNA species [1].

Nuclei-derived RNP as well as RNP derived from polysomes have been isolated recently by oligo (dT) cellulose chromatography, they behaving similarly with polyadenylated RNA [7], [5]. Elution of the retained material is made with a gradient of formamide, since washing with low salt buffer alone does not release all material from the column [4]. By using chromatography of polysome-derived RNP on microcrystalline cellulose as described by Kitos et al. [3] and by Schutz et al. [9], we show that it is possible to obtain a series of RNP subfractions by successive washing of the column with low salt buffer. Subfractions of RNP may be demonstrated in RNP derived from membrane-bound polysomes as well as in RNP from free polysomes, irrespective of the dissociation procedure used. Existence of such subfractions seems to be a general phenomenon in as far as it can be demonstrated for RNP derived from rat liver and tumor polysomes as well as from hen oviduct polysomes.

MATERIALS AND METHODS

The isolation of rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and free polysomes from hen oviduct, rat liver and rat tumor tissue was performed according to Schimke et al. [8] and Green et al., [2], respectively.

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Dissociation of RER into membranes, ribosomes and RNP was performed at 0.5 M KCl in the presence of 0.03 M EDTA. Free polysomes were treated either as shown above or incubated with 10^{-4}M puromycin at 0.5 M KCl.

In order to set free the entire amount of RER-bound polysomes. after the dissociation step the membranes were dissolved with SDS 0.1%. Part of the dissociated polysomes were recentrifuged over a 1.5 M sucrose cushion, for 60 minutes at 44.000 rpm. in the 8 × 11 ml rotor of a VAC-601 ultracentrifuge, to separate RNP particles from ribosomal subunits.

In parallel experiments, chromatography on Merck microcrystalline cellulose of the SER, dissociated in the same conditions as described for the RER, was performed. Cellulose chromatography of RNP particles was made according to Kitos et al. [3] and Schutz et al. [9]. Elution of material linked to the cellulose was performed with low salt buffer at 5 hours interval until no more material was washed off. Recovery of the sample was 98% and the column fractions were registered at 280nm with the use of a Beckman Spectrophotometer. In some cases the elution buffer contained 0.1% SDS. RNA was extracted from the eluted peaks according to Green et al. [2]. The RNA fractions were analyzed on 5—20% sucrose gradients spun in the swing-out rotor of a VAC-601 ultracentrifuge at 34.000 rpm for 5 hours. The proteins contained in different fractions of RNP obtained after cellulose chromatography were analysed on polyacrylamide gels according to the method of Laemmli [4].

RESULTS AND DISCUSSION

The binding of polysomes to the membranes of the endoplasmic reticulum is due to a number of factors, such as interaction of the larger ribosomal subunit with certain membrane areas [8], interaction of the mARN molecule with these membranes as well as interaction of the polypeptide chains synthesized and transferred into the vesicles of the endoplasmic reticulum (ER) with them. Polysomes may be released from the membrane of the ER either by incubation with EDTA and high salt (0.5 M KCl) or with puromycin and high salt in which case about 85% of the ribosomes are dissociated. In order to release all polysomal material the membranes of the ER have to be dissolved by the use of detergents, such as SDS [6].

As a source of membrane bound polysomes we used hen oviduct. RER isolated as shown under *Materials and methods* dissociated with EDTA and KCl was layered directly on a column of microcrystalline cellulose and eluted as described in the legend to Fig. 1. Treatment and chromatography of SER under the same conditions indicate that there is no material retained by the column, Fig. 2. That means, on the one hand, that the peaks which appear at the RER chromatography are not due to proteins dissociated from the membranes of the ER, and, on the other hand, that under the conditions used there was minimal contamination of the SER with polysomes. Almost identical elution profiles are obtained with polysomes dissociated with EDTA or puromycin (results not given).

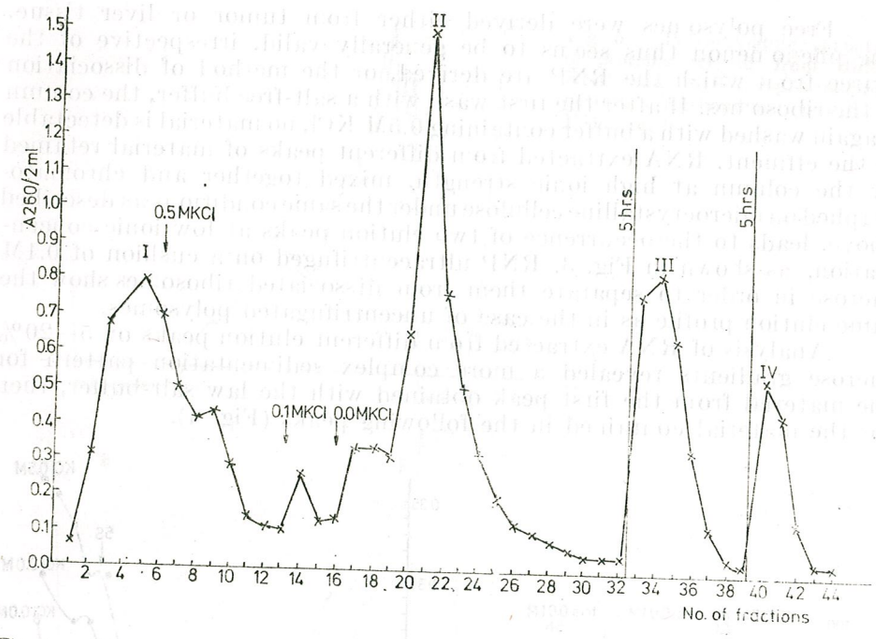

Fig. 1. — Chromatography of RER derived from hen oviduct, treated with KCl and EDTA and layered directly on a column of microcrystalline cellulose. The cellulose-bound material (RNP) was eluted stepwise, at 5 hours intervals with 0.01 M Tris pH 7.4.

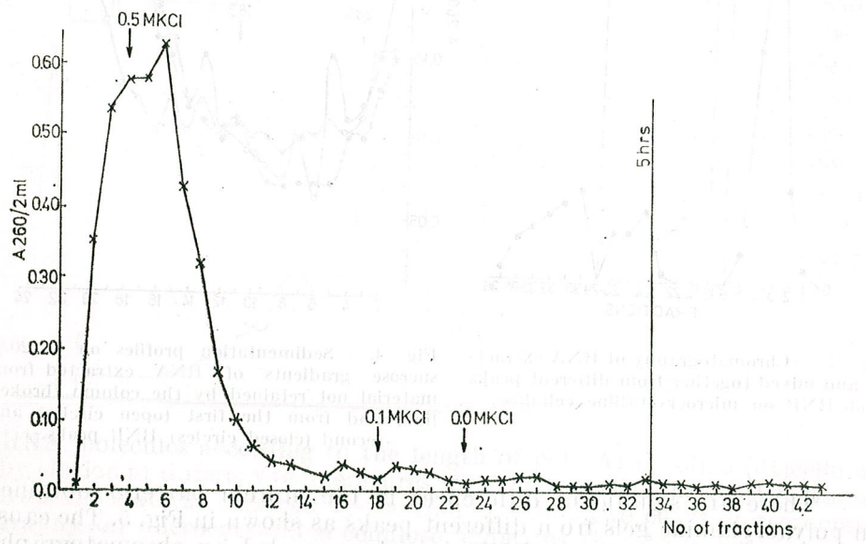

Fig. 2. — Chromatography of hen oviduct SER ; same conditions as in Fig. 1.

Free polysomes were derived either from tumor or liver tissue. The phenomenon thus seems to be generally valid, irrespective of the source from which the RNP are derived, or the method of dissociation of the ribosomes. If after the first wash with a salt-free buffer, the column is again washed with a buffer containing 0.5M KCl, no material is detectable in the effluent. RNA extracted from different peaks of material retained by the column at high ionic strength, mixed together and chromatographed on microcrystalline cellulose under the same conditions as described above, leads to the occurrence of two elution peaks at low ionic concentration, as shown in Fig. 3. RNP ultracentrifuged on a cushion of 0.1M sucrose in order to separate them from dissociated ribosomes show the same elution profile as in the case of uncentrifugated polysomes.

Analysis of RNA extracted from different elution peaks on 5—20% sucrose gradients revealed a more complex sedimentation pattern for the material from the first peak obtained with the law salt-buffer, then for the material contained in the following peaks (Fig. 4).

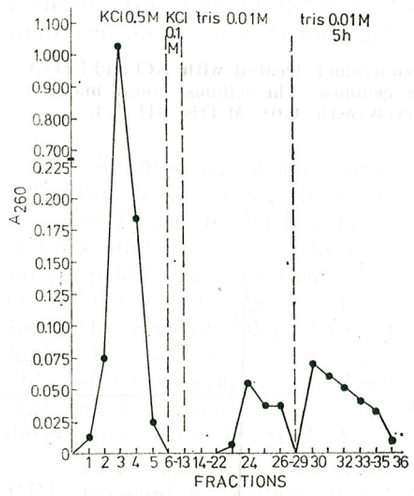

Fig. 3. — Chromatography of RNA extracted and mixed together from different peaks of RNP on microcrystalline cellulose.

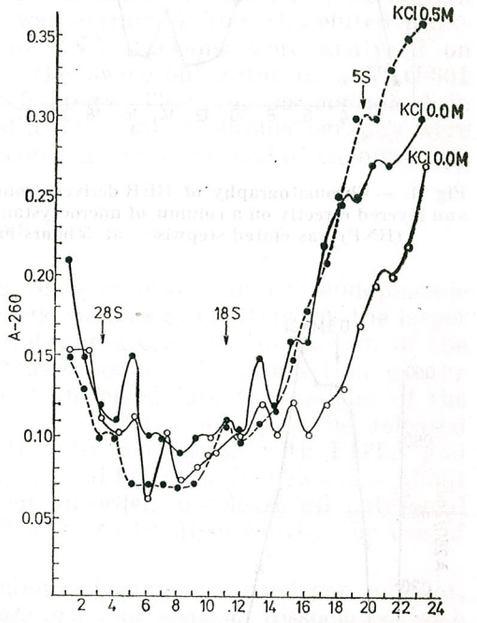

Fig. 4. — Sedimentation profiles on 5—20% sucrose gradients of RNA extracted from material not retained by the column (broken line) and from the first (open circles) and second (closed circles) RNP peaks.

There are significant differences in the protein patterns obtained on polyacrylamide gels from different peaks as shown in Fig. 5. The cause of this heterogenity in the RNP particles revealed by chromatography on microcrystalline cellulose is not clear. It is possible to isolate different

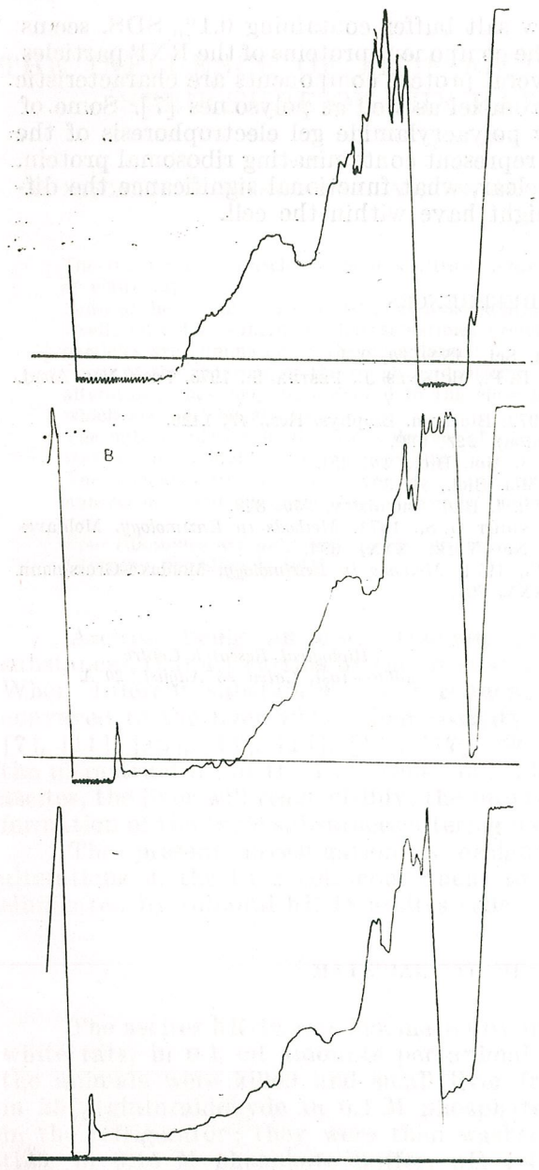

Fig. 5. — Polyacrylamide gel patterns of proteins derived from different RNP peaks; a, b, c, represent protein patterns corresponding to peak II, III, IV in Fig. 1

RNA molecules according to the length of poly(A) on oligo (dt)cellulose by elution at different temperatures [5]. The fact that the RNA extracted from the material retained by the column shows two peaks on recromatography on microcrystalline cellulose, indicates that to a certain extent the RNA moiety of the RNP particles may be responsible for the observed effect. On the other hand, the occurrence of a single elution peak when

wa shing the column with a low salt buffer containing 0.1% SDS, seems to suggest the involvement of the component proteins of the RNP particles in the binding mechanism. Several protein components are characteristic of RNP particles derived from nuclei as well as polysomes [7]. Some of the protein bands revealed by polyacrylamide gel electrophoresis of the different RNP fractions might represent contaminating ribosomal protein. Yet, at the moment it is not clear, what functional significance the different subfractions of RNP might have within the cell.

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THE INFLUENCE OF hR-18 ASCITES ON THE ULTRASTRUCTURE OF THE RAT LIVER CELLS

BY

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The tumour hR-18 ascites produces ultrastructural alterations in the liver cells of white rats.
Some of the cell nuclei appear to be diminished in size, strongly notched containing small, virus-like spherules, that sometimes present a double membrane; these particles are eliminated in the cytoplasm.
Some of the cisternae of the rough endoplasmic reticulum undergo advanced alteration; they may be observed in the close proximity of the mitochondria which are likewise altered.
The mitochondria exhibit various degrees of alteration up to their total transformation into a highly electron dense mass.
The nocuous effect of ascites may thus cause the appearance of a considerable number of secondary lysosomes and residual bodies in all the liver cells.

Free ribosomes are only casually encountered.
Glycogen depletion is total.

Ascites, being an open tumour, permanently releases catabolic substances, that are carried by the circulatory system of the host organism. When different substances are introduced into the organism they are conveyed to the liver where their toxicity is sometimes annihilated [1], [7], [11], [12], [14], [15], [16], [17], [20], [22], influencing, however, the ultrastructure of the liver cells; in a like manner, in the presence of ascites, the liver will react visibly, the intense activity deployed for transformation of the toxic substances altering its ultrastructure.

The present investigation is designed to follow ultrastructural alterations in the liver cells consequent to the action of the substances eliminated by tumoral hR-18 ascites cells.

MATERIAL AND METHOD

The ascites hR-18 was inoculated by intraperitoneal route to female white rats, in 0.1 ml amounts per animal. Ten days after inoculation, the animals were killed and small liver fragments collected and fixed in 25% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 for 2 hours in the refrigerator; they were then washed thrice for 60 minutes each time in 0.15 M phosphate buffer, pH 7.4 and kept overnight in 1% 0.15 M phosphate buffer, pH 7.4. The fragments were subsequently fixed in Os04 and embedded in Vestopal. The sections were cut in a Porter-Blum MT-1 ultratome, double stained with uranyl acetate and sodium citrate and examined in a Hitachi HU-11 electron microscope.

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RESULTS

The liver cell nuclei did not exhibit significant changes ; their contour and dimensions were maintained, but they were poor in chromatin, which was intermittently dispersed around the periphery (Fig. 1). In some instances however, the nuclei were reduced and of irregular shape, presenting notches and digitiform processes (Fig. 2). The cells containing such nuclei had a marked rough endoplasmic reticulum, the general aspect of the cytoplasm with all its organelles being similar to that of the cells with normal nuclei. The shape of the nucleus, peripheral chromatin and spherules present in the zones where the nucleolemma was not clearly outlined, suggested that diminution of the nucleus was brought about by a sustained metabolic activity, releasing into the cytoplasm numerous synthesis products. In the electron-dense areas of the nucleus, clear spherules could be seen, sometimes with a double membrane, the outer one merging with the electron-dense mass of chromatin. These spherules, with their particular content appeared to be eliminated into the cytoplasm, where they disaggregated. Lending support to this assumption was the presence of spherules in the apex of the longest digitiform process of the nucleus in Fig. 2 and those in the neighbouring invagination of this process. On the other hand, the spherules in the smaller nuclei closely resembled virus-like bodies or even viruses. They appear to have been observed at different moments in their development, with double membrane and nucleoid (Fig. 2). Multiplication of these particles and their elimination into the cytoplasm, where they disappeared, might account for diminution of the nuclear volume.

All the characteristic organelles of the liver cells were found in the cytoplasm but with marked structural alterations in some cases.

In none of the cells examined did we find the presence of glycogen granules or aggregations, that define the liver cells. At times (Fig. 3) granules larger than ribosomes, surrounded in some instances by small electron clear spaces and recalling glycogen granules, could be discerned at the periphery of the cells. Within their ambience, mitochrondrial profiles in advanced stages of disaggregation were noted. The presence of smooth endoplasmic reticulum vesicles was not observed.

Free ribosomes of imprecise form were difficult to discern. Polysomes were more frequently encountered presenting an aspect of spherules with an irregular contour (Fig. 3).

The rough endoplasmic reticulum consisted of long profiles running parallel both around the nucleus and between the mitochondria (Fig, 2, 3), as also described in the normal cell, although at certain places their membrane was altered, the contour of the cisterna section appearing to be incomplete. Over extensive areas the ribosomal granules were pulverized between the endoplasmic reticulum cisternae or disaggregated together with their support, i.e., the membrane of the endoplasmic reticulum cisternae (Fig. 2). Disaggregation of these cisternae and adjacent granules was often noted in the proximity of the mitochondria that were in advanced stages of alteration (figs. 2—4).

The mitochondria were numerous, spherical or elongated, showing all the signs of disaggregation ; the matrix consisted of a granular material

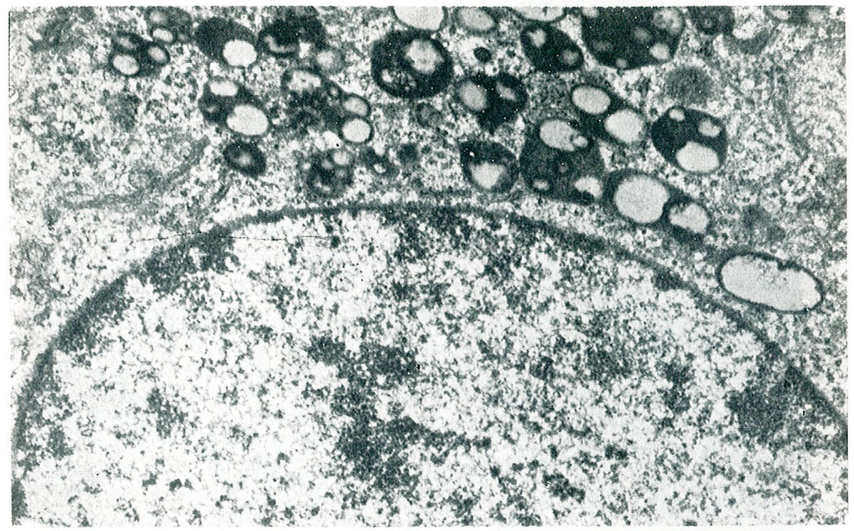

Fig. 1. — Liver cell fragment. Note the large amount of perinuclear secondary lysosomes (× 10.500).

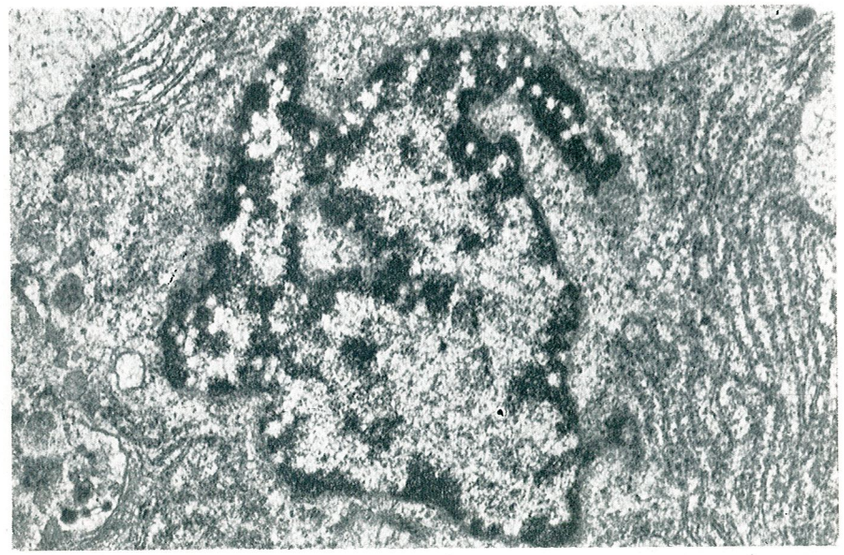

Fig. 2. — Liver cell with an irregular and reduced nucleus. Within the dense chromatin, electron-clear vesicles, sometimes taking on the aspect of virus-like bodies, can be discerned (× 14.500).

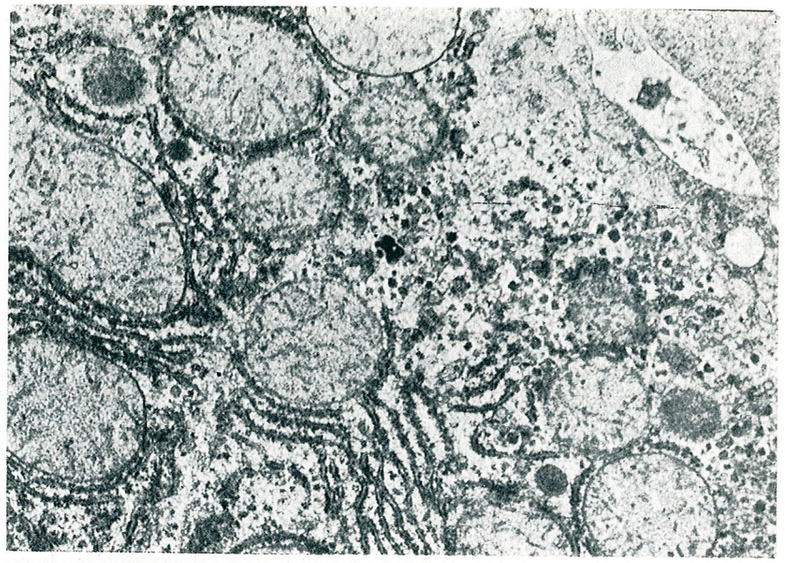

Fig. 3. — Mitochondria and rough endoplasmic reticulum in various phases of alteration. Glycogen-like granules may be observed in the vicinity of the bile canaliculus (× 16.000).

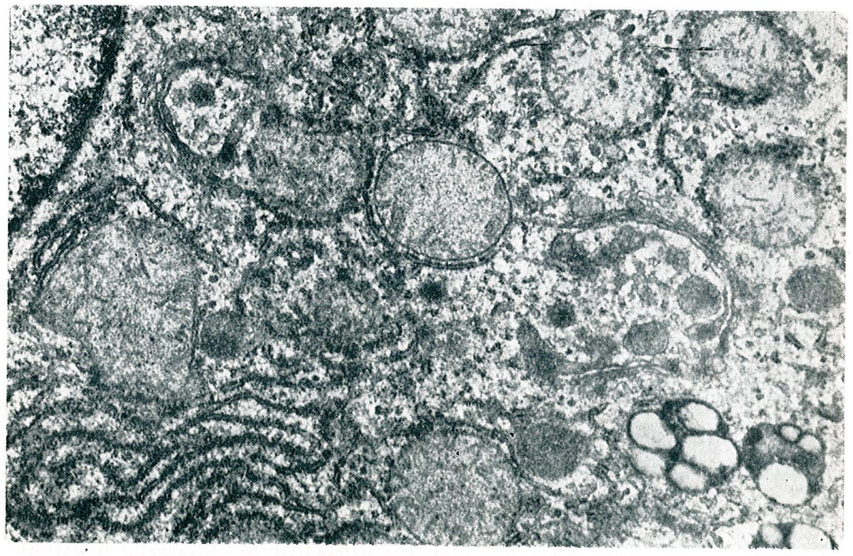

Fig. 4. — Fragment of a liver cell in which the dilated ends of the cisternae in a Golgi complex can be observed. In the concavity of the organelle, within the membrane, some vesicles containing an electron-dense material can be seen, similar to that in the dilatations of the Golgi complex. Note also mitochondria and rough endoplasmic reticulum in various stages of alteration (× 15.000).

containing the residues of crests with a modified structure. Seldom could crests with an integral structure be observed. This was also the case of the osmiophil mitochondrial membrane which, even when intact presented almost totally fused osmiophil laminae (Fig. 4, centre). Most of the mitochondrial membrane, however, had a hazy, weakly osmiophil contour, with altered portions. The mitochondria were as a rule strongly altered, surrounded by the rough endoplasmic reticulum cisternae that were likewise disaggregated, their content chiefly consisting of small electronodense spherules merging with the mitochondrial membrane (Figs 3, 4). The alteration of these organelles continued either by 'melting' of their remains in the cytoplasm (Fig. 4) or merging of the electronodense material into a single common ovoid mass, exceeding in size that of the organelles from which it derived (Fig. 4).

The Golgi complex was conspicuous in the ascitic animals within the cytoplasmatic areas in which they are normally found. The cisternae were compressed, convergent, an electronclear lumen. The extremities of some of the cisternae presented dilatations containing a material of medium electron density. In the concavity of the organelle and even around it, granules larger than the dilated ends of the cisternae and containing the same material, could also be discerned (Figs 3, 4). The content of the granules, their size and especially their single membrane prove that they are lysosomal. The proof of intense acid phosphatase production also stands in the effect of these enzymes, demonstrated by the presence of a large number of secondary lysosomes that occupy extensive areas in the cytoplasm of the liver cells (Figs 1, 4).

The existence of a large amount of lysosomal granules is one of the characteristics of the liver cells of ascitic animals (Fig. 1). In some instances, lysosomes may be observed in the final phase of digestion, in others, however, their activity appeared to be in the incipient phase. The multivesicular bodies may, in the last instance, be considered as fully active lysosomes (Fig. 4).

The Kupffer cells suggested a sustained functional activity. Some of the few cisternae of the rough endoplasmic reticulum exibited bifurcations, characteristic of this type of cell. In other Kupffer cells all the cisternae of the rough endoplasmic reticulum were dilated, without maintenance of their limiting membrane in all the cases.

The mitochondria presented different aspects of morphological alteration, similar to those of the liver cells, except for the content of the matrix which was not granular. In some cases the mitochondria disaggregated together with the surrounding rough endoplasmic reticulum cisterna, the same as in the liver cells.

The Kupffer cell cytoplasm contained voluminous electronodense granules with a diffuse contour or lysosomal electroncellular vesicles suggestive of an active phase of digestion of acid phosphatase.

DISCUSSION

Ascites hR-18 produces marked perturbations in the organism, finally leading to the death of animals. It is well known that the toxins eliminated by ascites cells cause intoxication of the organism. The liver

— among whose functions is also that of detoxification — undergoes marked ultrastructural alterations in the course of annuling the action of molecules of the nocuous substance.

Total glycogen depletion is the first sign of stress of the organism with a view to satisfying increased energy requirements, or the result of intense, local perturbations. Glycogenolysis often develops under the stress created by certain substances in the organism [14], but in most cases depletion of the glycogen reserves is not total, not even when the deleterious agent is carcinogen [7], [16], [20] or when the animals are starved [15]. Nevertheless, the granules in the vicinity of the bile canaliculi may be considered glycogen granules although no smooth endoplasmic reticulum cisternae profiles may be observed in their neighbourhood, and the presence of altered organelles might suggest that they are altered granulae to be eliminated through the bile canaliculi.

Alterations of the rough endoplasmic reticulum, ribosomes and polysomes represent evident signs of diminution of the synthesis capacity of the liver cells of ascitic animals, just as alteration of the mitochondria suggests change in the energy activity of the cells.

The lysosomes reveal the intense 'struggle' of the cell to annihilate the action of certain foreign, deleterious substances ingested by the liver cell, and their participation in the digestion of the altered organelles in the cell [1], [17]. The granules neighbouring the Golgi complex (Fig. 4) with a slightly electron-dense granular content included within the membrane, are certainly primary lysosomal granules elaborated by the Golgi complex [5], [13], [17], [21]. The increased stress upon acid phosphatases activates the phosphatase elaboration systems; J. B. Trump et al. [21] demonstrate that the elaboration of acid phosphatases starts in the endoplasmic reticulum and is completed in the Golgi complex where they can be first evidenced cytochemically. The presence of secondary lysosomes and postlysosomes or residual bodies in very high amounts (Fig. 1) as well as the presence of debris eliminated through the bile canaliculi, points to the increased consumption of acid phosphatases necessary for digestion and the elimination of toxic substances and of the organelles destroyed by the latter.

Our investigation did not demonstrate the presence of hepatic necrosis; this may perhaps be attributed to the short duration of ascites hR-18 in rats (10 days).

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THE ULTRASTRUCTURE OF THE GRANULOMA WITH *APLECTANA ACUMINATA* SCHRANK (OXYURIDAE, NEMATODA) IN *BUFO VIRIDIS*

BY

C. PRUNESCU, PAULA PRUNESCU and ELENA CHIRIAC

The mature granulomas with *A. acuminata* in *Bufo viridis* were constituted of macrophages, epithelioid cells and giant cells. A parallel between the granulomas described in *Bufo viridis* and the foreign body granulomas of mammals was established. The resemblances concerning the cellular composition and the development of granuloma and the differences concerning the interdigitating pattern of the cytoplasmic processes from the neighbouring cells in the granulomas were emphasized. The lack of the eosinophilic cell population around the parasite in the mature granuloma was noticed in the amphibian host.

Most of the individuals of *Bufo viridis* captured in the environs of Bucharest in the period 1973—1976, presented a heavy parasitose with *A. acuminata* Schrank (Oxyuridae, Nematoda). The young larvae were situated in the centre of small, spherical or ovoid formations — granulomas — localized in the stomach and cloaca walls, in the adventitial evaginations of the intestine wall, in the liver and in the mesentery [9].

The cellular composition of these granulomas consisted mainly of macrophages and giant cells. The ultrastructure of the typical cells in the granulomas with *A. acuminata* made the object of this paper. This was an attempt to provide a better understanding of the information about the composition and reactivity of granulomas in low vertebrates according to similar data on the structure and reactivity of granulomas in high vertebrates [1], [7], [8], [10], [12].

MATERIAL AND METHOD

A large number of localized granulomas from the liver, mesentery, intestine and stomach wall were dissected from 6 adult individuals of *Bufo viridis* and fixed for 12 hours in cold 4% solution of glutaraldehyde in phosphate buffer (pH 7.2). Specimens were postfixed for 2 hours in cold 1% solution of OsO_4 in phosphate buffer (pH 7.2), dehydrated in acetone and embedded in Lac 6611 (I.C.C.L.V. Bucureşti). Sections were cut on a Tesla ultramicrotome, stained with uranyl acetate, contrasted with lead citrate and examined on a JEM 7 electron microscope (80 MV).

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RESULTS

The granuloma which appeared as a reactive response round the larva of *A. acuminata* was initially composed of polymorphonuclear eosinophilic cells and monocytes. As the granuloma was constituted its cellular population became more uniform, most of the cells being large macrophages. Among the macrophages there were few lymphoid cells or polymorphonuclear eosinophilic cells.

The macrophages in the granuloma with *Aplectana acuminata* showed various degrees of maturation from young macrophages to epithelioid cells. Ultrastructurally, the macrophages displayed a large nucleus with an ovoid or sinuous outline on the section, with large pore complexes and a considerable amount of peripheral heterochromatin. The cytoplasm was dense, with several free ribosomes or polyribosomes. The mitochondria were relatively few in number, round or somewhat longshaped, with numerous short cristae and a condensed appearance. The Golgi complex was poorly developed. Lysosomal bodies were electrondense and irregular in form. The larger phagosomes contained circular or irregular electron-light vacuoles, inclusions and myelin-like material (Fig. 1).

As a rule, the epithelioid cells presented an eccentric nucleus but sometimes they had two nuclei. The epithelioid cell displayed a paranuclear position of Golgi complexes. In the more peripheral areas, the cytoplasm presented several tubules of rough endoplasmic reticulum, some condensed mitochondria and lysosomes. Some areas showed higher concentration of glycogen aggregates (Fig. 2).

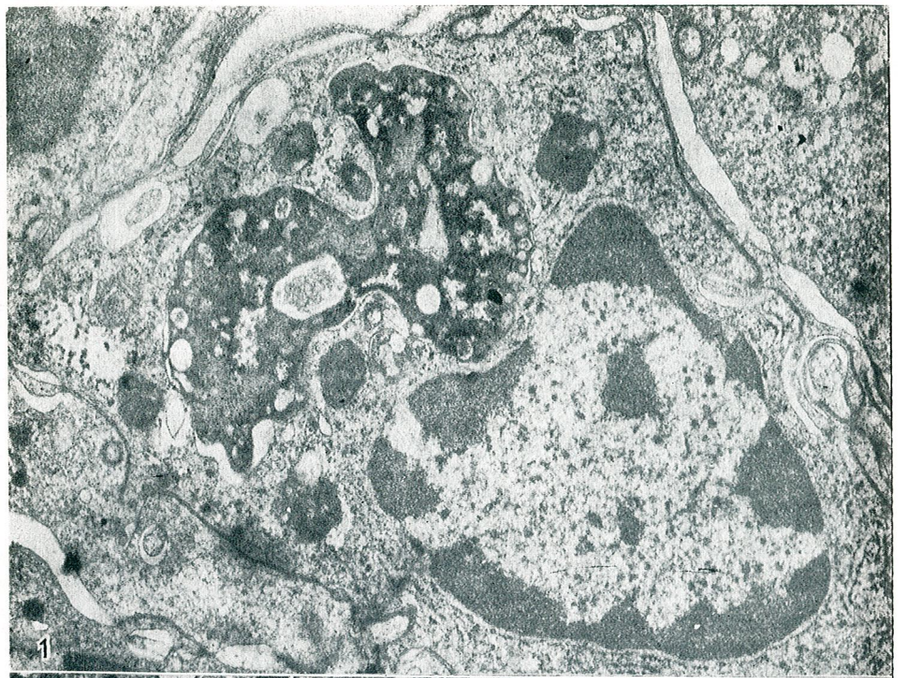
The most salient feature of the granuloma with *A. acuminata* was the existence of the complex plasmalemma relations between the macrophages or between the macrophages and the neighbouring epithelioid cells. On the ultrathin sections, various types of filamentous or lamellar cytoplasmic processes from two or several neighbouring cells, interdigitated with one another. Often, some of these processes penetrated deeply into the neighbouring cytoplasm, up to the nuclei (Fig. 2).

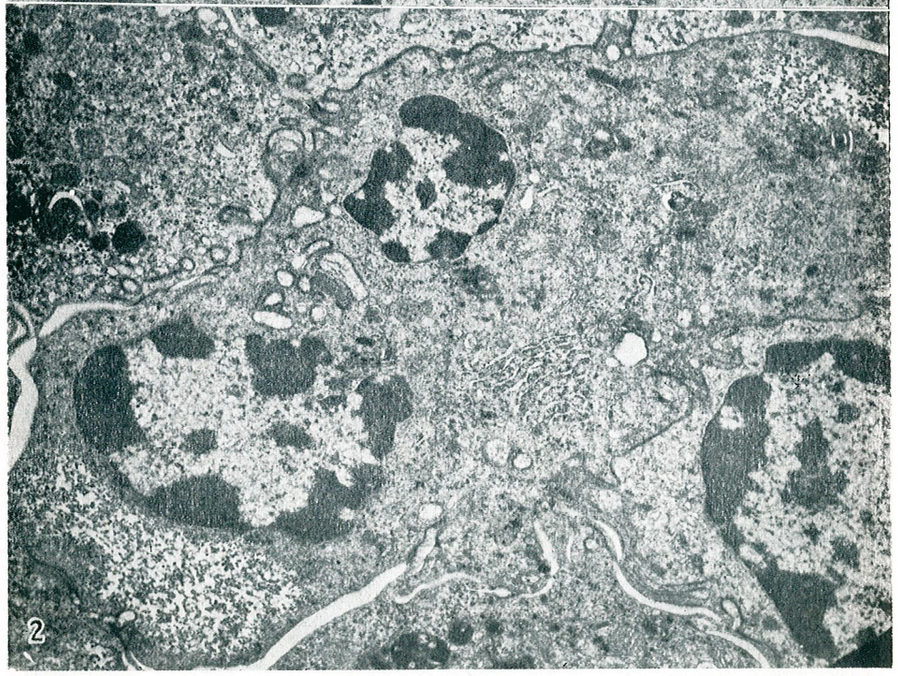
The macrophages which were in a close proximity to the parasite were morphologically similar to the ones situated at a certain distance from the central zone. Also, characteristic membrane relations at the surface of all granuloma cells were observed (Fig. 3).

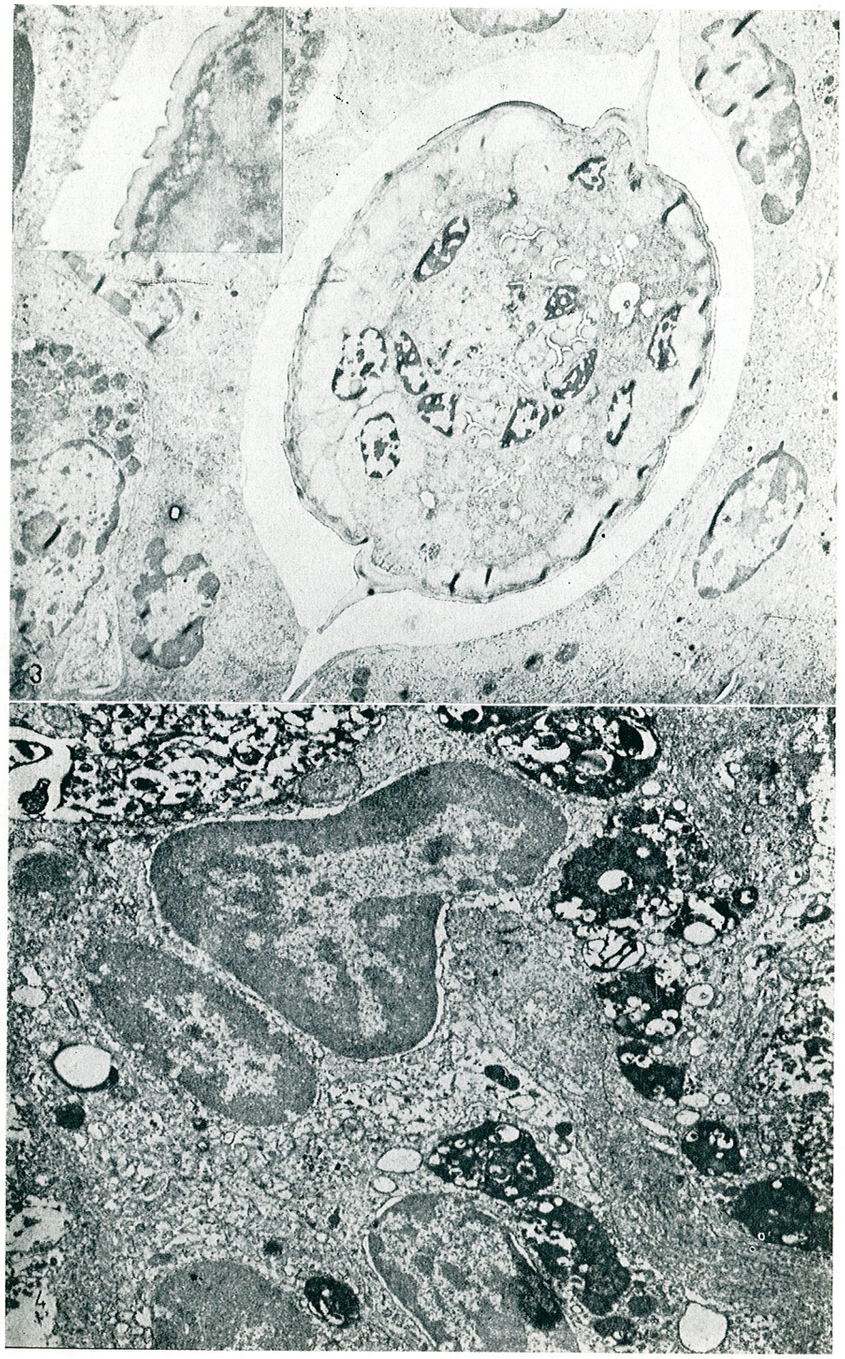
The cuticular peculiarities of the parasite were imprinted into the cellular body of every inflammatory cell situated near the larva (Fig. 3 inset). This may be an indication of the intimate contact between granuloma tissue and the parasite. However, a free space between the parasite and granuloma tissue was constantly observed on the electronmicrographs perhaps as a result of the electronmicroscopic processing, or simply because there existed a true space permitting the parasite slight movements.

Fig. 1. — Typical macrophage with a large phagosome in the granuloma with *A. acuminata*. Note the complex plasmalemma relations with the neighbouring cells. (19000x)

Fig. 2. — The epithelioid cell displaying two nuclei. Note some cytoplasmic processes from the neighbouring cell penetrated near the nuclei. (12000x)







The giant cells were arranged at a distance from the central zone, often to the peripheral zone of the granuloma. They were large syncytial structures more or less circular with a crown of nuclei at the periphery of the cell. The multinucleated giant cells contained from 4—5 to 20—30 nuclei on a histologic section [9]. The phagosomes were agglomerated inside the circle formed by the crown of nuclei. These phagosomes were very large, confluent, containing a spongy electron-dense material which seemed to represent the cellular remnants (residual body). The cytoplasm surrounding the phagosomes displayed bundles of fine fibrillar material arranged by the smooth membranes with a tortuous line. Such areas were composed almost exclusively of these microfilaments and displayed no other organelles (Fig. 4).

In the granuloma with Aplectana, there were some cells with a wealth of rough endoplasmic reticulum and swollen cisternae, very similar to mature plasma cells.

DISCUSSION

The granulomas constituted around the larvae of Aplectana as an inflammatory tissue response of the host, presented many similarities with the foreign body granulomas occurring in homeothermae in various pathological and experimental situations [1], [7], [10], [11]. A similar feature of both types of granuloma was the presence of the macrophages as the main component of this tissue. Both in the granulomas with Aplectana in the amphibian *Bufo viridis* and in the foreign body granulomas in birds or mammals, the evolution of the macrophages followed a determined line in which the mononuclear cells migrated from the circulatory flow, turned into the macrophages and then, into epithelioid and giant cells [6], [11].

But the plasmalemma relations between the macrophages in the granuloma with Aplectana were very different from the plasmalemma relations developed between macrophages or macrophages and epithelioid cells in the foreign body granuloma in birds or mammals. The later type of relationship was characterized by sheet-like cytoplasmic processes which mutually interdigitated in a nearly perfect symmetry producing cytoplasmic arcades [8]. In the granulomas with Aplectana in *Bufo viridis*, the cytoplasmic processes were less organized. They were long flexuous, lamellar processes, which interdigitated over large surfaces or were indented deeply into the cellular mass of the surrounding cells.

Fig. 3. — Ultrathin cross-section in a larva of *A. acuminata* situated in the centre of a granuloma ; the parasite is surrounded by macrophages.
(3500x)
inset : Detail on the close contact between granuloma tissue and the parasite. Note the complementary outline of both contact surfaces.
(14000 x)

Fig. 4. — A giant cell with four nuclei on the ultrathin section. The phagosomes are large, numerous and irregular, containing vacuolating residual bodies. Among the phagosomes there are some bundles of fine fibrillar material.
(7200x)

Another peculiarity of the mature granuloma with Aplectana in the *B. viridis* was the uniformity of the cellular type in the composition of this tissue and the rare occurrence of the new infiltrated cells. In mammals, in granulomas with parasite worms, an agglomeration of polymorphonuclear eosinophilic cells was usually noticed in the immediate proximity of the parasite [7], [12]. On the contrary, in *B. viridis* round the larva of Aplectana in the mature granuloma only mononuclear macrophage cells were noticed. In the proximity of the parasite, cellular necrosis phenomena were never seen.

The data about the cellular and humoral defense system in amphibians [2], [3], [4], [5] confirm the similitude of these defense mechanisms with those in birds and mammals. In Anura, lymphoid organs analogous to mammalian lymphoid ganglia [3], [4] were described. These small lymphoid organs, in addition to thymus, spleen, hematogenous marrow, kidneys etc., represented the morphological basis of the defense processes.

The granulomatous reactivity in *Bufo viridis* against the larvae of *Aplectana acuminata* demonstrated the cells possibilities of the defense system in amphibians.

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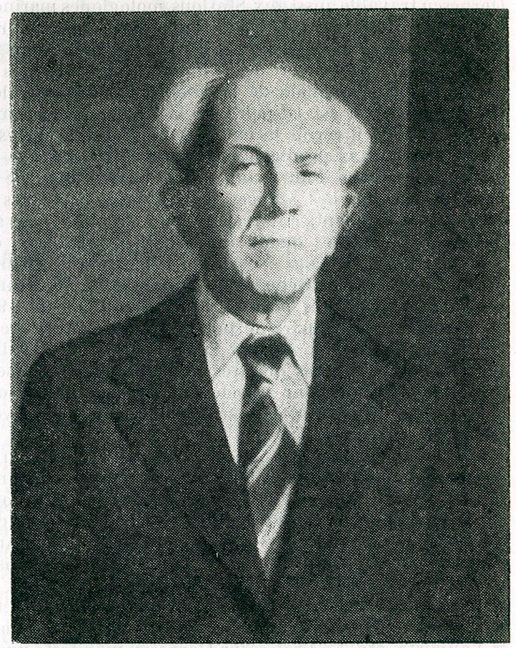
LE PROFESSEUR GHEORGHE T. DORNESCU À SON 80e ANNIVERSAIRE



Le 28 février 1978, au Laboratoire d'Anatomie comparée de la Faculté de Biologie de Bucarest, quelques élèves et amis, chercheurs ou universitaires de la Faculté, ont fêté le Professeur G. T. Dornescu pour ses 80 ans. Ce sont toujours presque les mêmes admirateurs du vénéré Professeur qui tous les 28 février des 10 dernières années se réunissent pour lui rendre leur respectueux hommage. Ces manifestations de profond attachement se déroulent chaque fois avec l'appui de son ancien élève, actuellement son successeur à la Chaire d'Anatomie comparée, le maître de conférences dr Dumitru Mișcalenco.

Malheureusement, l'espace réduit dont nous disposons ne nous permet de rappeler que les principales étapes de la carrière universitaire du Professeur Dornescu, de même que de mentionner les principaux problèmes abordés dans son activité scientifique.

Après avoir suivi les cours du Lycée « Petru Rareș » de Piatra Neamț (1917), il fait ses études universitaires à la Faculté de Sciences de Jassy, étant nommé préparateur (1920) et ensuite assistant (1924) au Laboratoire de Morphologie de Jassy, dirigé par le Professeur Paul Bujor. Dès le 1er avril 1927, il commence son activité au Laboratoire de Morphologie animale de Bucarest. Dans les périodes allant de 1927 à 1930 et du 1 juin au 15 novembre 1931, il travaille dans le Laboratoire du Professeur Champy à l'Hôpital Broca de Paris, dans celui du Professeur Guillermond de même qu'au Laboratoire d'Anatomie et d'Histologie comparée de Sorbonne avec le Professeur Wintrebert. Pour souligner l'excellente impression laissée par le Professeur Dornescu dans les cercles scientifiques français, nous allons citer les paroles de Wintrebert même quant au jeune chercheur roumain : « Au cours de ses recherches, M. Dornesco a fait preuve des plus brillantes qualités scientifiques, en même temps que d'une grande habileté dans un domaine où la perfection technique est de rigueur... Ce travail... a nécessité de la part de l'auteur une patience et une persévérance inlassables qui, jointes à des connaissances histologiques, cytologiques et physiologiques très remarquables, l'ont conduit à des résultats

et à des interprétations nouveaux et originaux ». D'ailleurs, grâce à une recommandation de son maître de Sorbonne, l'Université de Paris lui accorde une bourse Arconati-Visconti pour les années scolaires 1928—1929 et 1929—1930. Lors de son séjour en France, le Professeur Dornescu travaille aussi aux Stations zoologiques marines de Roscoff, de Concarneau et deMonaco.

En 1935, devant une commission formée par les Professeurs D. Voinov (Président), D. Călugăreanu et A. Popovici-Bâznoşanu (membres), il soutient sa thèse de doctorat intitulée *Recherches sur la structure des cellules nerveuses*. Depuis 1946, il est nommé Professeur à la Chaire de Morphologie animale de la Faculté de Biologie de Bucarest. Entre 1953 et 1968, l'année de sa retraite, il est titulaire de la Chaire d'Anatomie comparée, d'Histologie et d'Embryologie ; en 1964 il reçoit la haute distinction de Professeur émérite.

Pendant sa prodigieuse activité didactique, le Professeur Dornescu se distingue par ses inoubliables cours d'une haute tenue scientifique, de même que par son exigence à l'occasion des examens ou des séances de travaux pratiques au Laboratoire d'Anatomie comparée. Beaucoup d'étudiants ont préparé leurs diplômes sous sa direction, ayant ainsi largement profité de ses connaissances quant aux difficiles problèmes d'anatomie comparée et d'histologie. En collaboration avec Olga Necrasov, Professeur à l'Université de Jassy, membre correspondant de l'Académie de la République Socialiste de Roumanie, il rédige l'excellent traité d'*Anatomie comparée des Vertébrés*, paru en deux tomes (1968 et 1971). Un nombre de 25 candidats roumains ou étrangers ont préparé ou préparent leurs thèses de doctorat sous la direction du Professeur Dornescu. Tous ceux qui ont eu la chance de travailler dans son laboratoire ont été impressionnés par les hautes qualités humaines du Professeur Dornescu et, en premier lieu, par sa profonde affection pour ses élèves ; fort nombreux sont ceux qui ont bénéficié de ses savants conseils, de même que de son influence mise, en diverses occasions, au profit exclusif de ses collaborateurs ou élèves.

L'activité scientifique du Professeur Dornescu se traduit par plus de 80 travaux qu'il publie seul ou en collaboration dans des revues roumaines ou étrangères. A l'exception des études concernant la tératologie, il faut mentionner sa contribution relative à l'emploi de l'acide chromique pour la dépigmentation. Ses recherches de cytologie portent sur les cellules nerveuses (des Arachnides, des Crustacés, des Insectes et des Amphibiens), hépatiques (des Poissons, des Amphibiens, des Reptiles et des Mammifères) ou sur les éléments sanguins (des Crustacés Phyllopodes, des Poissons et des Amphibiens). Quant aux études d'histophysiologie, nous retenons ceux ayant comme sujet le système nerveux des Insectes pendant la métamorphose, la structure des branchie de l'Ecrevisse, la structure et la circulation du sang dans les podobranchies de la Languste, etc. Le Professeur Dornescu manifeste un intérêt tout particulier pour les recherches concernant les glandes tégumentaires des Phyllopodes anostracés, pour celles portant sur la structure des branchies des Téléostéens ainsi que pour l'étude des voies évacuatrices du foie et du pancréas chez les Poissons (Cobitidae), les Amphibiens et les Reptiles (Chéloniens, Lacertiliens et Ophidiens). En même temps, son attention est attirée par l'étude des glandes salivaires des Sauriens et par celui de l'évolution des arcs aortiques des Poissons (Cyprinidae et Cobitidae). Il signale pour la première fois une glande lacrymale chez les Urodèles ainsi qu'un nouvel organe lymphoïde chez les Téléostéens. Si nous ajoutons aussi ses importantes études sur le développement du neurocrâne chez les Cyprinidae et les Salmonidae et ceux sur la morphologie de la colonne vertébrale des Rongeurs, du crâne des Cervidés et des Pinnipèdes, nous nous rendons mieux compte de sa contribution considérable à l'avancement de l'anatomie comparée.

La feconde activité didactique de même que les remarquables réalisations dans le domaine de l'anatomie comparée et de l'histologie ne caractérisent que partiellement la personnalité complexe du Professeur Dornescu, l'un des plus brillants représentants de la biologie roumaine.

Dan Dumitrescu et Lucia Dumitrescu

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

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