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REVUE ROUMAINE DE
BIOLOGIE

BIOL. INV. 98

SÉRIE DE ZOOLOGIE

TOME 16

1971

N° 3

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REVUE ROMAINE DE
BIOLOGIE
REVUE DE ZOOLOGIE

COMPETITION AND ITS BEARING ON THE
FRESH-WATER FAUNAS

BY

PETRU BĂNĂRESCU

The main dominant and competitive groups of fresh-water animals are: Ostariophysi among fishes, crabs among decapoda, Parabathynellidae among Syncarida, Unionacea (except Hyriidae) among mussels, Diptomidae among Calanoid Copepoda and Streptocephalidae among Anostraca. South-East and East Asia were the main centers of evolution for these groups; North America was a secondary center for crayfishes and mussels, South America a second center for Ostariophysi. Archaic groups, eliminated by competitive ones, survive mainly in southern continents, especially Africa for fishes, Australia for Invertebrates. Some archaic and non-competitive groups lived once also in northern continents, while others seem to have dispersed through direct contact between southern continents.

In analysing the zoogeography of fresh-water fishes and terrestrial vertebrates, Darlington [3] concluded that one main feature of their distribution pattern is the presence of a few dominant and competitive families or higher taxa, all or most of which center in the Tropics of the Old World, showing the tendency to spread over the whole Megagea (e. g. Eurasia, Africa and North America) and to eliminate progressively the older groups, which survive mainly in peripheral areas. He explains in the same manner [5] the distribution of some old groups of Invertebrates, occurring in Australia, New Zealand and the southern top of South America.

One can distinguish not only among fishes, but among several higher taxa of fresh-water Invertebrates, dominant and competitive groups on one hand, archaic and non-competitive on the other, and their distribution reflects the effects of competition and of partial elimination of the last-named by the first ones.

Fresh-Waters Fishes. As generally accepted by Darlington and other students, the Ostariophysi (excluding the Gonorynchiformes, recently included by Rosen & Greenwood [15] as a distinct series, Anotoptysi, within Ostariophysi) are the dominant and most competitive group of fresh-water fishes. Their distribution indicates two main centers of differentiation and radiation: South-East and East Asia for Cyprinoidei and a few families of Siluriformes, tropical South America for Characoidei and other Siluriformes. A few families (Anabantidae, Mastacembelidae a.o.), original from South-East Asia, accompanied the Cyprinoidei in their dispersal, while all other orders and families of primary fresh-water fishes proved less competitive than Ostariophysi and were "vanquished" by them. The most important among the "vanquished" groups are the Osteoglossomorpha, which were once the dominant group and had a world-wide range; the Jurassic East-Asian and Siberian *Lycopera* belonged to them [6]. At present the Osteoglossomorpha are richly represented only in Africa, but a few survive also in South America, South Asia, Australia, even in North America. Among the other non-competitive fresh-water fishes are: the Percopsiformes, Amiidae and Lepisosteidae in North America (formerly also in Europe), four African families and the Holarctic Esociformes and Percidae.

One general conclusion of this distribution pattern is that primitive non-Ostariophysi were almost completely eliminated in the two dispersal-centers of Ostariophysi, and survived mainly in Africa, then in North America, a few also in Europe (Fig. 1).

Secondary fresh-water fishes are less competitive than primary ones, being abundant only in Central America, where primary fresh-water fishes are recent intruders [11] and in some special habitats, such as inland water bodies with special chemical features from arid areas and even great lakes (the African Cichlidae!).

Even less competitive are peripheral fishes, quite scarcely represented in continental areas, but abundant in islands where there are no, or only quite few primary, secondary and vicarious fresh water fishes (Madagascar, Lesser Sunda islands, New Zealand) as well as in parts of continents under very cold conditions, where the former primary fresh-water fauna was decimated (northern Siberia and Canada, probably cold South America too).

Higher Crustaceans. The only primary fresh-water Decapoda are the Crayfishes: Astacidae (Holarctic) and Parastacidae (southern continents, except Africa). Unlike primary fresh-water fishes, crayfishes proved less competitive than secondary fresh-water crabs (former Potamonidae; according to the recent studies by Bott [1] [2] a.o. several families, each of them having distinct marine ancestors) and peripheral fresh-water prawns. The Parastacidae were totally eliminated from Africa (where they probably lived once) and tropical South America. Because of the competition of crabs, the Astacidae were not able to colonize tropical Asia (or were eliminated here, if once present). Among the Astacidae, the primitive subfamilies, Cambaroidinae and Astacinae have a disjunct range (North East Asia, Europe, western North America), having been

eliminated in eastern North America by the more competitive Cambarinae. This subfamily proved even able to compete in Central America with fresh-water crabs (Fig. 2).

The distribution pattern of fresh-water Isopoda and Amphipoda doesn't reveal the effects of competition between families or genera. Both groups live mainly in temperate and even cold areas; their absence or scarcity in tropical areas may be due to the competition by prawns.

One peculiar group of fresh water Isopoda, the Phreatoicoidea lives in Australia, New Zealand, India and the southern top of Africa. This distribution too seems to reflect elimination, at least from tropical Africa. Like Parastacidae, the Phreatoicoidea occur in northern Australia, where the competition by modern groups is much feebler than in other tropical areas.

The effects of competition are very evident in the distribution of the mainly hypogeous Syncarida [12], [13]. The most primitive order, Anaspidacea, is exclusively Australian, the second, Stygocaridacea, lives in southern South America and in New Zealand, while within the last order, Bathynellacea, the family Bathynellidae has a disjunct, bipolar range, occurring in the temperate zone of both hemispheres; it has been eliminated in the tropical zone by the more competitive Parabathynellidae [12].

Entomostraca. The effect of competition is evident also in the geographical distribution of the two exclusively fresh-water families of Calanoid Copepoda: Boeckellidae and Diaptomidae. The first family ranges in Australia (without reaching its northern corner), in New Zealand and southern South America and adjacent sub-antarctic islands; one species is isolated in Mongolia. The Diaptomidae are more differentiated in East and South-East Asia; they occur throughout Megagea and in tropical and warm-temperate South America; two species (none of them endemic) reach northern Australia. Some genera are Holarctic, but only one of them, *Arctodiaptomus* has a wider range in America, reaching its warm-temperate parts; the other Holarctic genera are mainly Palaearctic, a few of their cold-adapted species living also in Alaska and northern Canada.

It is quite reasonable to conclude that Boeckellidae had once a much wider range, being eliminated later by the Diaptomidae from the whole Megagea and from tropical South America; the single Mongolian species is a relict in a part of the former range (Fig. 3).

Among the Anostraca, one family, Streptocephalidae, seems to be in an expansive stage, radiating from tropical Africa. The range of Branchinectidae (Holarctic and cold-temperate South America) may suggest a former wide range and subsequent elimination from the tropics; yet this cold-adapted family could also have reached temperate South America by slow dispersal along the Andean high plateaux.

Fresh-water Mussels. The main distribution pattern of fresh-water mussels is the occurrence of Unionacea (except Hyriidae) in the Megagea, with maximum of differentiation in East- and South-East Asia (not in India!) on one hand, in North America on the other, the occurrence of Hyriidae, a family of Unionacea, in South America, Australia (with New

Guinea) and New Zealand and of Mutelacea in South America, Africa and India. The Megageic Unionacea are evidently a competitive group. There is no evidence of a former occurrence of Mutelacea outside their present range; yet one can assume that Unionacea entered relatively recently in India and Africa and already began to eliminate the Mutelidae from these countries. The South American Hyriidae are apparently offshoots of North American immigrants which arrived here at the end of Cretaceous-beginning of Palaeocene [14]; the genera to which they belong became extinct from North America, where they may have been eliminated by more modern Unionacea (Fig.4).

The distribution pattern of fresh-water Prosobranchiates does not indicate clear effects of interfamilial competition.

Plecoptera. Like other aquatic insects (and also like fresh-water mites), the stone flies have terrestrial origin and the adults have the possibility to cross water-sheds; I consider them therefore less significant for fresh-water zoogeography than primary aquatic animals. Their general geographical distribution was presented by Illies in several papers [7-9]. The most primitive families have an "amphinotic" range, occurring in Australia, New Zealand and cold South America, while the modern families are Megageic, with maximum of differentiation in East- and South-East Asia and to a less degree in North America; quite few stone-flies live in Africa. Illies explained this distribution accepting a Gondwanian origin, respectively a Trans-Antarctic dispersal of the primitive families. But this distribution reflects also the effects of competition: in South America the primitive families were eliminated from the tropics by the modern families (or these ones prevented the northwards dispersal of the former), while the absence of modern families in Australia allowed the survival of the primitive ones.

★

Darlington [3], [4] concluded that all or most of the dominant families within the five classes of continental Vertebrates dispersed from "the Old World tropics". The distribution pattern of the few fresh-water groups discussed above proves that not all the Old World tropical zone was the dispersal center of the competitive groups, but only South East Asia (including partially also warm-temperate East Asia but excluding India). This is quite evident in the case of Cyprinoidei (which reached Africa only during the Pliocene), of Unionacea, of Diaptomidae and of modern Plecoptera; the only dominant group which apparently dispersed from Africa was the Anostracan family Streptocephalidae, while the dispersal center of secondary fresh-water crabs and prawns seems to have been the whole tropical zone, the Old and the New World families of fresh-water crabs having evolved independently from distinct marine ancestors [1], [2] and the same is true for prawns.

Far from having been a dispersal center for dominant fresh-water groups, Africa was colonized rather recently by these groups (e.g. Cyprinoidei and Unionacea) and has retained many archaic groups, especially among fishes (in no other continent do the Osteoglossomorpha and other archaic groups survive in such a great number as in Africa); also the Mutelacea live in Africa and in its southern top a few Phreatoicoidea; only the Parastacidae became extinct.

The dominant groups which evolved in and radiated from South-East Asia dispersed over the whole Megagea, a few ones (e.g. the Diaptomid Copepoda) over most of the World, including northern Australia. For some dominant groups of fresh-water Invertebrates of probable South-East or East-Asian origin, temperate eastern North America represents a center of secondary evolution and radiation: e.g. for Unionacea and for Cambarine crayfishes.

The Ostariophysi, which are the dominant group of fresh-water fishes, had a second center of radiation, quite independent from the South Asian: tropical South America (for Characoidei and 13 families of Siluriformes). But while the Cyprinoidei dispersed from South-East Asia throughout the whole Megagea (except Madagascar), the Characoidei reached only Africa and Central America. Already the wider range reached by the fish taxa original from South East Asia suggests that these are more successful than those of South American origin. This is confirmed by the fact that in Africa the Characoidei remained less speciose than the Cyprinoidei, in spite of their much older age. A quite different situation occurred in Central America, which was colonized rather recently by primary fresh-water fishes, both from South and from North America [11]; there are, in Central America, only 3 fish species of North American origin, as against 101 of South American descent [10]. The South American fish fauna proved, in Central America, to be much more competitive than the North American, which is a branch of the Megageic fauna, having thus a remote South Asian origin. This may be explained by the geographical position of Central America which lies very close to the radiation center of the South American fish fauna and quite far from South Asia. The North American fish fauna is only a pale reflexion of the very competitive South Asian one; although dominated by Ostariophysi (Cyprinoidei and Ictaluridae) of remote Asian origin, North America is, after Africa, the continent which retained the greater number of archaic fish families (Hiodontidae, Percopsiformes, Amiidae, Lepisosteidae). The aquatic Invertebrates of Central America have, on the contrary, North American affinities: all genera of fresh-water mussels, the crayfishes, apparently also all or most snails. This is a consequence of the above mentioned fact that North America is a center of secondary radiation for crayfishes and fresh-water mussels, but not for fresh-water fishes. The fresh-water crabs of Central America belong to two families occurring also in South America, one of which, Trichodactylidae, seems to be of Central American origin and to have colonized South America later on [2], like Poeciliidae and perhaps other secondary fresh-water fishes.

Most dominant and competitive groups of fresh-water animals have thus a Megageic or Holarctic distribution, with maximum of differentiation in South-East or East Asia. The non-competitive, "vanquished" groups range on the contrary mainly in southern continents; the present range of many of them is "Gondwanian" or "Notogeic" (= Transantarctic). The greatest number of archaic fresh-water fish families live (or survive) in Africa, only two of them in Australia, one also in tropical South America, none in Madagascar, New Zealand or cold South America (except eventually the Percichthyidae, if recognized as distinct family and not included within the marine Serranidae). The archaic groups of fresh-water

Invertebrates survive mainly in Australia (even in its tropical corner), New Zealand and cold South America, with maximum of differentiation in Australia (Phreatoicoidea, Parastacidae), quite few of them in Africa and India (Mutelacea, Phreatoicoidea).

In debating the conditions which favour the evolution of dominant animal groups, Darlington [3], [4] concluded that both tropical climate and large areas were favorable to this evolution. A combination of both conditions is present (and was present in Tertiary times) in the Old World tropics. Yet, as shown above, the evolution center for dominant fresh-water groups was only South East Asia (excluding even India) which is not exceedingly large but had (contrary to Africa) favorable tropical climate since the Mesozoic. Also tropical South America (the second evolution center for Ostariophysi) is rather large and had constantly a favorable climate, while eastern North America is the largest warm-temperate area on the northern hemisphere.

Many dominant groups of fresh-water animals actually need warm or at least temperate climate: for ex. Ostariophysi, fresh-water mussels, crayfishes became extinct or diminished under cold climate in areas in which they were once richly represented, such as Siberia, northern Canada, etc; fresh-water crabs became extinct even in Central Europe. Also Parabathynellidae are confined to the tropical and warm-temperate zone, while Streptocephalidae are mainly tropical and their representants in the temperate zone develop, unlike most other Anostraca, during the hot months. The Diptomidae seem to be the only dominant group of fresh-water animals living in arctic conditions, but its adaptation to these conditions develops quite slowly: Palaearctic species able to live in cold climate colonized North America, their offspring adapted to temperate, then to tropical climate, reaching tropical and partially temperate South America, but has not yet re-adapted to the cold climate of southern South America.

Many archaic, "vanquished" groups survive in temperate or cold conditions: lampreys, Boeckellidae, Parastacidae (partially!), archaic Plecoptera, even Esociformes and Percidae (both groups lived in Europe and North America also under tropical or subtropical conditions, but most of the recent species are cold-adapted).

★

The role of competition in the genesis of recent faunas was emphasized by the supporters of the permanence of continents, who believe that all animal groups at present confined to the southern land masses were once distributed also on the northern continents, where they were later eliminated (Dahl's "Verdrängungstheorie"). This view was adopted in recent years mainly by Darlington [3] [4], even when accepting continental drift for a remote period [5]. He thinks that the present range of these taxa does not reflect Mesozoic Palaeogeography, showing that some groups now confined to southern, mainly cold areas, have fossil representatives in northern continents (*Nothofagus*) and he believes the same is true for groups without fossil record.

At least one group of fresh-water animals whose distribution is mainly "Transantarctic" has left a relict colony in North East Asia: the

Boeckellidae. Osteoglossomorpha too have a few recent and fossil representatives on the northern hemisphere, in spite of their predominant Gondwanian range. The distribution of both groups can thus be explained by dispersal through the North. But Parastacidae, Mutelacea, the snail family Ampullaridae (South America, Africa, South Asia, with fossil record only within the present range) indicate rather a Gondwanian, or at least an Africano-Brasilian dispersal route. For Phreatoicoidea and for the Amphipod family Hyalellidae (Australia, New Zealand, South America, with infiltrations in North America) one can assume independent colonization from the sea. Transantarctic dispersal can be assumed for archaic Plecoptera, for Stygocaridacea and eventually for Hyriidae. The last named family may have reached South America from the North, as accepted by Parodiz [14], but in Australia it probably arrived from South America, by an Antarctic route (a dispersal from Asia is quite improbable, because of the absence of fresh-water mussels in the Lesser Sunda islands). Myers [11] demonstrated that Characidae are recent intruders in Central America and their occurrence in South America and Africa cannot be explained by an independent colonization from the North: being a competitive group, they could not have become extinct in Central America, if once present there. I have an objection in this problem. The sister-group of Characoidei are the Cyprinoidei, whose dispersal-center was not India, but South East and even East Asia, e.g. areas which originally belonged to the northern land-mass, not to Gondwana. The common ancestors of Characoidei and Cyprinoidei must once have lived also in the northern continents, dispersed over most of the world (except Australia), became extinct on most of range, except South America (or Africa) and South East Asia, where they evolved in Characoidei and Cyprinoidei, getting in the same time the characters which made them competitive. Remote, non competitive ancestors of Characoidei may once have lived also in Central America. But the recent Characoidei from South America and from Africa are too closely related for accepting an independent origin from some remote, not competitive northern ancestors and a direct dispersal, by contact between both continents, is quite probable.

Among the animal groups with mainly or exclusively "Gondwanian" range, some probably dispersed by direct continental contact (e.g. continental drift), others may have been eliminated in the north by more competitive groups. Both categories are archaic, noncompetitive groups: it is natural that Mesozoic groups (the only which could use continental drift) cannot compete with modern groups which evolved during Tertiary times in South East Asia. I think the indisputable fact that the modern groups, with South-East or East-Asian origin have the tendency to colonize the whole world, eliminating the older groups, is not incompatible with a Gondwanian, respectively an Africano-Brazilian or Notogeic origin and dispersal of some (not all!) archaic groups. It is even possible that in Mesozoic times Gondwana was the main center of differentiation and dispersal for dominant animal groups.

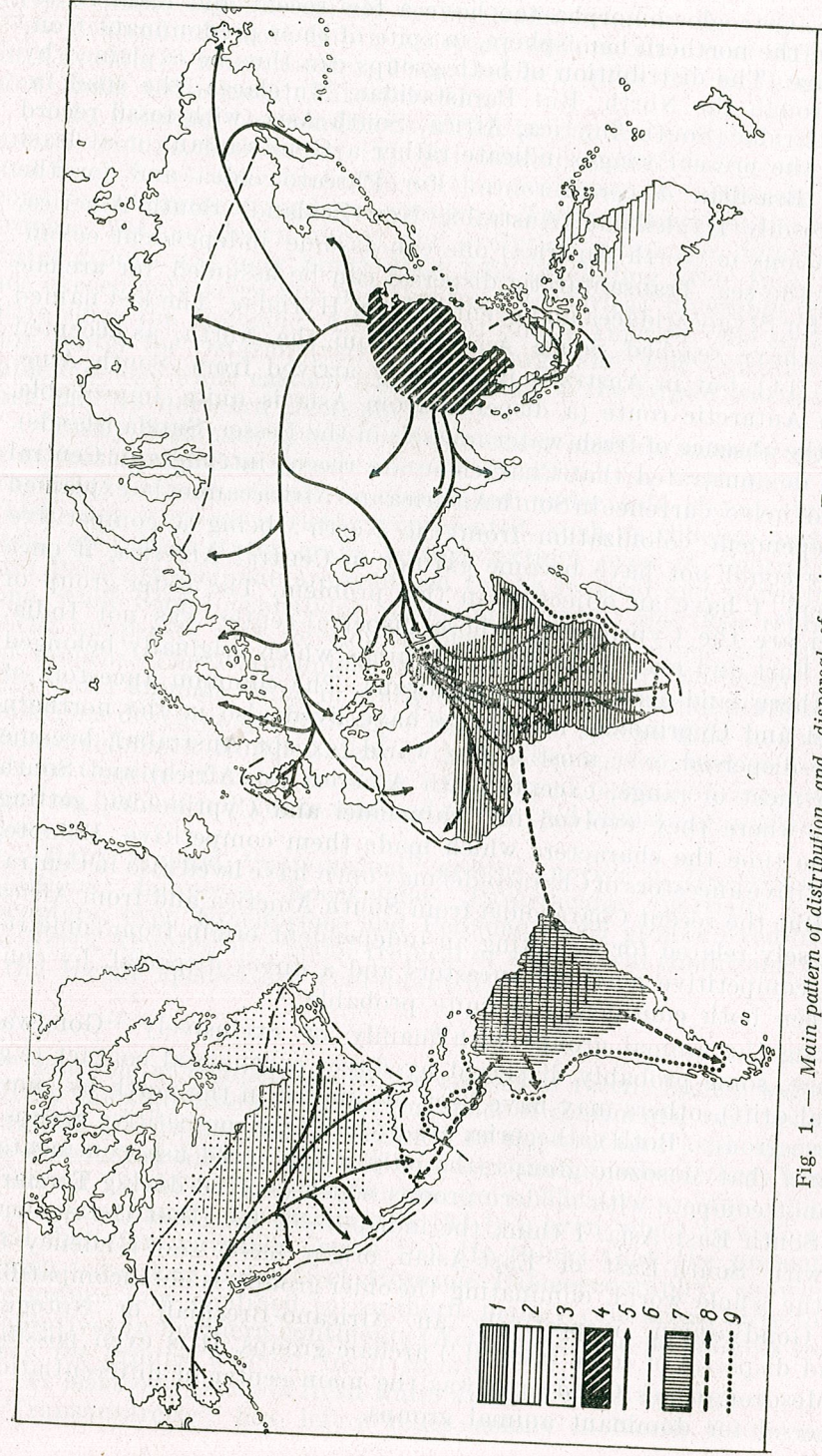


Fig. 1. — Main pattern of distribution and dispersal of primary Fresh-water Teleosts. 1. Osteoglossomorpha, numerous; 2. Osteoglossomorpha, rare; 3. Other archaic Teleosts (Denticipitidae, Kneriidae, Phractolaemidae, Umbridae, Percopsiformes); 4. Dispersal center of Cypriniformes; 5. Dispersal routes of Cypriniformes; 6. Limit of Cypriniformes; 7. Dispersal center of Characiformes; 8. Dispersal routes of Characiformes; 9. Limits of Characiformes.

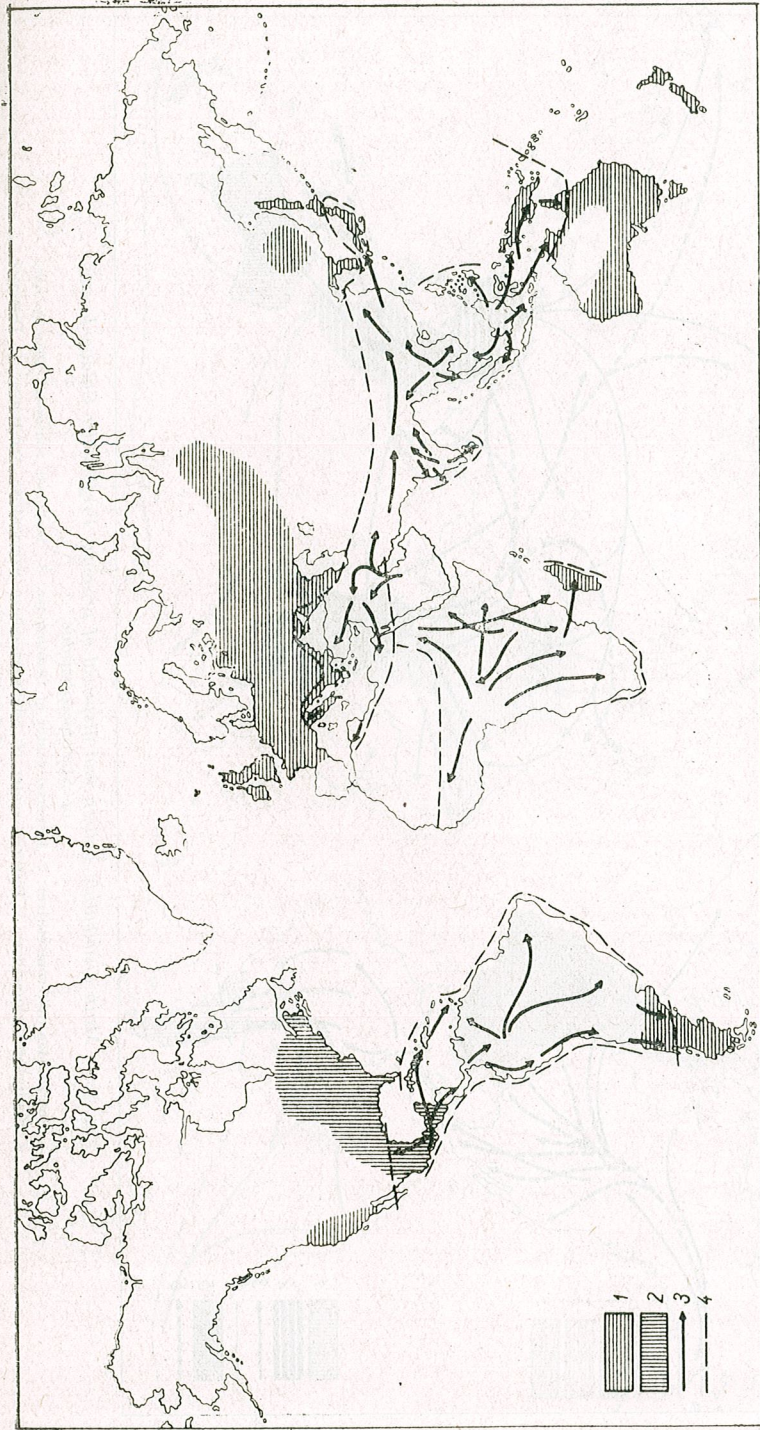


Fig. 2. — Distribution and dispersal of Crayfishes and of Fresh-water Crabs. 1. Non-competitive Crayfishes (Astacinae, Cambaroidinae, Parastacidae); 2. Competitive Crayfishes (Cambarinae); 3. Dispersal routes of Freshwater Crabs; 4. Limits of Freshwater Crabs.

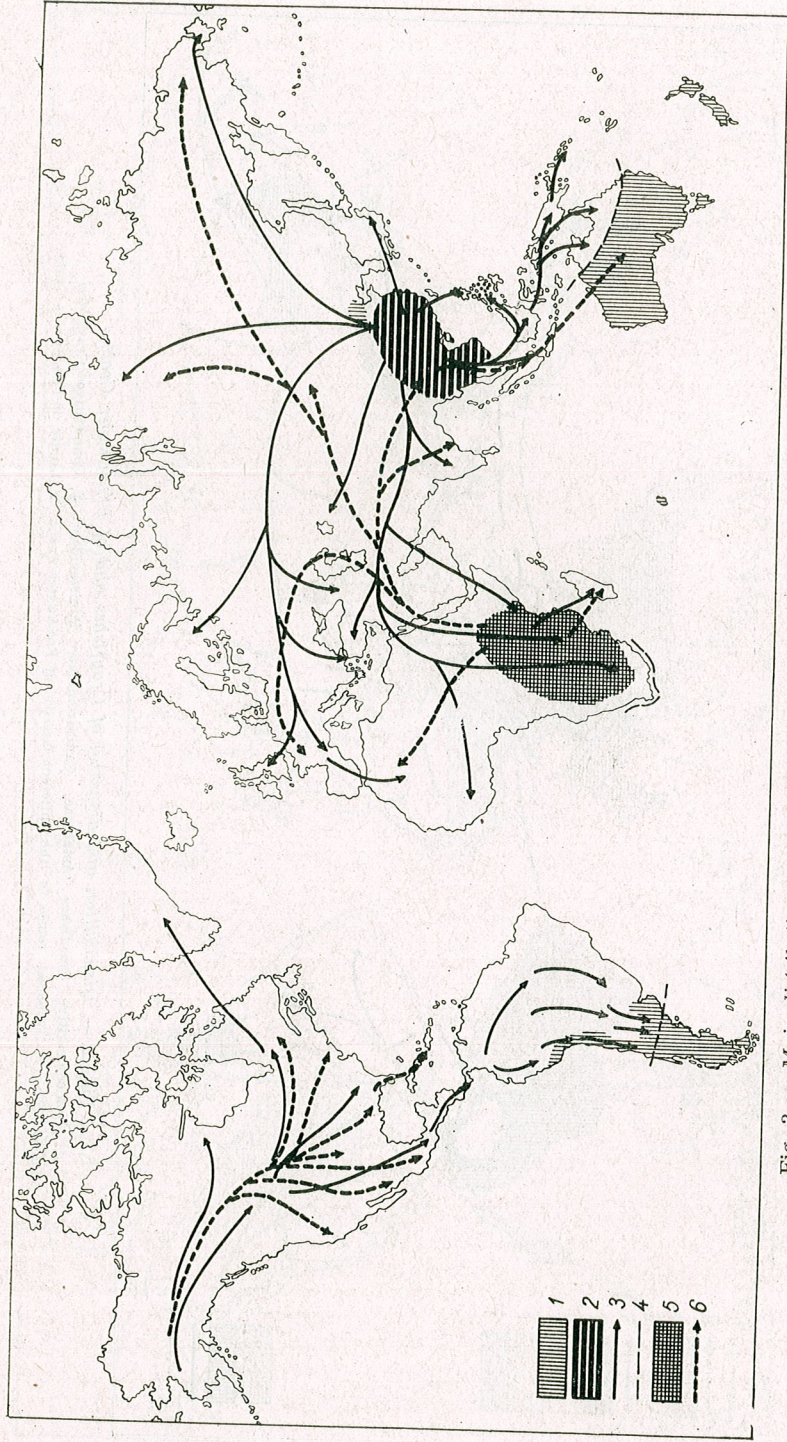


Fig. 3. — Main distribution pattern and dispersal of primary Fresh-water *Catanoidea* and of *Streptocephalidae*.

1, Boeckellidae (non-competitive); 2, Dispersal center of Diaptomidae; 3, Dispersal routes of Diaptomidae; 4, Limits of Diaptomidae; 5, Dispersal center of Streptocephalidae (competitive Anostraca); 6, Dispersal routes of Streptocephalidae

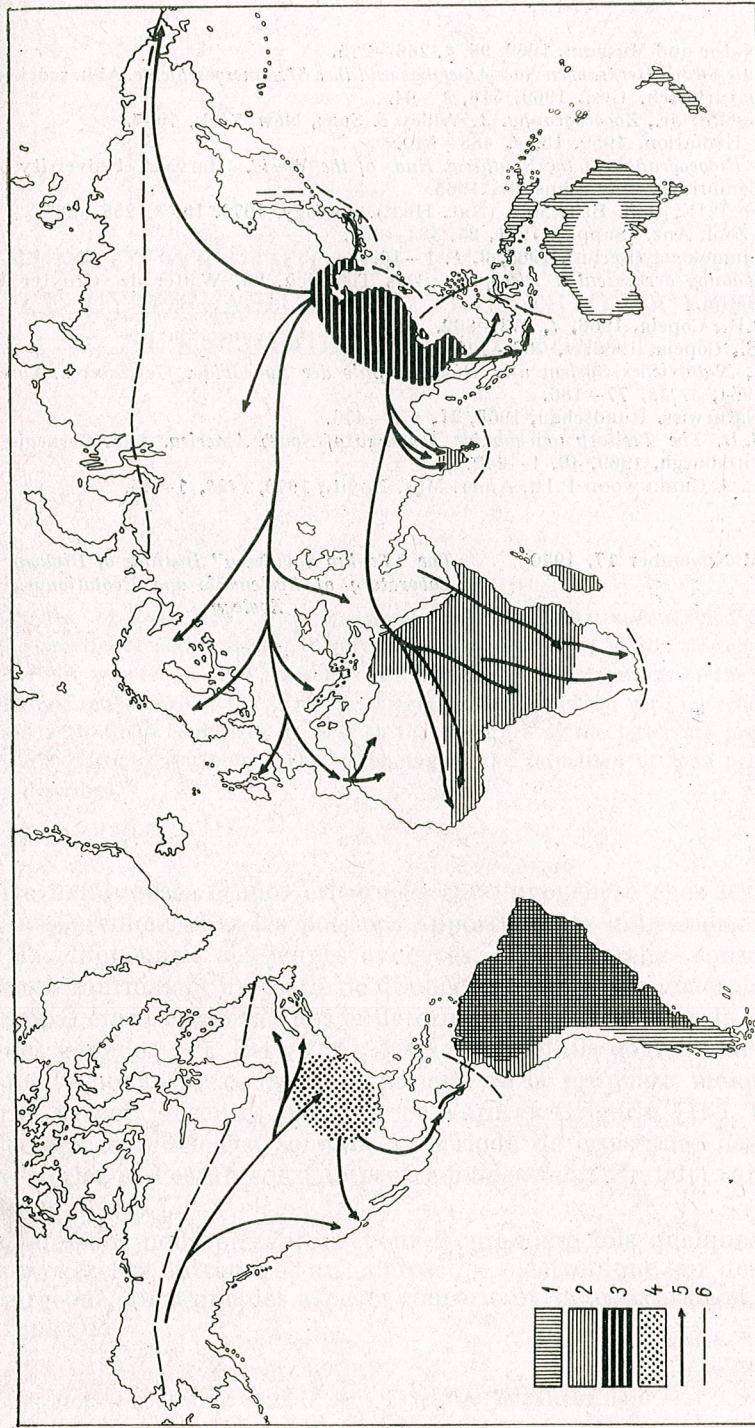


Fig. 4. — Distribution and dispersal of Fresh-water Mussels
1, Hyriidae (non-competitive); 2, Murelidae (Murelidae and Aetheriidae, non-competitive); 3, Main dispersal center of competitive Unionacea; 4, Secondary dispersal center of competitive Unionacea; 5, Dispersal routes of Unionacea; 6, Limits of competitive Unionacea.

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Received November 17, 1970

The "Traian Săvulescu" Institute of Biology
Laboratory of Systematic and Evolutionary
Zoology

DONNÉES CYTOCHIMIQUES ET ULTRASTRUCTURALES SUR LE CYTOPLASME DES JEUNES OVOCYTES CHEZ L'ESTURGEON *HUSO HUSO* L.

PAR

MARIA CALOIANU-IORDĂCHEL

Oocytes of *Huso huso*, with a diameter of 150—250 μ were studied during the previtellogenetic period. An intensive accumulation of alkaline proteins and of RNA was found. The ribosomes are not bound to formations of the endoplasmic reticulum but free. The enrichment of the cytoplasm with mitochondria and with Golgi complexes as well as the structure of the latter are probably characteristic of sturgeons. The beginning of the formation of zona pellucida is described.

De nombreuses études effectuées sur l'ovogenèse chez les vertébrés et particulièrement chez les poissons apportent des indications précieuses sur le développement des jeunes ovocytes. Les recherches consacrées aux esturgeons fournissent une série de données histologiques générales, l'objectif principal étant constitué par la détermination des étapes de développement des gonades [4], [24], [25], [26]. Des détails concernant les modifications du noyau au cours de la période de la prophase méiotique chez la sterlet furent présentés par Ionescu-Varo et Grigoriu [16]. Une étude détaillée sur les nucléoles pendant la période de croissance des ovocytes chez le sterlet et l'esturgeon (*Acipenser güldenstädti* Brandt) fut effectuée par Raikova [21].

Ci-dessous nous présentons pour la première fois quelques résultats de nos recherches entreprises au microscope électronique sur des ovocytes des esturgeons, ainsi que des aspects concernant la constitution chimique du cytoplasme.

MATÉRIEL ET MÉTHODE

Des portions d'ovaire ont été prélevées des exemplaires de grand esturgeon (*Huso huso* L. Fam. Acipenseridae) de différents âges soit de l'embouchure du Danube, soit de la Mer Noire, à une distance de quelques kilomètres du littoral. Pour l'étude histologique et histo-chimique des jeunes ovocytes on a utilisé les fixateurs classiques : Bouin, Sussa, Zenker, Carnoy, Champy, formol neutre.

Pour l'étude au microscope électronique la fixation fut réalisée soit au tétr oxyde d'osmium directement, soit après une préfixation à la glutaraldéhyde dans le tampon au cacodylate ou tampon phosphaté. L'inclusion a été faite dans le vestopal W ou dans l'araldite. Les coupes recueillies sur des grilles sans membranes, colorées au citrate de plomb (Reynolds, 1963), furent étudiées au microscope JEM-7.

RÉSULTATS

Chez le grand esturgeon, les jeunes ovocytes sont disposés uniformément dans le stroma de l'ovaire (fig. 1) ayant comme caractéristique des étapes semblables de développement.

Les jeunes ovocytes au diamètre de 150—200 μ présentent un noyau grand, vésiculeux, à contour régulier et riche en nucléoles. Le nucléoplasme clair est coloré faiblement à «Azan» et légèrement en violet, à picro-indigo-carmin. La réaction Feulgen est négative. Les nucléoles, de dimensions différentes, orientés dans leur majorité au long de la membrane nucléaire, sont APS positifs (fig. 2, 3) et présentent une réaction intense au bleu bromophénol, Millon acétique, Millon sulfurique, à l'alloxane-Schiff, réaction spécifique aux protéines. Les colorations des nucléoles et du cytoplasme avec de l'hématoxyline, bleu de toluidine, vert-méthyl-pironine, vérifiées aussi par le traitement avec de la ribonucléase, marquent une présence abondante en ARN (fig. 4.5).

Au microscope optique le cytoplasme, intensément coloré à l'azocarmin, est uniforme et à aspect faiblement granulaire. Une série de réactions spécifiques (Millon, bleu bromophénol, bleu bromophénol mercurique, fast green FCF, jaune naphтол) mettent en évidence une basophilie intense du cytoplasme due en grande mesure aux protéines basiques. De même, au microscope électronique le cytoplasme présente de nombreux ribosomes dispersés, non liés à d'autres structures. Les organites cellulaires sont faiblement représentés. De petites mitochondries arrondies, pauvres en cristae, ainsi que les corps de Golgi sont dispersés dans le cytoplasme. À ce moment, d'autres formations du réticulum endoplasmique sont absentes. La membrane nucléaire présente des pores régulièrement disposés et de dimensions égales (fig. 6). Dans le cytoplasme périnucléaire on remarque une série de membranes parallèles à la membrane nucléaire avec laquelle elles viennent en contact direct (fig. 7). À la surface de l'ovocyte, de petites évaginations sans orientation radiaire forment des plages discontinues étroitement disposées au-dessous de la surface des cellules folliculaires (fig. 8). Les cellules folliculaires sont peu nombreuses et très aplaties. À l'extérieur elles sont délimitées par la couche périfolliculaire. Fortement

développée, cette couche présente une réaction APS intensément positive. Au-dessus on distingue des cellules conjonctives qui forment la thèque (fig. 9).

Le contour du noyau des ovocytes, au diamètre de 200—250 μ , est irrégulier. Dans la zone périnucléaire du cytoplasme, les membranes lisses, semblables à la membrane nucléaire, sont nombreuses. Autour de la zone périnucléaire, les mitochondries rondes ou fortement allongées (fig. 10) forment des groupes compacts. Dans cette zone on trouve aussi des dictyosomes. Vers la périphérie de l'ovocyte, on observe dans le cytoplasme l'apparition d'un grand nombre de vésicules formant deux zones concentriques spécifiques (fig. 11). L'augmentation en organites du cytoplasme de l'ovocyte apparaît très évidente dans la zone corticale (fig. 12). Les mitochondries disposées à la proximité de la périphérie de l'ovocyte ont encore un nombre relativement réduit de cristae, marquant en même temps dans leur matrice un enrichissement en granules osmiophiles. Les corps Golgi se multiplient considérablement et présentent des formes variées. Dans le cytoplasme on ne rencontre pas, à ce stade, des formations tubulaires du réticulum endoplasmique.

Entre la surface de l'ovocyte et celle des cellules folliculaires, dont le volume continue à augmenter, apparaissent de petites plages ou des microvilli qui baignent, dans une masse de substance homogène, la substance péllucide. Le cytoplasme des cellules folliculaires accuse également un enrichissement en organites. À cet état de développement de l'ovocyte apparaissent dans le cytoplasme les premières inclusions. Les inclusions, légèrement osmiophiles, sont rondes et disposées soit à la périphérie de la zone périnucléaire, soit dans la zone corticale du cytoplasme (fig. 13). Elles sont situées à la proximité des paquets de mitochondries ou dans le cytoplasme dénué d'organites. Présentant, en même temps, une réaction positive aux protéines ainsi qu'aux lipides, on conclut qu'elles sont des complexes lipoprotéiques.

DISCUSSIONS

Les ovocytes au diamètre de 150—200 μ , dont nous avons fait la remarque, appartiennent au stade où les modifications caractéristiques à la prophase méiotique sont achevées et où le processus d'accroissement cytoplasmique s'accroît.

La dispersion des chromosomes dans le noyau, ainsi que la réaction Feulgen négative sont considérées par Ghinzburg [13], Grégoire [14], etc. comme le moment de passage des ovocytes du petit accroissement au grand accroissement. D'autres auteurs le définissent comme un passage de la période synaptique à celle d'accroissement cytoplasmique [19] ou de prévitellogenèse [22]. Nous optons pour la terminologie de Raven.

Caractéristique pour les jeunes ovocytes, au début de la période de prévitellogenèse, est une intense basophilie du cytoplasme, due à l'accumulation de l'ARN et des protéines basiques. Ce processus représente un phénomène général, observé chez les ovocytes de divers groupes d'ani-

maux : nombreuses espèces de Mollusques, de Prochordés, des Mammifères (souris) [6], des Amphibiens [6], [10], [17], des Échinodermes [3], [9], [12], [23] et des Crustacés [1]. Chez les esturgeons, ainsi que chez d'autres groupes des invertébrés et des vertébrés, il résulte que les jeunes ovocytes sont riches en protéines basiques. Si l'ARN n'est pas, au préalable, enlevé, leur blocage par désamination n'est que partiel, ce qui signifie la liaison des protéines basiques à l'ARN dans un complexe au niveau de ribosomes. Selon Afzelius [1] et Kemp [17] la basophilie du cytoplasme est attribuée au réticulum endoplasmique sur lequel sont fixés de nombreux ribosomes. Chez le grand esturgeon, le réticulum est très réduit et les ribosomes sont dispersés dans le cytoplasme. La variation de la concentration en ARN et en protéines basiques du cytoplasme des jeunes ovocytes est la même, autant dans le cas des esturgeons que chez d'autres groupes d'animaux. Cette variation peut être poursuivie conformément à la première et la seconde périodes établies par Lechenault [18], résultats de ses études cytophotométriques chez les Oligochètes et les Némertiens. En considérant l'intensité de la réaction des jeunes ovocytes d'esturgeons, par l'abondance des protéines basiques, on constate que la réaction s'approche plutôt de celle de certains invertébrés : Ascidies, Mollusques, certains Échinodermes (Crinoïdes et Oursins irréguliers) [7], [8], [9].

Un phénomène généralement rencontré pendant le développement des ovocytes c'est l'enrichissement du cytoplasme en organites et leur migration vers la périphérie. Chez le grand esturgeon on observe une grande agglomération des corps golgiens dans la zone corticale de l'ovocyte, lesquels peuvent être rencontrés tout aussi bien dans le reste de la masse du cytoplasme en formes diverses et à différentes phases de division. Les mitochondries deviennent moins rondes, plutôt allongées ou vésiculeuses et accumulent des granules intensément osmiophiles dans leur matrice claire. Différemment des téléostéens, le cytoplasme de l'ovocyte d'esturgeon est pauvre en formations réticulaires. La présence des paquets de mitochondries et des corps golgiens dans l'entier cytoplasme, au cours de la période de prévitellogenèse, constitue la remarque d'une structure différente, observée par Raikova [21] et Votinov [27] sur des coupes étudiées au microscope optique, mais restée sans interprétation. La formation d'une zone riche en vacuoles golgiennes et réticulaires, délimitée dans le cytoplasme de l'ovocyte est tout aussi caractéristique.

Les premières inclusions lipoprotéiques apparaissent, chez le grand esturgeon, dans le cytoplasme de l'ovocyte. Leur formation n'est pas liée à aucun élément structural comme chez *Rana* [17] et d'autres groupes d'invertébrés [22]. Leur apparition est interprétée comme le résultat du processus de condensation et de groupement des lipides, lesquels se trouvaient antérieurement dispersés dans le cytoplasme. Chez les esturgeons, le développement des ovocytes a lieu au cours d'une période assez étendue pendant laquelle l'organisme accumule un riche matériel énergétique. Ceci se reflète au niveau de la gonade par l'accumulation d'une quantité appréciable de graisse, fait qui détermina plusieurs chercheurs [24], [25], [26], etc. à différencier le II^e stade gras dans l'échelle du développement des gonades.

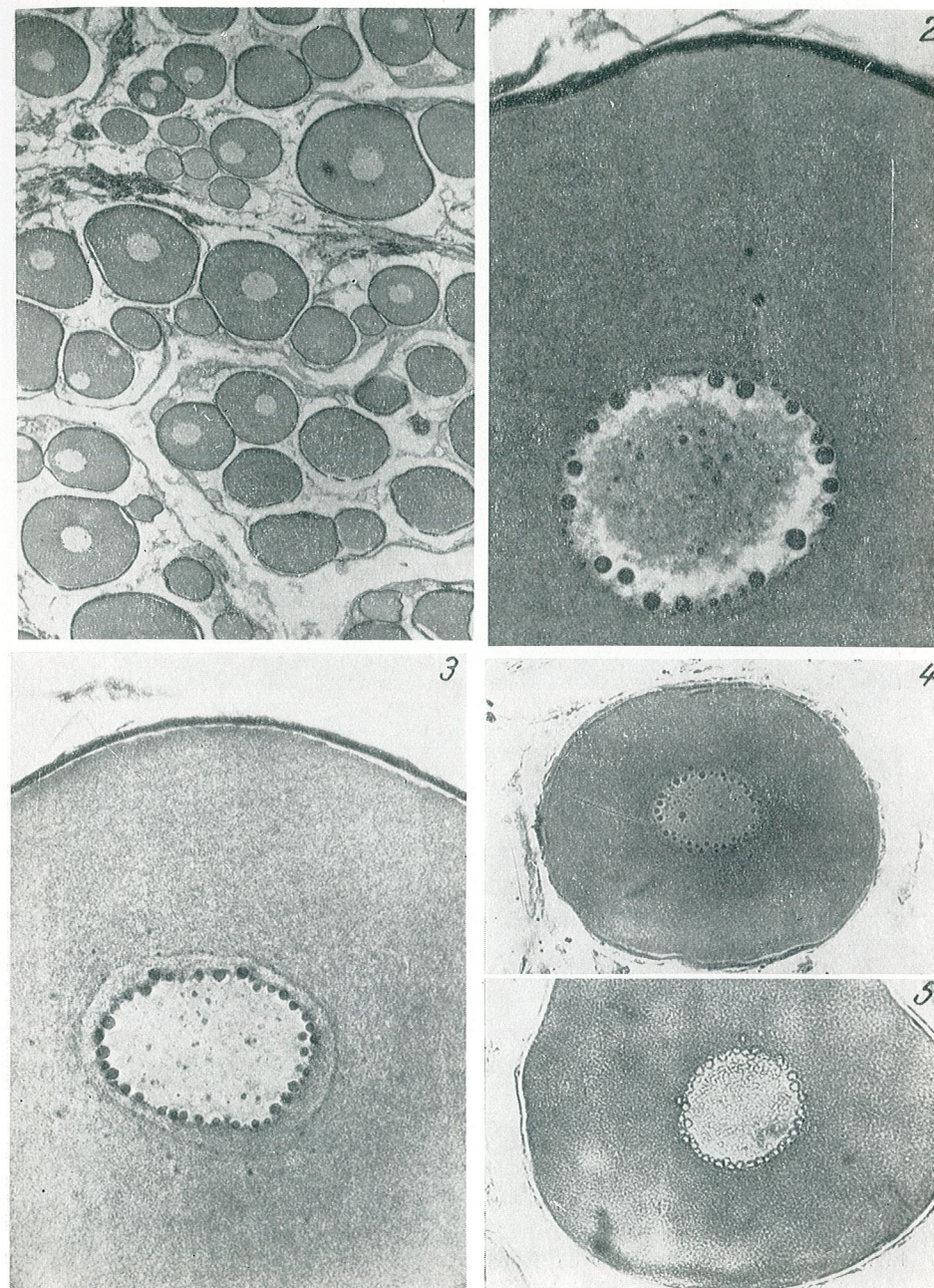


Fig. 1. — La structure de l'ovaire de grand esturgeon dans la période de prévitellogenèse. APS picro-indigo-carmin. Oc. 8, Ob. 6,3.
 Fig. 2. — La disposition périphérique des nucléoles. APS, picro-indigo-carmin. Oc. 6, Ob. 65.
 Fig. 3. — La formation de la zone périnucléaire. APS picro-indigo-carmin. Oc. 6, Ob. 65.
 Fig. 4. — Nucléoles colorés au vert-méthyl-pironine (VMP). Oc. 6, Ob. 40.
 Fig. 5. — Aspect des nucléoles et du cytoplasme colorés au VMP, après le traitement à la ribonucléase. Oc. 8, Ob. 40.

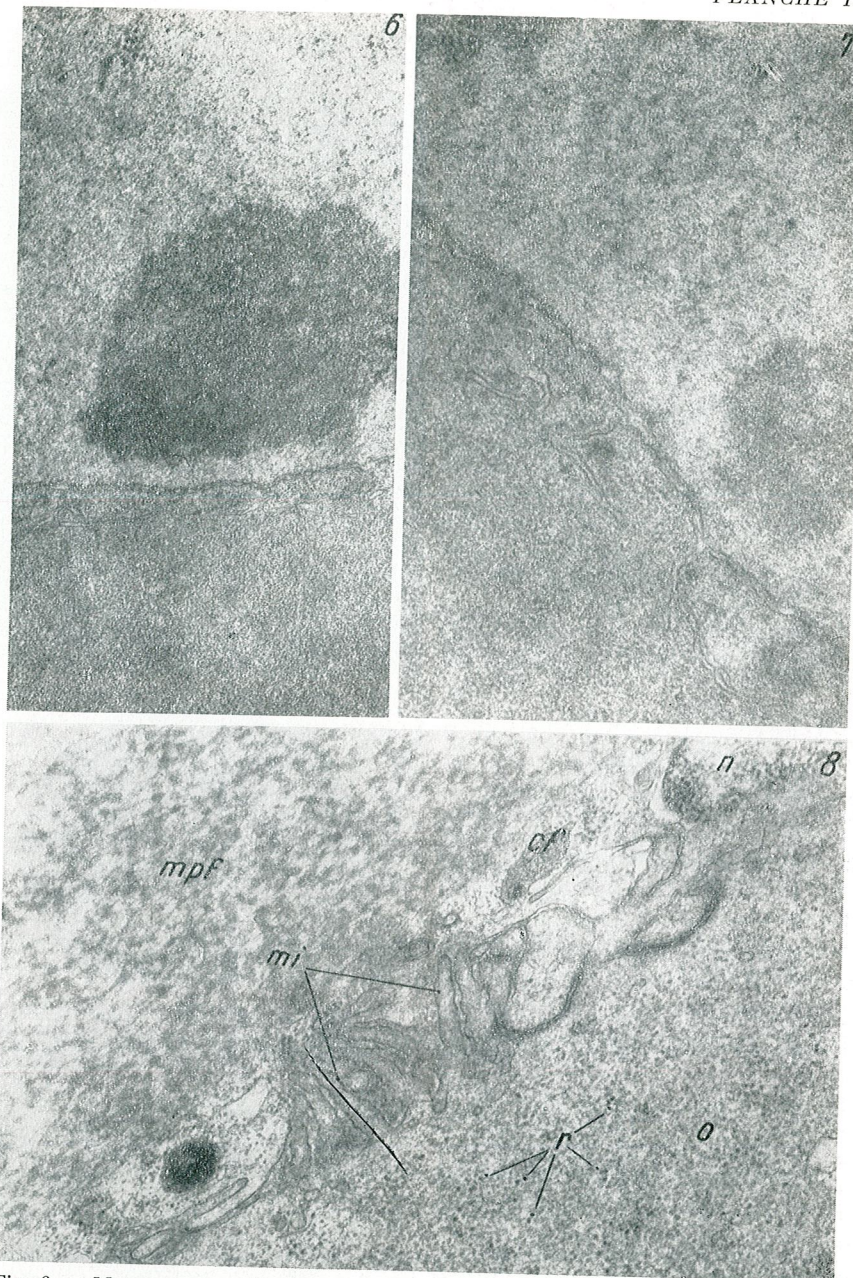


Fig. 6. — Membrane nucléaire de jeune ovocyte. On remarque le cytoplasme pauvre en organites cellulaires; seulement autour du noyau se forment des membranes pareilles à celle de la membrane nucléaire. \times 49800.
 Fig. 7. — Membranes lisses ayant des liaisons directes avec la membrane nucléaire. \times 52500.
 Fig. 8. — La zone corticale du cytoplasme de l'ovocyte \times 32800.
 Abréviations.
 cf, cellule folliculaire; g, corps golgiens; i, inclusion; m, mitochondries; mi, microvilli; mpf, membrane pérfolliculaire; n, noyau; o, ovocyte; r, ribosomes; ti, thèque; v, vacuoles golgiennes.

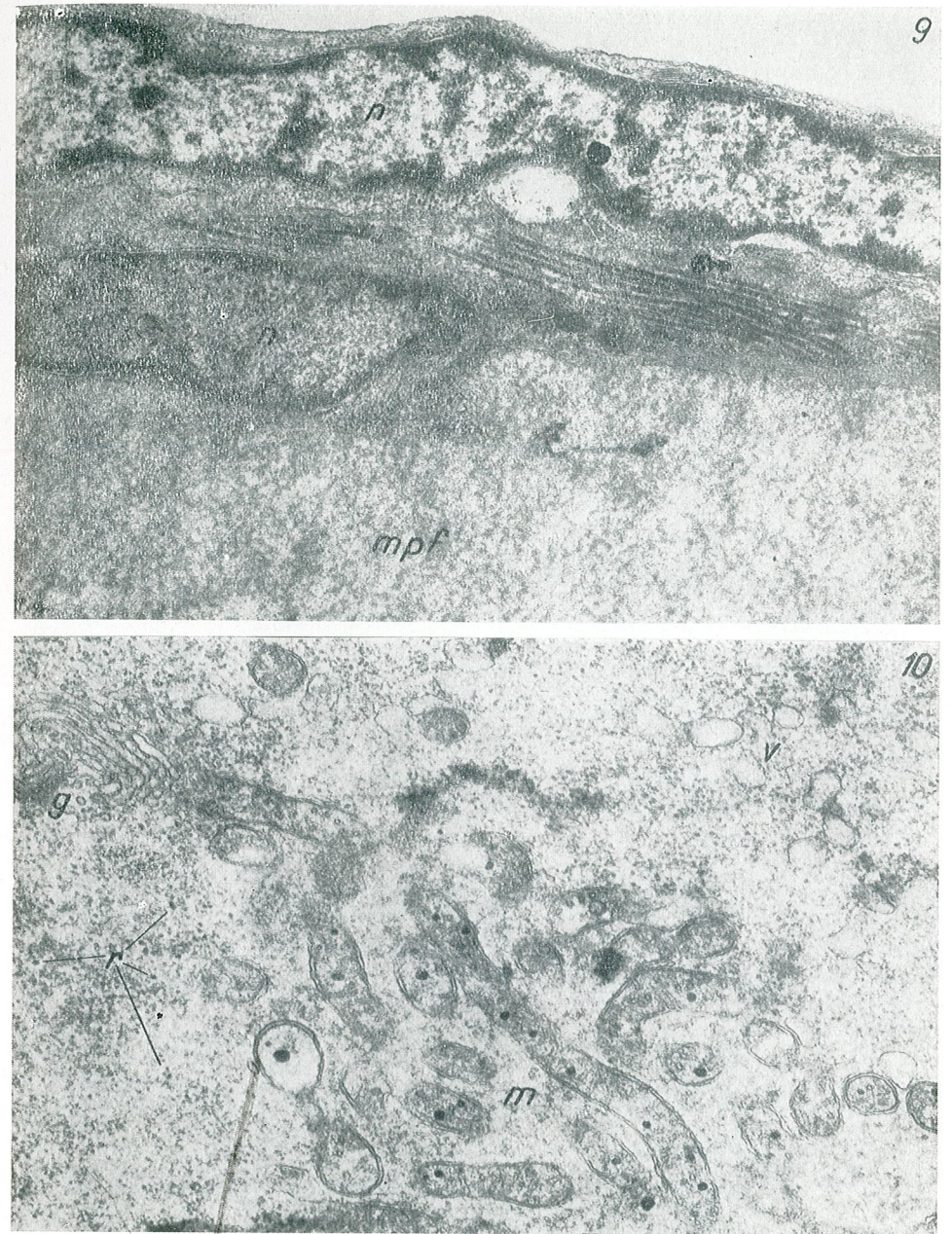


Fig. 9. — La structure fine des membranes externes de l'ovocyte. \times 37400.
 Fig. 10. — La disposition compacte des organites cellulaires à la proximité de la zone périnucléaire. \times 30500.

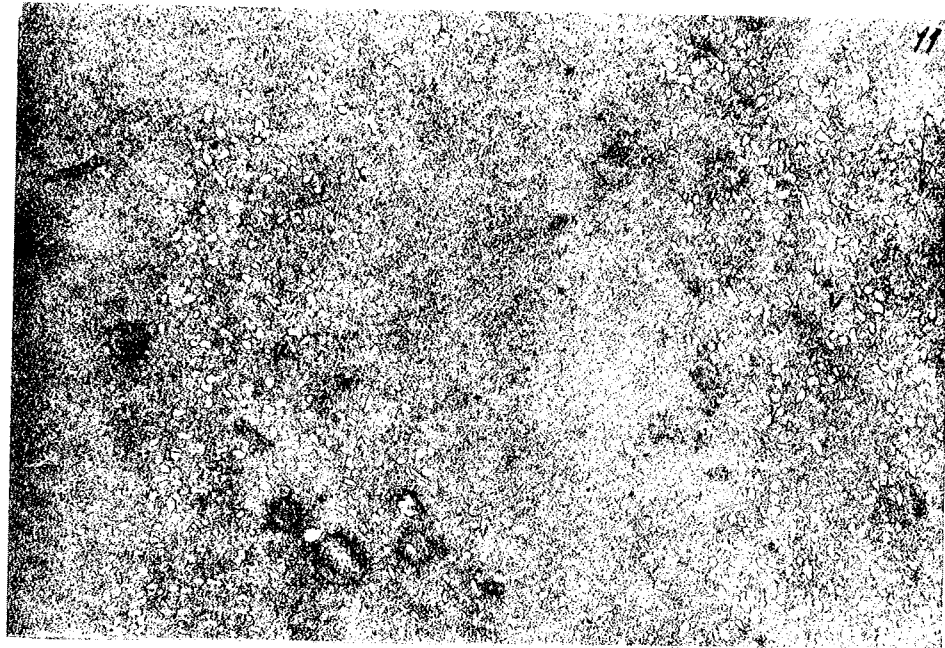


Fig. 11. — Cytoplasme d'un ovocyte pauvre en organites. On remarque des ribosomes libres et des vacuoles golgiennes qui ont une répartition spécifique, ainsi que des inclusions dans le cytoplasme. $\times 10\ 800$.
 Fig. 12. — L'enrichissement en organites du cytoplasme cortical de l'ovocyte. $\times 18\ 000$.

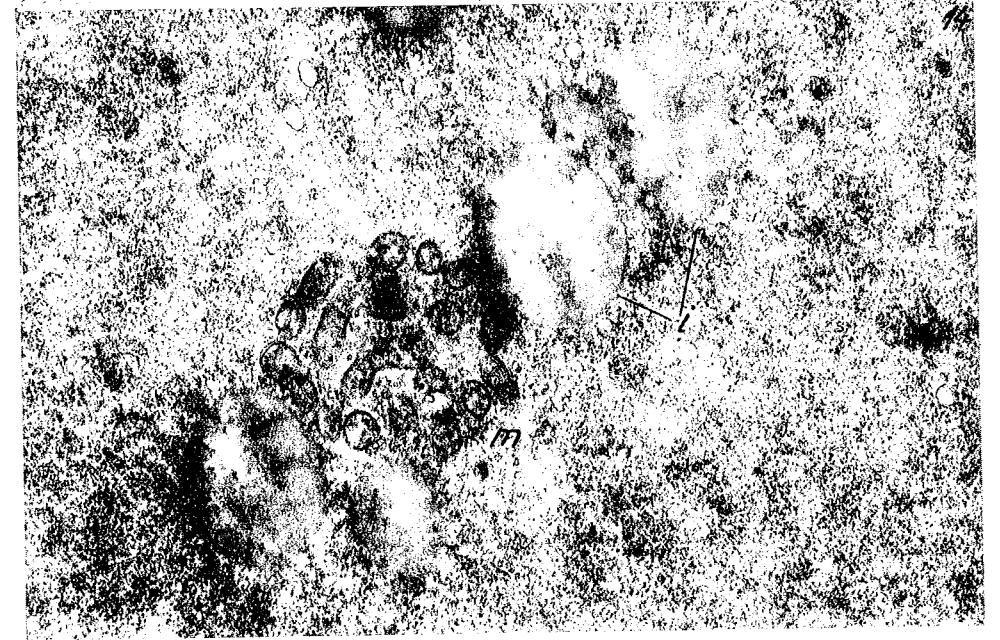
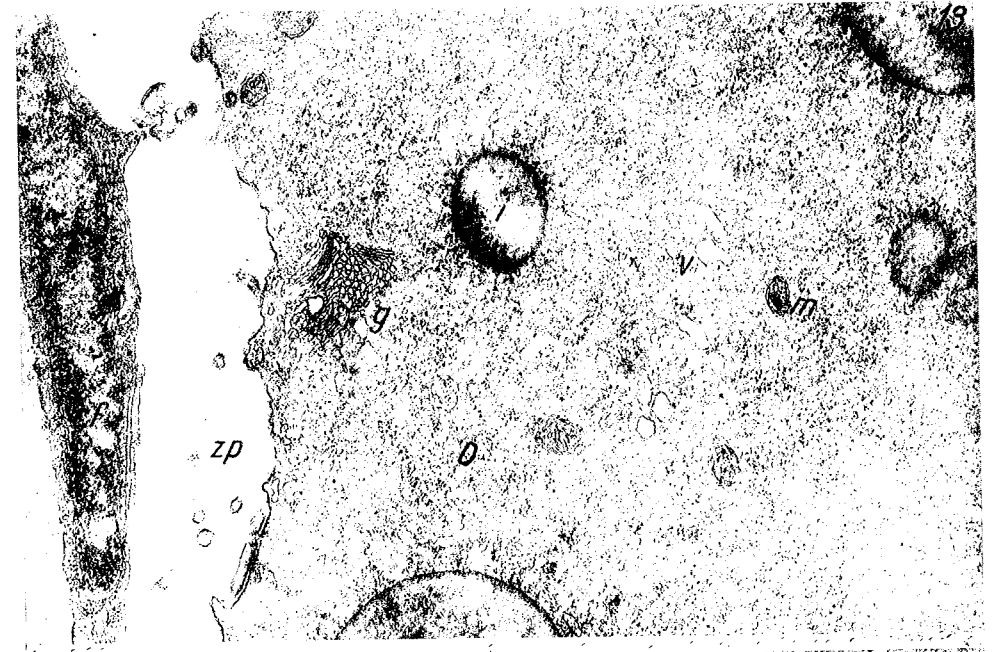


Fig. 13. — La zone corticale de l'ovocyte à un stade de développement plus avancé. Le cytoplasme de l'ovocyte qui continue à avoir peu d'organites; on remarque l'apparition de la zone pellucide. $\times 165\ 000$.
 Fig. 14. — Inclusions dans le cytoplasme profond de l'ovocyte. $\times 20\ 000$.

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Reçu le 23 décembre 1970

Institut de Biologie
« Traian Săvulescu »

L'APPAREIL SÉRICIGÈNE CHEZ *PARDOSA LUGUBRIS*
(LYCOSIDAE, ARANEAE)

PAR

ELENA TRACIUC

In this study of the sericigen apparatus in *Pardosa lugubris*, four types of sericigen glands and their histological structure during the egg-laying period are described.

La littérature offre peu de données sur les glandes séricigènes chez les Lycosidae (Richter [3], [4]); c'est justement ce qui nous a imposé les études ci-présentes. Par contre, la littérature de spécialité comprend un grand nombre de mémoires concernant les glandes séricigènes de certaines familles d'Araignées, dont [1], [2], [3] offrent des données particulièrement amples sur les glandes séricigènes surtout chez les araignées bâtissant leur toile.

MATÉRIEL ET MÉTHODES

On a utilisé des individus de *Pardosa lugubris* capturés dans la forêt de Pustnicu (Bucarest). Des exemplaires femelles ont été fixés ou bien après la mue suivant l'accouplement, ou bien pendant la construction du cocon. Les fixations ont été faites dans du Zenker et Bouin. Les sections sériées, de 5-7 μ d'épaisseur, ont été colorées à Azan trichromique et hémalun-éosine-orange G. D'après les sections sériées on a reconstitué la forme des glandes, le trajet des canaux et le type de filières sur lesquelles ils s'ouvrent. En tant que contrôle, on a fait des dissections suivies par la clarification au lactophénol pour mieux observer les fusules. La structure de chacun des types de glandes est illustrée sur des microphotographies. Les photographies des filières ont été prises dans du lactophénol selon une méthode originale.

DESCRIPTION DES GLANDES SÉRICIGÈNES

L'appareil séricigène consiste en deux parties : le corps des glandes avec leur canaux excréteurs et les filières. Nos études traitent du complexe séricigène tout entier. Les types de glande ont été considérés selon. M. Dumitresco (1941). Chez l'espèce étudiée on a trouvé quatre types de glandes :

- glandes ampullacées
- glandes tubuliformes } grandes
- glandes aciniformes } petites
- glandes piriformes

Les glandes agrégées et les glandes lobées manquent chez l'espèce étudiée.

LES GLANDES AMPULLACÉES

Ont la forme caractéristique, consistant en une portion basale dilatée qui se continue par un court prolongement. Chez *P. lugubris* les glandes ampullacées sont en nombre de huit, tout en se distinguant par leur grandeur et par leur position. Les deux glandes latérales ont un corps glandulaire grand et un canal long. Elles dépassent le milieu de l'abdomen. Les autres six glandes sont médianes, ayant le corps glandulaire plus réduit et les canaux plus courts.

Le corps glandulaire est constitué par un épithélium prismatique (Pl. I, 1). Les canaux de ces glandes sont formés d'un épithélium prismatique qui délimite une lumière plus vaste que celle des autres types des glandes séricigènes. L'épithélium glandulaire ne produit qu'un seul type de sécrétion, notamment une sécrétion basophile. Ces glandes ont, après la dernière mue des femelles et jusqu'à la fabrication du cocon, une grande taille et la sécrétion permanente.

LES GLANDES TUBULIFORMES

Chez les Lycosidae, ces glandes appartiennent à deux classes, distinctes aussi bien par leur forme que par leur structure. L'un des types consiste en glandes placées sur la ligne médiane de l'abdomen et s'étendant jusqu'à la proximité de la fente génitale. Ce sont des glandes tubuliformes grandes, à épithélium plat (suivant une sécrétion intense), dont la lumière est particulièrement dilatée et la sécrétion très abondante pendant la fabrication du cocon, période où elles sont les glandes les plus actives du complexe séricigène (Pl. I, 2).

Latéralement par rapport à ce groupe de glandes tubuliformes grandes, on rencontre deux groupes de glandes tubuliformes dont le corps glandulaire est plus petit, les canaux plus courts. Elles sont ondulées de forme et d'emplacement latéral et postérieur par rapport aux glandes tubuliformes

grandes. Elles sont formées d'un épithélium prismatique et d'une lumière restreinte (Pl. I, 3).

En sus des différences de forme et de structure, ces deux types de glandes diffèrent aussi par l'affinité de leur sécrétion pour les colorants. Ces glandes manquent d'activité jusqu'à la fabrication du cocon. Chez les deux types de glandes, l'épithélium sécrète le même type de sécrétion tout au long du trajet glandulaire.

LES GLANDES ACINIFORMES

On en rencontre un grand nombre, en deux groupes symétriquement placés par rapport à la ligne médiane de l'abdomen, en dessous du tégument ventral. Le corps glandulaire est ovoïde (du type aciniforme). Chez la plupart, la sécrétion est de deux types : la partie apicale de la glande se caractérise par une sécrétion éosinophile, tandis que la partie basale par une sécrétion basophile (Pl. I, 4). Quelques-unes des glandes aciniformes ont un seul type d'épithélium dans leur corps glandulaire et un seul type de sécrétion à affinité pour l'éosine (Pl. I, 5). Les canaux de ces glandes présentent la particularité suivante : ceux-ci n'étant pas uniformes en longueur, les glandes ont une disposition en échelle, les unes se trouvant dans les filières, tandis que d'autres s'étendent jusqu'au bon milieu de l'abdomen (Pl. I, 4-5).

L'activité de ces glandes est permanente. Les femelles étudiées pendant toutes les périodes (même pendant les stades de nymphe) présentaient ces glandes en état de fonction.

LES GLANDES PIRIFORMES

Elles sont en nombre plus petit que les glandes aciniformes et de taille très réduite, groupées à proximité des filières et s'ouvrant par des canaux courts. On devrait souligner le fait que ces glandes ne sont piriformes qu'en forme, la structure en est aciniforme. Leur activité maximum a lieu pendant la formation du cocon. La sécrétion de ces glandes diffère de celle des autres glandes séricigènes de *P. lugubris* par leur faible affinité pour les colorants.

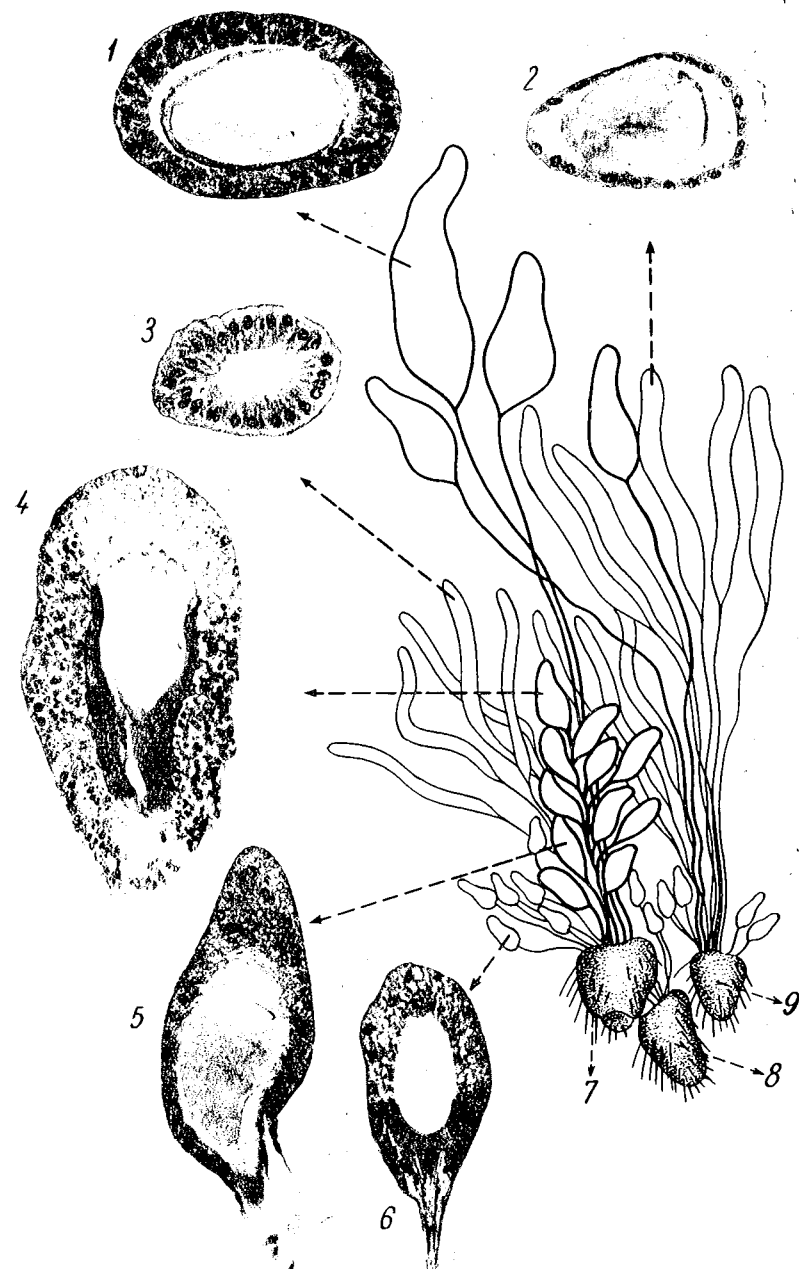
LES FILIÈRES

Elles sont des formations adaptées à l'évacuation des produits sécrétés par les glandes séricigènes. Chez les Lycosidae il y a six filières (Pl. I, 7, 8, 9), dont deux antérieures, deux médianes, plus réduites, et deux postérieures. Dans les filières antérieures s'ouvrent quatre glandes ampullacées, deux latérales et deux médianes. Toujours dans les filières antérieures s'ouvrent les glandes aciniformes (Pl. II, A, D). Dans les

filières médianes s'ouvrent quatre glandes ampullacées petites (Pl. II, B, E) ainsi que les deux types de glandes tubuliformes et les glandes piriformes. Dans les filières postérieures s'ouvrent les glandes piriformes et tubuliformes de petite taille (Pl. II, C, F).

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Pl. I — Schéma de l'appareil séricigène chez *Pardosa lugubris* (on a figuré seulement une moitié du complexe). Coupes histologiques des glandes séricigènes. 1. glande ampullacée — vue d'ensemble et coupe transversale; 2. glande tubuliforme grande — vue d'ensemble et coupe transversale; 3. glande tubuliforme petite — vue d'ensemble et coupe transversale; 4. glande aciniforme présentant 2 types d'épithélium — vue d'ensemble et coupe longitudinale; 5. glande aciniforme présentant un seul type d'épithélium — vue d'ensemble et coupe longitudinale; 6. glande piriforme — vue d'ensemble et coupe longitudinale; 7. filières antérieures; 8. filières moyennes; 9. filières postérieures.



CHANGES IN SOME MORPHOLOGICAL INDICES OF THE ADRENAL GLAND DURING THE DIFFERENT STAGES OF THE SEXUAL CYCLE IN *PHALACROCORAX CARBO L.*

BY ANCA PETRESCU-RAIANU

The biometrical studies performed on the adrenal glands in *Phalacrocorax carbo L.* indicate the interrenal cords thickness, the diameters of nuclei, the cell height and the nucleo-cytoplasmic ratio as valuable indices of the functional state of the gland. They undergo significant changes during the sexual cycle. A parallelism was also found to exist between the activity of the adrenal glands and the reproductive activity. The maximal activity of the adrenal glands occurs during the mating period, while the minimal one, during the sexual repose.

The studies performed on adrenal glands in Vertebrates in different functional states revealed noteworthy seasonal variations depending upon the sexual cycle. In Reptiles and Mammals the changes so far reported consist in gland volume and weight alterations, as a result of the secretory tissue hypertrophy or involution [6] [16] [25] [26] [29] [38], of quantitative alterations in some of the chemical constituents of the cell [3] [5] [10] [13] [18] [19] [29] [33], as well as of changes in vascularisation [16].

As to the Birds, in 1961, Hartman and Brownell [15] analysing the adrenal weight in 249 species, belonging to 49 families, failed to reveal any difference between the values found for egg laying period and the sexual repose. However, in 1923, Riddle reported an increase in size of the adrenal glands in pigeons during ovulation [35]. Several other authors, however, found a certain parallelism between sexual activity and interrenal cells activity [4] [9] [14] [23] [31] [43] [44]. As these studies were unfortunately dealing mainly with male individuals, the estimation

Pl. II — Filières clarifiées au lactophéno. Coupes histologiques des canaux excréteurs des glandes séricigènes dans la région de leur aboutissement. A. filière antérieure. — micro-fusules; B. filière moyenne. Macro-fusules et différents types de micro-fusules; C. filière postérieure. Micro-fusules; D. Canaux excréteurs des glandes aciniformes de la filière antérieure; E. Glandes ampullacées et leur canal excréteur aboutissant dans la filière moyenne; F. Canaux excréteurs des glandes tubuliformes, petits, aboutissant dans la filière postérieure.

of the functional state was based upon a rather limited number of parameters (in some cases only upon the fractional interrenal volume) and as the results are partly in disagreement with one another, the present paper reports the study of the morphological variations of the adrenal glands in another species, namely *Phalacrocorax carbo* L. The estimation of the functional state of the adrenal gland was based upon the values of the interrenal cords thickness, diameters of nuclei, height of the cells and nucleo-cytoplasmic ratio for interrenal cells.

MATERIAL AND METHODS

Adrenal glands from 27 males and 32 females of *Phalacrocorax carbo* L. were examined at different times of the sexual cycle (mating, egg laying — hatching, sexual repose) as shown in Table 1.

Table 1

Number of male and female individuals killed in different stages of the sexual cycle		
Stages	Number of males	Number of females
Mating (March)	7	8
Egg laying—hatching (April-May)	12	15
Sexual repose (September-October)	8	9

The adrenal glands were fixed in Bouin-Hollande mixture, included in paraffin and sections of 5μ in depth were stained with haemalaun and eosin.

Measurements of the diameters of nuclei, height of the cells and interrenal cords thickness were performed using an eyepiece micrometer and nucleo-cytoplasmic ratio was calculated. Separate measurements were carried out for interrenal cords and cells in the peripheral zone and in the central one. Each value is the average of 120 measurements. The values obtained

for the three stages studied were compared and statistically treated by the limit-difference test, to establish the degree of significance of the differences found.

RESULTS

Interrenal cords thickness was studied in the peripheral zone of the gland as well as in the central one. In the peripheral zone, in both sexes, the highest values of this parameter were scored in the mating period and a very significant decrease of these values was found for the egg laying period. With the setting up of sexual repose, the thickness of these cords shows a very significant decrease in males and a significant increase in females though without reaching the high level from the mating period (Table II and Fig. 1).

As to the interrenal cords thickness from the central zone, a very significant decrease occurred in both sexes from the mating period to the sexual repose with no but one exception: in males, between the mating and the hatching periods only a distinctly significant difference occurs.

Table 2

STAGE	SEX	Cords thickness (μ)		Diameters of nuclei (μ)		Cells height (μ)		N/C ratio	
		P.Z.	C.Z.	P.Z.	C.Z.	P.Z.	C.Z.	P.Z.	C.Z.
Mating	♂ ♀	39.15 ± 0.39	61.97 ± 0.93	6.44 ± 0.06	5.99 ± 0.08	14.03 ± 0.29	30.3 ± 0.82	0.1441 ± 0.010	0.0084 ± 0.0005
		38.20 ± 0.37	55.60 ± 0.43	6.58 ± 0.15	6.07 ± 0.08	13.11 ± 0.34	29.1 ± 0.36	0.1704 ± 0.010	0.0098 ± 0.0003
Egg-laying hatching	♂ ♀	33.52 ± 0.46	55.96 ± 0.58	5.89 ± 0.07	5.78 ± 0.08	13.81 ± 0.19	26.1 ± 0.21	0.1001 ± 0.005	0.0114 ± 0.0005
		34.06 ± 0.40	52.25 ± 0.55	6.34 ± 0.09	5.88 ± 0.05	13.33 ± 0.30	21.9 ± 0.68	0.1466 ± 0.010	0.0263 ± 0.0020
Sexual repose	♂ ♀	29.89 ± 0.32	46.71 ± 0.94	6.43 ± 0.10	5.53 ± 0.07	15.30 ± 0.46	20.1 ± 0.29	0.0948 ± 0.007	0.0232 ± 0.0010
		35.52 ± 0.30	50.04 ± 0.26	6.39 ± 0.04	5.78 ± 0.10	14.38 ± 0.36	23.9 ± 0.72	0.1309 ± 0.010	0.0157 ± 0.0010

P.Z. = Peripheral zone of the adrenal gland
C.Z. = Central zone of the adrenal gland

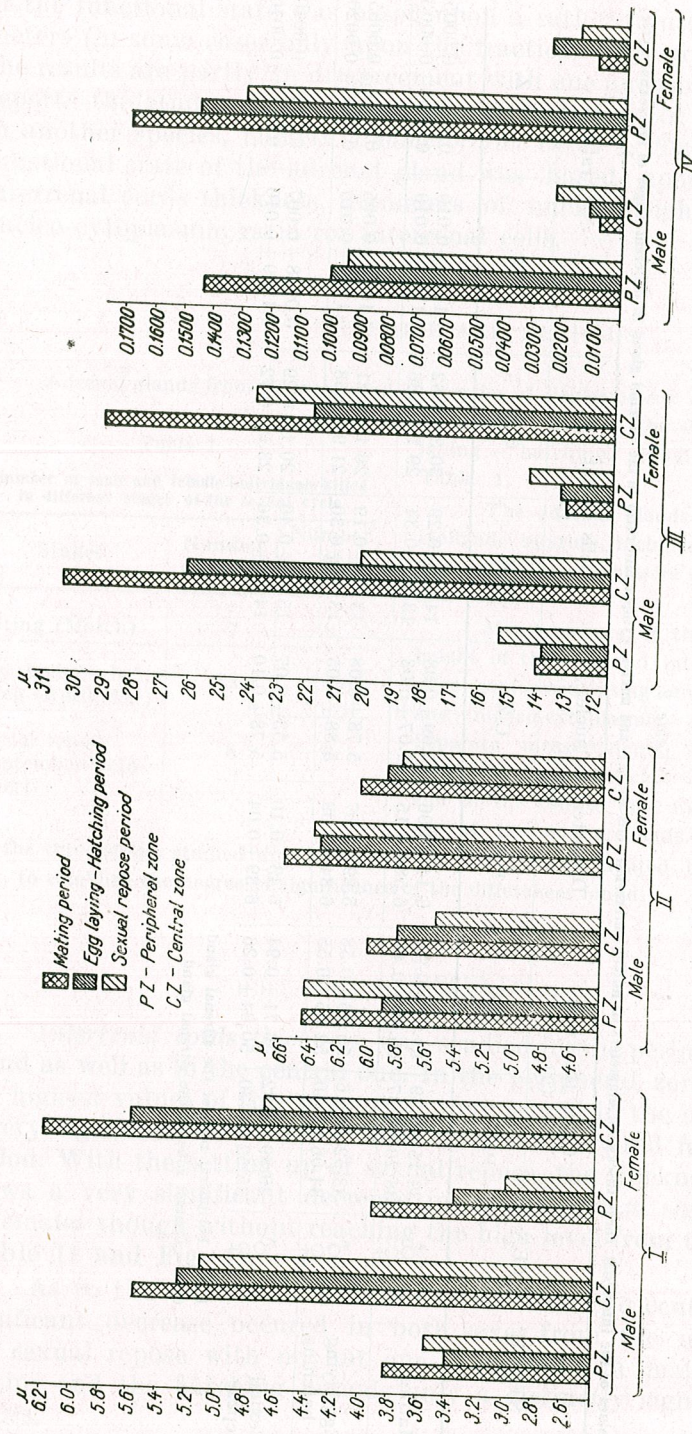


Fig. 1. — Variation of some morphological indices of the adrenal gland during different stages of the sexual cycle in *Phalacrocorax carbo L.* I, Variation of interrenal cords thickness in the two zones as a function of the sexual cycle stages. II, Variations of the diameters of nuclei of the interrenal cells in the two zones as a function of the sexual cycle stages. III, Variation of the interrenal cells height in the two zones as a function of the sexual cycle stages. IV, Variation of the nucleo-cytoplasmic ratio of the interrenal cells in the two zones as a function of the sexual cycle stages.

The diameters of nuclei from the peripheral zone display only small variations during the sexual cycle, mainly non-significant ones. In both sexes, the largest values for the diameters of nuclei were found in the mating period, followed by a decrease during the egg laying — hatching period (very significantly in males and non-significantly in females). During the sex repose a non-significant increase occurs in females and a very significant one in males.

In the central zone, the diameters of nuclei reach the highest values during the mating period, which differ very significantly in males and distinctly significantly in females from the values found for the sexual repose. Between the mating period and the egg laying — hatching one no significant decrease in the diameter of nuclei was found neither in males, nor in females.

Cell height is a parameter showing very marked changes during the sexual cycle. In males, as well as in females, the height of the interrenal cells from the peripheral zone takes the highest values during the sexual repose. Smaller differences were found in females than in males, namely significant differences between the mating and the egg laying — hatching periods on the one hand and the sexual repose on the other hand. In males, these differences are very significant or distinctly significant.

More marked differences occur in the central zone, as compared to the peripheral zone, in both sexes. The males display very significant differences between the three stages, the highest values being those from the mating period, and the lowest one those from the sexual repose. In females too, a very significant decrease occurs in the cell height from the mating period to the sexual repose.

Nucleo-cytoplasmic ratio shows very different values for the cells belonging to the two zones of the adrenal gland, the values from the peripheral zone being much higher than those found for the cells from central zone.

In the peripheral zone, the highest values were found in both sexes during mating. These values gradually decreased, more abruptly in males (distinctly significant) than in females (nonsignificant). The decrease in the values of the ratio lasts through out the sexual period, so that the differences between the repose values and those from the mating period become significant in females as well as in males.

In the central zone, the lowest values of the nucleo-cytoplasmic ratio were scored in both sexes during the mating period. A gradual increase occurred in males through the sexual repose period, the differences between the values being distinctly and very significant. However in females a noteworthy increase in the values of the nucleo-cytoplasmic ratio occurs after the mating period throughout egg laying — hatching period, followed by a subsequent decrease towards the sexual repose period, though without reaching the low value from the mating period.

DISCUSSIONS

In recent works, the assumption of the structural alterations of the cells, tissues and organs as a result of functional alterations, seems to be generally accepted. Among the structural changes, some may be of a striking appearance and consequently easily to detect. However, several other changes may be revealed only at thorough examinations. The parameters used in the present paper are of the latter type.

A rapid examination of the structure of adrenal gland in birds in different physiological states, fails to reveal any marked morphological alteration. However, the biometrical analysis of the interrenal cords thickness, diameters of nuclei, cell height and nucleo-cytoplasmic ratio shows that these parameters might be valuable morphological indices of the functional state.

Karyometrical measurements are frequently used as indices for the physiological state of the cells. Palkovits, Stark et al [30] [46] performed special investigations with the aim of estimating the actual reliability of the nuclear volume measurements in characterising the functional state of the cells. Many successful applications of this index in the estimation of the adrenal activity are reported for Mammals [1] [2] [8] [18] [22] [27]. In Birds, although several works are concerned with the behaviour of the adrenal glands in different physiological states, the karyological studies were not frequently used [14] [20] [21] [36].

Our studies in *Phalacrocorax carbo* revealed a specific pattern of the changes in the diameters of nuclei during the mating egg laying — hatching and sexual repose periods. In both zones, the highest values occur during the mating, which suggests that the most intensive activity of the interrenal cells proceeds during this period. This fact is in good agreement with the high values of the nucleo-cytoplasmic ratio (as a definite index of the metabolic activity of the cell), as well as with the values of cells height and interrenal cords thickness.

The activity of the cells, as judged by the variation in the parameters studied by us, significantly decreases in *Phalacrocorax carbo*, during the egg laying — hatching period in most cases, as compared to the mating period. The values found for the egg laying — hatching period are generally intermediate between those from the mating period and sexual repose.

The morphological variations reported in the present paper are in agreement with a great bulk of data in the literature which support the idea of some relationships between adrenal glands and gonads. These data cover a large field and were reported in many papers which, depending upon the aspects approached, might be classified into four classes:

I. Data which rule out the occurrence of a sexual dimorphism of the adrenal gland.

II. Data concerned with seasonal variations of the adrenal gland depending upon the sexual cycle.

III. Data concerned with experimentally — induced alterations in the adrenal glands, by means of gonadectomy or sexual hormones treatments.

IV. Data on the alterations in the reproductive system or reproductive processus resulting from alterations in the function of the adrenal gland (adrenalectomy, treatments with corticosteroid hormones or ACTH).

The results of our study in *Phalacrocorax carbo* are related only with the first two aspects, so that the following discussion will be confined to these.

The occurrence of sexual dimorphism of the adrenal glands seems to be ruled out in several Mammals, namely in rodents as due to the presence of the X zone [39], as well as to weight differences [12], [26], [42], [48], [50] and quantitative differences in several chemical constituents like: pigments [39], glycogen [32], lipids [42], enzymes [7], [47], progesteron [49].

In Birds, no obvious sexual dimorphism of the adrenal glands was so far revealed. Neither Hartman and Brownell [15], which measured absolute and relative weight of the adrenal glands in 249 species belonging to 49 families, nor Sinha et al [44], which compared the ratio of interrenal and chromaffin tissues in pigeon, did obtain significant differences between the two sexes.

The analysis of our data concerning the interrenal cords thickness, the diameters of nuclei, cell height and nucleo-cytoplasmic ratio in the interrenal tissue of *Phalacrocorax carbo*, does not seem to indicate any sexual dimorphism of the adrenal glands in this species. Moreover, the variation patterns of these parameters are quite parallel, which points out a similar function during the annual cycle in females as well as in males. The graph in fig. 1 shows that the variations in interrenal cords thickness in the central zone of the gland, in the diameters of nuclei in both zones and in the nucleo-cytoplasmic ratio proceed in the same direction in both sexes.

The relationships between adrenal glands and gonads were demonstrated in all classes of amniotic vertebrates, either experimentally (gonadectomy, treatments with sexual hormones) or by comparing the structure of the adrenal gland in different stages of the sexual cycle. More detailed and extensive studies were performed in Mammals. Many reports are dealing with variations of the adrenal weight, which occur in different physiological states related with reproduction [6], [18] [37] [40] [41]. In addition to the weight changes, histological and histochemical changes, vascularisation changes, mitotic activity changes and changes in the relative proportions of the cortical and medullar tissues were also described. [5] [16] [17] [18] [33] [45] [50].

As far as the Birds are concerned, the work is less extensive and the results often contradictory. Hartman and Brownell [15] did not find significant differences between the weight of the adrenal glands prelevated during repose and egg laying for none of the 249 species examined. Other investigators report changes in the adrenal glands depending upon the stages of the sexual cycle [4] [9] [14] [23] [31]. However, while Fromme — Bouman [9], Bhattacharyya and Ghosh [4], and Hall [14] in *Turdus merula*, *Passer domesticus* and respectively *Platycercus eximius* report a coincidence between the maximal activity of the adrenal glands and the maximal activity of the testes, Lorenzen and Farner [23] found in *Zono-*

Arichia leucophrys gambelii just the reverse situation. Our data show that in females as well as in males the activity of the adrenal glands superimposes the reproductive activity. The maximum of the adrenal activity occurs during the mating period, while the minimum during the sexual repose.

CONCLUSIONS

1. The studies performed on the adrenal glands in *Phalacrocorax carbo* L. indicate the interrenal cords thickness, the diameters of nuclei, the cell height and the nucleo-cytoplasmic ratio as valuable indices of the functional state of the gland.

2. These parameters undergo significant changes during the sexual cycle.

3. A parallelism was found to exist between the activity of the adrenal glands and the reproductive activity: the maximal adrenal activity occurs during the mating period, while the minimal one during the sexual repose. The same holds for the both sexes.

4. As far as the morphological indices used by us are taken into account, no structural difference between the two sexes appears for the adrenal glands of *Phalacrocorax carbo*.

5. The parameters examined take different values in the two zones of the adrenal gland and the direction in which they change is sometime different. This fact is a further support for the morphological and functional zonation of the adrenal gland in *Phalacrocorax carbo* L.

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Received February 1st, 1971

The "Traian Săvulescu" Institute of Biology
Department of Animal Morphology and Taxonomy

NUCLEIC ACIDS IN THYMUS INVOLUTION AND REGENERATION

BY

V. TOMA, A. D. ABRAHAM and E. A. PORA.

Researches carried out on male white rats weighing 100 g show, that after administration of 7.5 mg/100 g b.w. of hydrocortisone, the maximum thymus involution occurs within 3 days. In this moment the relative weight of thymus decreased by 67 per cent, the DNA and RNA content 52 respectively 34 per cent. The thymic DNA appears to be most affected since after 24 h. The decrease of its quantity is statistically significant and remains so till the 16th day. After 30 days the thymus regeneration is complete, all the followed indices being integrated within the limits of the controls.

The reaction of the thymus in stress that is the action of corticoids on this organ, has been the subject of numerous investigations. Dougherty et al [4] have observed lymphocytokaryorhexis in the lymphoid structures of the thymus after hydrocortisone administration. It is characterized by an inhibition of mitoses and decrease of the nucleic acid synthesis and proteins one, as well as by the characteristic destruction of small thymocytes.

It is interesting to note the modifications brought about corticoids in the thymus that do not depend on the continued presence of these hormones at the level of the gland. The labelled hydrocortisone can no longer be detected after 100 min. of administration. Bellamy et al [2] showed that hydrocortisone caused nucleic acid degradation in the thymus after 1-16 h of administration.

Based on this data, we have also extended our investigations on the dynamic modification of nucleic acids in the thymus during involution and regeneration.

MATERIAL AND METHODS

The experiments were carried out on male white rats weighing 100 ± 10 g. Hydrocortisone (C.I.F.) was injected i.m. in a unique dose of 7.5 mg/100 g b.w. As long as the experiment lasted (April and May 1969) the animals underwent an identical living regime. After injections the rats were sacrificed by decapitation at the rate of 8, 24 h and 3, 8, 16 and 30 days. A lot of animals considered controls, were injected with an equal quantity of liquid without hydrocortisone. The concentration of the total nucleic acid was determined by Spirin's differential spectrophotometric method [11] and the DNA one by Ogur and Rosen's technique modified by us [1]. The difference enabled us to establish the RNA content.

RESULTS AND DISCUSSION

Our results exposed in the table confirm the precociousness of hydrocortisone action upon the thymus gland [5] [6]. Under the above mentioned working conditions the statistically significant differences appeared after 24 h of hydrocortisone administration. The lowest relative weight and that of nucleic acid content was noted at the third day after administration. (Table I). After 30 days the value of all investigated indices becomes practically integrated within the control limits. This fact showed the complete regeneration of the gland (Fig. 1).

Considering the data in the literature [3] [9] [10] [12] [14] it becomes clear that the involution phenomenon and that of the regeneration of the thymus, under the influence of cortisol, are particularly complex.

Table 1

Variation of the concentration of the total nucleic acid, DNA and RNA, as well as that of the relative weight of thymus during its involution and regeneration in white male rats treated with 7.5 mg of hydrocortisone/100 g.

Group	Total nucleic acid	DNA	RNA	Relative weight
Number of rats ()	mg/g fresh tissue			mg/g h.b.w.
Control (8)	25.151 ± 0.935	18.612 ± 0.953	7.662 ± 0.696	151.5 ± 6.6
8 hours (7)	26.514 ± 1.122	18.204 ± 0.839	8.311 ± 1.207	130.2 ± 5.3
24 hours (6)	20.149 ± 1.028^x	11.074 ± 0.774^x	8.520 ± 1.073	$99.8 > 3.0^x$
3 days (7)	14.466 ± 1.456^x	9.286 ± 0.648^x	5.029 ± 0.780^x	49.3 ± 7.0^x
8 days (6)	17.743 ± 0.836^x	10.887 ± 0.458^x	5.766 ± 0.310^x	70.8 ± 4.1^x
16 days (7)	23.663 ± 0.509	14.020 ± 1.072^x	8.214 ± 1.024	119.2 ± 2.3^x
30 days (7)	25.504 ± 1.612	18.619 ± 1.216	6.996 ± 0.456	134.8 ± 6.6

$x = p < 0.5$ against control group

They include structural, biochemical and ponderal modifications which have a certain selective preponderance during the various phases of these phenomena.

In this respect, it is characteristic the thymic DNA sensitiveness to the action of hydrocortisone. This modification can be explained on

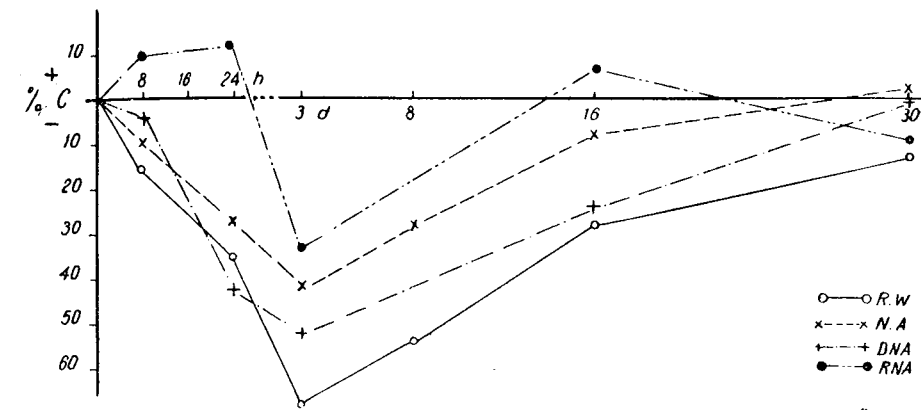


Fig. 1. — Percent differences of the weight of the thymus (relative weight=R.W.) and the quantity of the DNA, RNA and total nucleic acids (N.A.) after the administration of 7.5 mg hydrocortisone against control values.

the base of the hydrocortisone action on the thymus cell nuclei. Regeneration of the gland following the action of hydrocortisone occurs according to Ito and Hoshino [5]. Thus, the thymus becomes enriched in lymphatic elements and its biochemical statement is characterized accordingly the given ontogenetic stage. Thymus involution, being characteristic to stress states, may have biological signification. According to Jusfina [6] the fact that thymic epithelium becomes free from thymocytes would constitute the cause of the release of the epithelial glandular secretion.

Thus the number of Hassal corpuscles in the involution brought about by the involutive agent, as well as that of the depolymerisation of the nucleic acids take place and the mucopolisaccharide fractions increased, which play an important role in the formation of the Hassal's corpuscles [15] [16]. Milcu [7] [8] showed that the nucleic acid and mucopolisaccharide content of the blood increased after hydrocortisone administration and this phenomena can be in relation with the thymus involution.

As a conclusion, we can state that administration of a single dose of hydrocortisone brings about a thymus involution which touches its upper limit the third day when the content of the nucleic acids and the relative weight of organ are significantly decreased. 30 days after, regeneration of the thymus is complete due to the recovery phenomena.

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Received February 17, 1971

University of Cluj
Department of Animal Physiology

VITAMIN C AND GLUCOSE METABOLISM IN THE EARLY STAGES OF EMBRYOGENESIS IN *SALMO GAIRDNERI*

BY

VLAD ARTENIE, RODERICH BRANDSCH, ECATERINA DUCA,
ELENA HEFCO, GABI LINK and MATILDA JITARIU

Studies were carried out on eggs of *Salmo gairdneri* in the early stages of embryogenesis.

The authors find a correlation between the metabolism of vitamin C and that of free glucose in the egg. The ascorbic acid-dehydro-ascorbic acid system might ensure NADPH₂ oxidation, which is the essential condition for achieving the the pentose-phosphoric cycle, by which glucose is catabolized immediately after fertilization, up to the final appearance of the blastula.

Metabolic processes which occur in the period of embryonic development in fishes are still little known, as we have already mentioned in a previous paper [5]. The works in this field involve different metabolism aspects, in different fish species, with a very varied ecology. The results obtained in the study of the same metabolic process in the same genus, yet living in different media, will contribute to the more rapid elucidation of the most important problems of the biology of embryonic development.

In the present work, experiments were carried out on eggs of *Salmo gairdneri*, starting with the matured but nonfertilized ovule, then on eggs after 8 and 28 hours from fertilization, and eventually on eggs after 3 and 7 days from the incubation.

Quantitative variations of free glucose [1], lactic [2] and pyruvic [3] acid, ascorbic and dehydroascorbic acid [6], as well as oxygen consumption (Warburg method), were determined, in correlation with histological analyses for the establishing of the embryonic development stages when analyses were carried out.

Our results point to the existence of a glucide metabolism in fertilized eggs of *Salmo gairdneri*. Fertilization releases a glucose catabolization probably by the pathway of the pentose-phosphoric cycle. Glucose utilization in the first hours from the fertilization is so intense that about 3/4 of its totality disappears (Fig. 1). The proof of its utilization in this

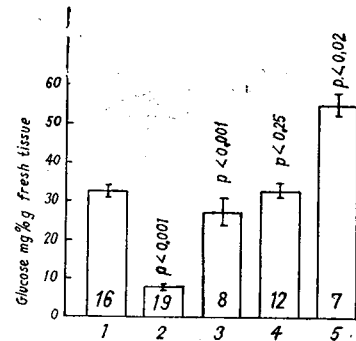


Fig. 1. — Variations of glucose amount in the eggs of *Salmo gairdneri*, in the first stages of embryonic development. 1, Control eggs (unfertilized); 2, 8 hs fertilized eggs; 3, 28 hs fertilized eggs; 4, 3 days incubation; 5, 7 days incubation.

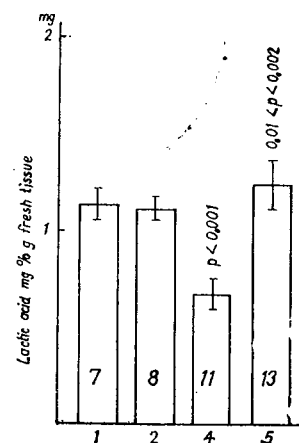


Fig. 2. — Variations of acid lactic amount in the eggs of *Salmo gairdneri* in the first stages of embryonic development. Same legend as figure 1.

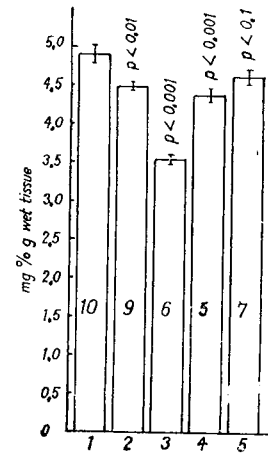


Fig. 3. — Variations of pyruvic acid amount in the eggs of *Salmo gairdneri*, in the first stages of embryonic development. Same legend as figure 1.

way is, among others, the almost constant amount of lactic acid in this period and the concentration of pyruvic acid, which though significantly decreasing is not proportional to the amount of oxidized glucose (Figs 2 and 3).

Our data agree with Boulekbache's results [4], who analysed the activity of lactate-dehydrogenase and glucose-6-phosphate-dehydrogenase and reached the conclusion that glucose is used on the pentose-phosphoric pathway in the first stages of embryogenesis in *Salmo irideus*.

Vitamin C behaves as an oxidoreducing system, since the ascorbic acid significantly decreases immediately after fertilization, while the dehydroascorbic acid almost significantly increases (Fig. 4). A significant oxygen consumption is also found (Fig. 5).

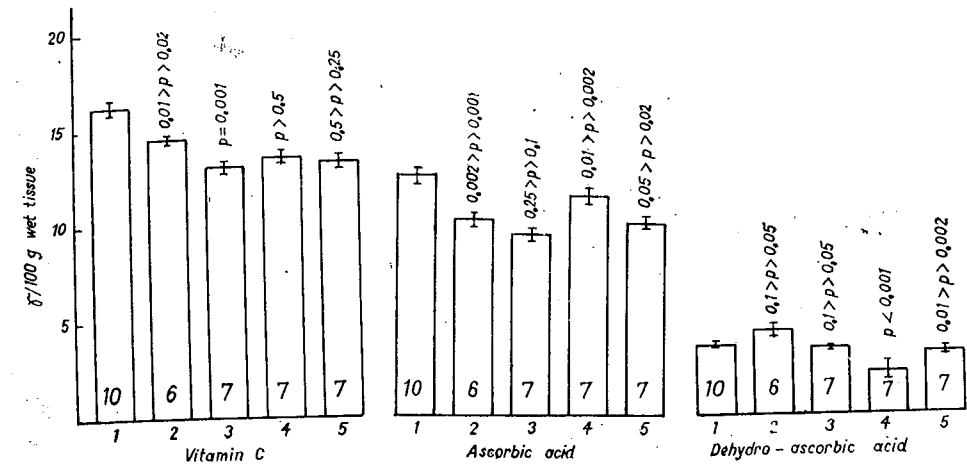
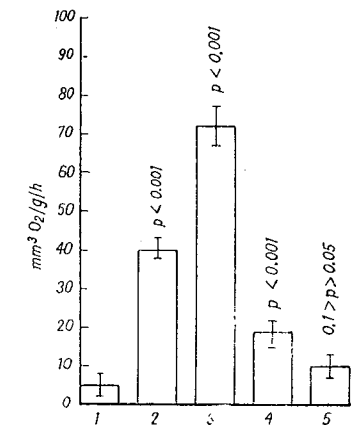


Fig. 4. — Quantitative variations of vitamin C in the eggs of *Salmo gairdneri*, in the first stages of embryonic development. Same legend as figure 1.

Fig. 5. — Variations of oxygen consumption in the eggs of *Salmo gairdneri* in the first stages of embryonic development. Same legend as figure 1.



Within 28 hours from the fertilization, segmentation was over and the blastule was completely formed. In this stage, glucose is partly recovered (there is an amount of 27.7 mg % g fresh tissue). The origin of this glucose cannot be specified but it may be supposed that one of the substrates is acid lactic: three days after incubation (after 28 hours, determinations could not be carried out), it presents a significant increase as against the amount existing 8 hours after fertilization.

Pyruvic acid goes on decreasing during the first 28 hours. Taking into account the very high oxygen consumption, we may suppose that pyruvic acid could catabolize on the oxidative pathway, releasing energy which accumulates especially as ATP and may be used in the intense biosynthetic processes of this period. Pyruvic acid could also be used in synthetic processes.

Our data point out the fact that in the blastule stage, ascorbic and dehydroascorbic acid do not behave as an oxidoreducing system but as a glucose source or a source of other synthesis processes. Anyhow, both ascorbic and dehydroascorbic acid amount decrease, and the lowest value is reached by the ascorbic acid. At the same time, pyruvic acid reaches the lowest value of all the period studied by us (Figs 3 and 4).

A comparison of these data with those obtained by investigating the first stages of embryonic development in *Salmo trutta fario* [5], points out that immediately after fertilization up to the final blastule formation, there is a similarity in the metabolic processes of *Salmo trutta fario* and *Salmo gairdneri*'s eggs. In both species, fertilization releases glucose metabolism on the pentose-phosphoric cycle pathway, which is a pentose source for nucleic acid synthesis, necessary in this segmentation period. Oxygen consumption increases, as recorded by Trifonova [7] [8] in the embryogenesis of *Perca* eggs. This author has found a decrease in oxygen consumption in the epiboly period, after the initial increase which lasted till blastulation. The same phenomenon appears also in *Salmo gairdneri* eggs. Three days after incubation, when epiboly is over, a significant decrease of oxygen consumption is found by us.

The low value of pyruvic acid amount in the blastule stage of both species cannot be definitely accounted for. It may only be supposed that it was used either as energy source or as substrate for some anabolic processes.

After the blastule stage, the available *Salmo gairdneri* eggs no longer permitted the further study of the same embryogenesis stages as in *Salmo trutta fario*.

Three days after incubation, embryogenesis in *Salmo gairdneri* is in the stage of complete epiboly, with a non-significantly increased glucose amount and a significant use of the lactic acid.

A reduced oxygen consumption corresponds to a significant decrease of pyruvic and ascorbic acid. The latter can be recovered from the dehydroascorbic acid, which significantly decreases, and also from glucose.

Seven days after incubation, the embryo already has 6 somites and the whole egg has a significantly increased glucose and lactic acid amount, concomitantly with a pronounced decrease of oxygen consumption and an almost constant amount of pyruvic acid. Vitamic C again behaves like an oxidoreducing system (Figs 1-5).

Results obtained by us on eggs of both *Salmo trutta fario* and *Salmo gairdneri* reveal a close correlation between the metabolism of glucose and of vitamin C. The ascorbic acid-dehydroascorbic acid system seems to ensure NADPH₂ oxidation — in our opinion. This oxidation is a compulsory requirement for the functioning of glucose-6-phosphate-dehydro-

genase and, hence, of the pentosephosphoric cycle, whose role particularly increases in intense cell divisions. Further investigations will have to bring evidence in favour of this hypothesis.

Acknowledgment. Thanks are due to Dipl. eng. Paul Decei, for his courtesy in making available the material necessary for this work.

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Received February 5, 1971

*The Biological Research Centre
Department of Animal Physiology
Jassy*

CONTRIBUTION TO THE STUDY
OF THE HYPOGLYCEMIC ACTION OF ACTH

BY

V. HEFCO, P. JITARIU, and P. ROTENBERG

Both ether and formol induce hypoglycemia in the adrenalectomized animals. This may be prevented by the lesion of the median eminence or after dexamethasone pretreatment. ACTH (5IU/Kg b.w.) induces hypoglycemia in sham-operated or adrenalectomized rats. Chlorpromazine results in persistent hyperglycemia in the intact animal and has no effect in the adrenalectomized one. In alloxan-diabetic rats, ACTH emphasizes diabetes and in alloxanized or depancreatized and adrenalectomized rats it exerts a hypoglycemic action. It may be concluded that the hypoglycemic action of ACTH could form a physiological mechanism and that other mechanism(s) which do not require insulin participation are involved in ACTH induced hypoglycemia, beside the mechanism which involves the activations of insulin secretion.

Literature data, remarkably reviewed by Engel and Lebovitz [5] and by Koranyi et al. [13], have shown that beside the specific ACTH action on the adrenal, there are a series of extraadrenal actions, like adipokinetic action *in vivo*, hypoglycemia and improved glucose tolerance by the increase of insulin secretion [4], increase in the adipose tissue glycogen [6], diabetogenic action in force-fed, cortisone maintained adrenalectomized rats [16] etc. Many of the extraadrenal actions observed with pituitary ACTH extracts were also confirmed by the use of synthetic ACTH. This does not mean, however, that endogenous ACTH should also exert the same effects. As shown by Engel and Lebovitz [5], one may object to the used doses, in other words one should followed up whether these extraadrenal effects represent a physiological mechanism or are the result of the used pharmacological doses. Also, the mechanism by which ACTH induced hypoglycemic action cannot be considered as solved.

The present work was first aimed at finding out whether endogenous ACTH variations, which may occur in an organism in different natural and experimental conditions, result in modifications in the blood glycemia level and whether these modifications have a physiological significance, also controlling the action of exogenous ACTH. Second, this paper represents a modest attempt at accounting for the hypoglycemic phenomenon, observed after sudden variations of endogenous and exogenous ACTH content.

MATERIAL AND METHODS

Female rats weighing about 200 g, maintained at constant temperature and Mac Collum's standard diet (17), were used for the experiment. Adrenalectomy was carried out under ether anaesthesia, and then the animals were kept on 1% saline solution. Ether stress was performed by animal exposure for 2 minutes in an ether-vapour saturated area and formol stress was performed by i.m. injecting 0.1 ml 5% solution. Chlorpromazine (0.5 mg/100g b.w.) was i.m. administered deeply. The lesion of the median eminence was made on each side of the junction of the pituitary stalk, with the help of a radiofrequency current, supplied by the TUR GH4 apparatus. Glass-insulated NiCr electrodes 0.3 mm in diameter were used. Animals were used in the experiment, 72 h after the lesion. Dexamethasone (400 μ g/100 g) were injected i.p. and after 2½ h, formol was administered in order to study glycemia. Alloxan diabetes was induced by i.v. injection of 50 mg recrystallized alloxan/kg b.w., after a previous 24–28 hour starvation. For the next 3 days 4 U/Kg/day insulin (Biofarm) was administered subcutaneously. ACTH (5 I.U./Kg b.w.) dissolved in saline solution was administered i.p. Glycemia was tested by Asatoor and King's method [3]. The determination of studied indices was done after a previous 14-hour starvation. Statistical evaluation was carried out by Student's test, and significations were calculated according to time zero.

RESULTS

As observed in figure 1, in 8-hour adrenalectomized rats ether stress results in an ACTH output, liable to induce obvious hypoglycemia 60 minutes after stress. Five days after adrenalectomy, rats exhibit a significant hypoglycemia of longer duration, secondary to ether treatment. The same phenomenon, more pronounced however, is also observed in rats stressed with formol (Fig. 2). Chlorpromazine does not induce significant modifications in adrenalectomized animals; conversely in sham-operated rats it leads to a hyperglycemia manifested also 2 hours after the treatment (Fig. 3). After dexamethasone pretreatment or lesion of the posterior portion of the median eminence, formol stress no longer induces hypoglycemia but, in exchange there occur a significant hyperglycemia 30 minutes after formol injection. In sham-operated animals, under the same experimental conditions, the used dose of dexamethasone does not induce glycemia modifications (Fig. 4). ACTH injection in adrenalectomized rats produces a significant hypoglycemia as early as 30 minutes after the injection; hypoglycemia is maintained even for 2 hours. In control animals hypoglycemia appears only after 1 hour and after 2 hours there occurs a nonsignificant decrease (Fig. 5). ACTH injected

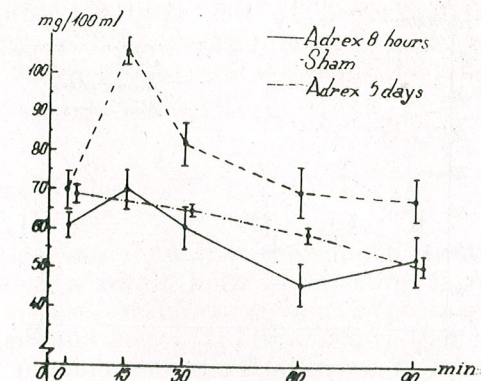


Fig. 1. — Effect of ether stress on blood sugar level in sham-operated, 8-hour or 5-day adrenalectomized (Adrex) rats. Values are mean \pm SE.

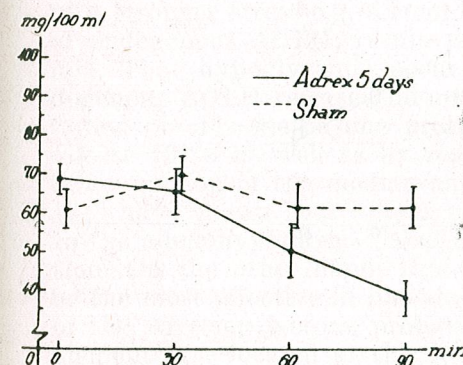


Fig. 2. — Changes of blood glucose level in sham-operated or adrenalectomized rats following formalin stress.

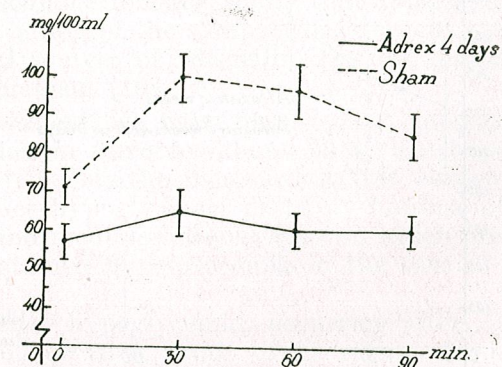


Fig. 3. — Changes in blood glucose level in adrenalectomized or sham-operated rats after chlorpromazine treatment.

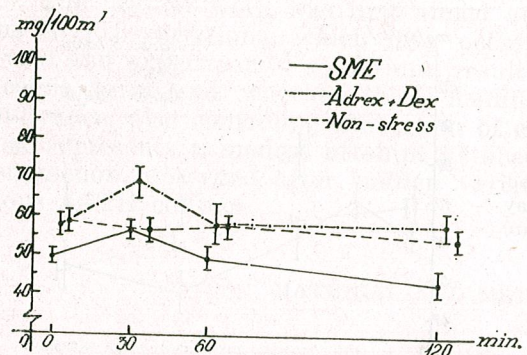


Fig. 4 — Blood glucose level in adrenalectomized-dexamethasone pretreated or with stalk-median eminence lesioned rats following formalin stress. Non-stress = adrenalectomized-dexamethasone treated rats.

Fig. 5. — Blood glucose level in sham-operated or adrenalectomized rats after ACTH treatment.

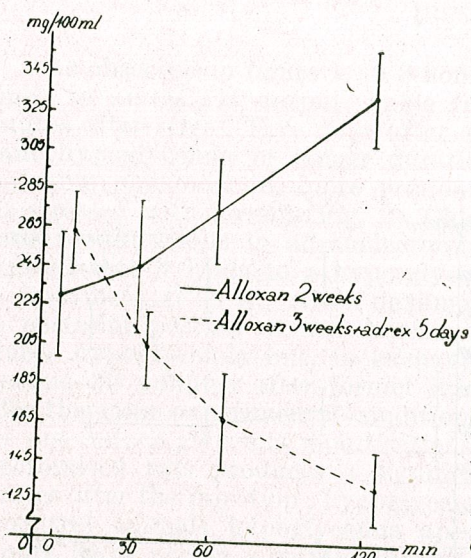
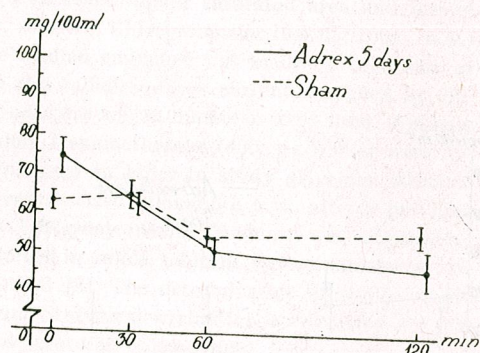


Fig. 6. — Effect of ACTH on 2-week alloxan-diabetic or 3-week alloxan-diabetic-adrenalectomized rats.

in 2-week alloxan after diabetic rats produces a continuous hyperglycemia which seems significant 2 hours after the treatment. In 3-week alloxan-diabetic rats, 5 days-adrenalectomy, ACTH has a significant hypoglycemic action after 30 minutes, with a more pronounced effect for the 2 hour-time of experiment (Fig. 6).

DISCUSSION

The results of present investigations show that the sudden variation of the circulating ACTH amount—as a result both of injecting it from the outside and of its endogenous release—results in obvious hypoglycemia in adrenalectomized animals. Hodges and Jones [10] have shown that mild stress factors-e.g. ether-induce a pronounced ACTH increase in adrenalectomized rats and this pituitary overactivity increased progressively with prolongation of the time interval between adrenalectomy and stress. This fact is also confirmed by our experiments on ACTH hypoglycemic action, which is more prolonged in animals adrenalectomized 5 days before, as against those adrenalectomized only 8 hour before. The use of a stronger stress-e.g. formol-leading to the release of a higher amount of endogenous ACTH, results in a more obvious consecutive hypoglycemia. That hypoglycemia occurring under these conditions is due to endogenous ACTH release and not to other factors, is shown by results obtained on dexamethasone pretreated rats—a powerful inhibitor of ACTH release [2]—as well as by results obtained after the lesion of the posterior portion of the median eminence. J. Porter's investigations of 1969 [21] have shown that lesions of this kind do not result in modifications in the adenohypophysis blood supply, measured 2 days after the intervention. On the other hand, this level of the median eminence proved to be the most effective in blocking ACTH release, as a result of the action of the stressing factors, probably because of the fact that CRF-secreting elements are located at this level. The absence of the hypoglycemic response after formol-treatment in animals with such lesions entitle us to believe that the occurring hypoglycemia is actually due to ACTH and not to the growth hormone or TSH, which in acute injections proved to be hypoglycemic, too [15]. The increase of glycemia 30 minutes after formol injections, observed in adrenalectomized-dexamethasone-treated rats, or having lesions of the median eminence, shows that hyperglycemia may also occur after the direct action of the sympathetic nervous system or may be due to extraadrenal sources of adrenalin, the rat, probably, is not an adrenal-dependent organism [19].

After chlorpromazine treatment, we have observed an increase in adrenal corticosteroid production in vitro by about 20%, which points to an increased ACTH release [8]. Yet, the increased ACTH content, in the present case, does not induce hypoglycemia probably because of the activation of the sympathetic and medullo-adrenal system, which prevails ACTH action, leading to a sustained hyperglycemia in the case of normal animals.

It is worth mentioning that hypoglycemia, occurring after ACTH administration from the outside or as a result of its release from the

pituitary, secondary to stress, does not appear immediately but after a rather long interval (usually more than 30 minutes), though ACTH release in high amount occurs in a very short lapse of time. This point out that the resulting effect is not due to the direct ACTH influence on glycemia but that it is an indirect effect. Oshawa et al. [18] has observed that ACTH administration in the intact dog results in increase insulin output. This phenomenon has a very fast onset and lasts little. Sussmann and Vaughan [24] also noticed an increase in circulating insulin after ACTH perfusion of isolated rat pancreas. However, the mechanism by which ACTH stimulates insulin secretion is not known. Grasso et al. [7] shows the importance of essential amino acids; while glucose alone induces a slight insulin increase, the infusion of essential amino acids was followed by a high and fast enhance in serum insulin content. Adrenal steroids also play an important role in ACTH — induction of hypoglycemia. This effect can be a direct or permissive one. The influence of corticosteroids on the endocrine function of the pancreas was demonstrated by Pora and Madar [20] and by Houssay et al. [11]. This is probably a direct action, since Malaisse et al. [14] have observed that insulin secretion by pancreatic slices under the glucose influence is increased *in vitro* in cortisol-treated animals.

Rappaport et al. [22] studied the effect of hormones and of changes in the blood flow irrigating the pancreas and observed that between the volume of pancreatic blood flow and insulin output there is a direct proportional relation. Yet, Kaneto et al. [12] have observed that neurohypophyseal hormones induce an increase in insulin secretion and their intervention is not accompanied by an increase of the blood volume irrigating the pancreas.

However, our experiments point to the possible existence of other mechanism(s) to obtain a hypoglycemic response, beside the mechanism which implies a more pronounced insulin secretion. This is suggested by the fact that ACTH has an obvious hypoglycemic action in adrenalectomized and alloxan-diabetic rats. Indeed, we have not carried out the histological study of the pancreas to find out the degree of the beta cells destruction, but the fact that experiment animals had a marked diabetes even after 3 weeks from alloxanization suppose a high destruction of beta cells. In spite of this, the hypoglycemic ACTH level is not reduced. Modifications on the same way were observed in three adrenalectomized-pancreatectomized rats to the extent in which it is possible to surgically remove the rat pancreas. Our supposition that ACTH might induce hypoglycemia using another mechanism than that of insulin is based also on the fact that insulin output induced by ACTH occurs very rapidly (in the dog it reaches a maximum after 1-minute and it returns to the initial level after 5–15 minutes [18]), while significant hypoglycemia in the rat is usually observed after 30 minutes and maintained also after 2 hours. The fact that repeated ACTH injections do not maintain the enhanced insulin level induced by the first injection [18] is bringing evidence to the same effect.

In diabetic animals with adrenals, ACTH has a clearly hyperglycemic action, of enhancing the diabetic state. However, since ACTH injection results in hypoglycemia in the normal animal and in hyper-

glycemia in alloxanized rats with adrenals, we are entitled to think that the mechanism of insulin secretion increase is also involved in this process, beside the hypoglycemic action of ACTH, by the use of other links not related to insulin. In the rat as against the dog, insulin modifications induced by ACTH are probably of a longer duration.

It thus appears that whenever ACTH content increases suddenly, there occurs a hypoglycemic effect in the adrenalectomized animal and—to a lesser extent—in the intact animal. But, in the rat, no hypoglycemia is observed when the amount of circulating ACTH is increased gradually and not suddenly. Thus, in a previous paper on the diurnal variation of the glycemia level and of the production of corticosteroid hormones—as a test of the circulating ACTH amount—no hypoglycemia was observed at 4 PM even if ACTH content was net enhanced [9]. Moreover, after, certain types of hypothalamic deafferentations resulting in an increase of the circulating ACTH amount, no decrease of the glycemia level was observed by us [8]. This might be account e.g. for by an insufficient ACTH amount, not able to induce such modifications. By comparing the action of ACTH, ether stress and diurnal variation on the plasma corticosterone level—as a test for the plasma ACTH content—Allen and Kendall [1] have noticed a corticoid increase at 4PM three times as high as the level of 8 AM; two-minute exposure to ether resulted in a 6-time increase; after 100 mU ACTH the increase was five-fold. It results that the ACTH amount at 4PM is about twice as low as that following ether stress. This difference decreases, however, in animals adrenalectomized 8 hours before ether stress, in which there appears, however, a significant hypoglycemia. The normoglycemia observed in rats at 4 PM or after hypothalamic deafferentations might be due e.g. to the increased adrenal activity which counterbalances the hypoglycemic action of ACTH. Thus the ACTH hypoglycemic action appears like a physiological necessity, aimed at maintaining blood homeostasis. It is very possible that the pancreas also present a diurnal rhythm of its activity in the same direction as the activity of hypothalamo-pituitary-adrenal system. Causes that might determine this rhythmical activity could be the same as for the activity of the above-mentioned system, or ACTH, in its turn might be the factor which induces such modifications.

In conclusion we consider that ACTH hypoglycemic action might form a physiological mechanism worthy of note, even if in normal conditions the presence of an increased amount of ACTH has a diabetogenic action in rats, too. Yet, this diabetogenic action is indirect and it is exerted especially by glucocorticoids secreted by the adrenal gland.

We consider that hypoglycemia induction by ACTH might be achieved also by other mechanism(s) which should not require insulin participation, beside the mechanism which involve increase of insulin secretion by the pancreas.

ACKNOWLEDGEMENT

Dexamethasone was a gift from Sigma Chemical Company.

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Received December 25, 1970

"Al. I. Cuza" University
Department of Physiology Jassy

VARIATION OF THE MATURATION RATE OF THE HEMATOPOIETIC MARROW CELLS, CONSECUTIVE TO THE INTERACTION OF THE BIOFIELD AND ARTIFICIAL ELECTROMAGNETIC FIELD

BY

P. JITARIU, C. SCHNELLER-PAVELESCU
and ELENA CHERA

By employing the method of calculation meant to establish the curve variation in mathematical analyses, we assimilated the slope of the graphs used in our previous papers with the speed of maturation of cytological medullar elements. The erythrocytic and leucocytic ranges were studied and the following data were ascertained:

1. *In the erythrocytic range*: with the control group the speed of maturation increased during the first stage and went down during the second stage. The speed variation was quite the same as after 5 and 10 experiments respectively.
2. *In the leucocytic range*: with the control group the speed increased during the first stages, and went down during the last stages. After 5 experiments the speed variation was unsteady showing a lack of equilibrium in the process of maturation.

The examination of previously presented graphs, showing the dynamics of medulohematopoietic cytologic element maturation, pointed to the possibility of estimating, by calculation, the rate of this process. These graphs are not continuous curves, as they should be if the process could be followed in its numerous evolution stages. These stages cannot be followed by us, as we notice only macroscopically different cells from a morphological standpoint. It is not yet possible to know the numerous forms which differ from a biochemical and biophysical standpoint. When this fact is possible, the graph might become a curve and its systematic analysis will be undertaken.

Because of the above mentioned technical imperfections, graphs are made up of a series of straight lines, forming a broken line. They actually are a series of rectangular triangles: each segment of the graph, an element of the above broken line, is the hypotenuse of a triangle and one of the cathets is the abscissa pace, representing the time elapsed between two morphological forms. The other cathet represents the difference of height between the two ends of the segment. For clarity's sake we present in a figure one of these triangles. The hypotenuse, having at its extremities the figures 587 and 861, represents the percentage of myelocytes and metamyelocytes, two successive stages in the graph of the dynamics of granulocytic neutrophil maturation. It is noticed that AB, the vertical cathet, may be considered as equal in size to the difference between 8.61 and 5.81, i.e. 2.74, and AC, the horizontal cathet, is equal to the constant pace on the abscissa, 2 cm.

If we consider $\sphericalangle \alpha = \sphericalangle BCA$, we notice that the ratio of the two cathets, i.e.

$$\frac{\overline{BA}}{\overline{CA}} = \frac{\text{height difference}}{\text{abscissa pace}} = \frac{2.74}{2} = \frac{\sin \alpha}{\cos \alpha} = \operatorname{tg} \alpha,$$

represents the value of tangent of angle α , since we considered $\overline{BA} = \sin \alpha$ and $\overline{CA} = \cos \alpha$. As the abscissa pace is permanently considered to be 2 cm in all graphs, the relative magnitude of $\cos \alpha = 2$.

Yet, mathematical analysis shows that $\operatorname{tg} \alpha$ always represents speed, i.e., the I derivative of space depending on time; in our example, $\operatorname{tg} \alpha = 1.37$.

In our study this derivative represents the variation rate at the intersection point of two elements of the broken line of the graphs. Namely, at the end, where the slope of the line segment is changing. The variation rate during the respective stage is represented by this derivative.

This calculus was repeated for all rectangular triangles of the broken line. This method was applied only to cellular series where defined cytological stages could be marked on the abscissa and results were examined in two ways:

a) for each of the three groups of results i.e. 1/ control, 2/ after 5 experiments and 3/ after 10 experiments, maturation rates were compared stage by stage,

b) the rates of the same stage were compared in each experimental group.

This method was applied only in the erythroblastic and granulocytic series. We shall give below the tables of results of our calculations, carried out by the above mentioned method.

This table records percentage values of cells from the erythroblastic series and successive differences were calculated, to obtain the relative value of $\sin \alpha$.

Table 1
Erythroblastic series

	Proerythroblasts	I	Polychromatophil erythroblasts	II	Orthochromatophil erythroblasts	III	Orthochromatophil normoblasts	
Control	2.32	—	2.25		5.74	—	4.37	%
		0.07		3.49		1.37		sin
After 5 exp.	3.20		3.14		5.40		2.10	%
		—		2.26		—		sin
After 10 exp.	2.75		3.49		5.68		5.05	%
		0.74		2.19		0.63		sin

To find the value of $\operatorname{tg} \alpha$, we shall divide by $\cos \alpha$, whose relative value is 2 and which is recorded in table 2: Table 3 gives the value of $\operatorname{tg} \alpha$.

Table 2

Value of $\operatorname{tg} \alpha$

Group	Stage	I	II	III
	Control		0.035	1.745
5 exp.		0.03	1.13	1.65
10 exp.		0.37	1.095	0.315

Table 3

Value of angle α

Group	Stage	I	II	III
	Control		358°	60°50'
5 exp.		358°15'	48°30'	301°35'
10 exp.		20°15'	47°25'	342°32'

The examination of the maturation rate figures of the erythroblastic series, according to the data of these tables, points out the following:

Figures presenting maturation rate in stages — in the erythroblastic series we delimited three evolutive stages — are observed to be similar, in general: all experimental groups (controls, after 5 experi-

ments and after 10 experiments) present a decrease of the rate, which is more pronounced after 5 experiments and compensated after 10 experiments. (Fig. 1).

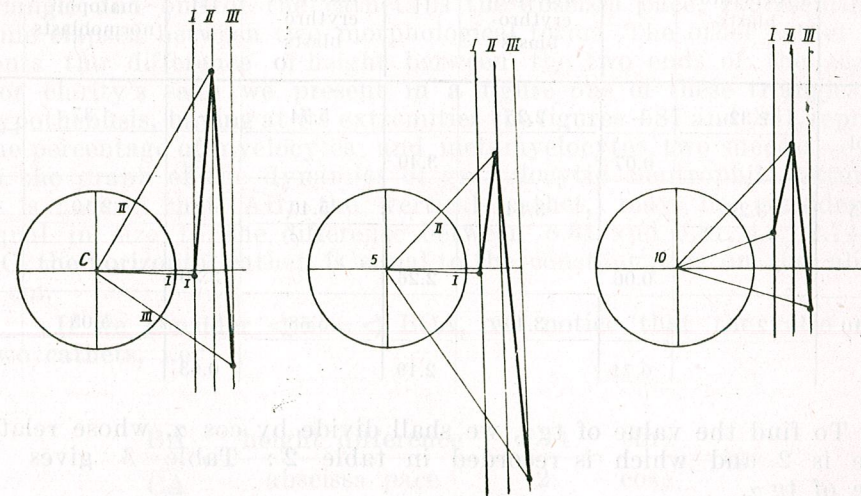


Fig. 1.—Maturation rate of the cells of the erythroblastic series by experimental groups. C, control groups; 5, after five experiments; 10, after ten experiments.

We must add, however, that since tangent variation has discontinued on the graphs, we presented the calculated angle corresponding to this trigonometric line and whose value steadily ranges from $0^\circ - 2^\circ$.

In all experimental groups, the rate increases in the second stage and then decreases. Erythroblastic cytodiasis should also be taken into account, leading to the conclusion that erythrodiabasis exceeds cytological maturation and plays a preponderant role in this stage (Fig. 2)

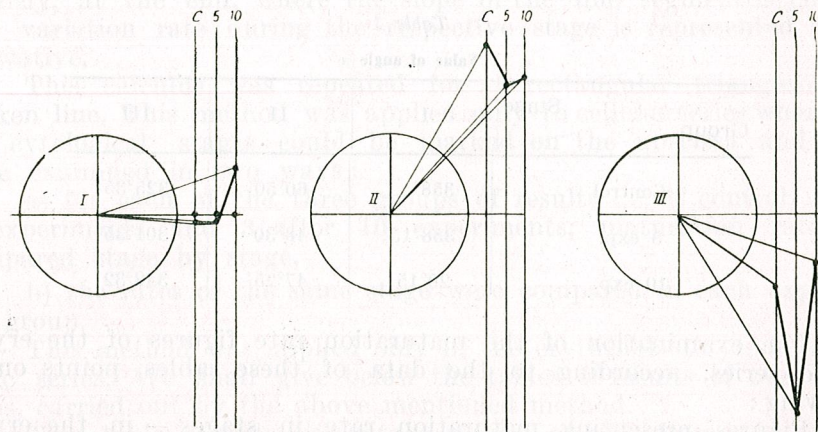


Fig. 2.—Maturation rate of the erythroblastic cells by evolutive stages. C, control group; 5, after five experiments; 10, after ten experiments; I, II, III, evolutive stages.

Table 4
Neutrophil Granulocytic Series

Stage Group	Cell Type	% sin		
		Control	5 exp.	10 exp.
I	Myeloblast	2.34%	3.00	3.48
II	Promyelocyte	3.60%	4.70	3.32
III	Neutroph. myelocyte	5.87%	6.66	7.66
IV	Neutroph. metamyelocyte	8.61	7.10	5.70
V	Neutroph. nonsegm. leucocyte	13.38	12.10	10.25
VI	Neutroph. segm. leucocyte	12.83	10.20	13.01

The same calculation method was used to the neutrophilic granulocytic series. We present further tables containing figures resulting from calculations, necessary to plot graphs for drawing conclusions (Table 4).

In the following table, the value of $\text{tg } \alpha$ is given according to the presented method (Table 5).

Table 5
Value of $\text{tg } \alpha$

Stage \ Group	I	II	III	IV	V
Control	0.63	1.135	1.37	2.385	0.275
5 exp.	0.85	0.945	0.44	2.50	0.95
10 exp.	0.08	2.17	0.98	2.275	1.38

Table 6 contains the value of angle α .

Table 6
Value of angle α

Stage \ Group	I	II	III	IV	V
Control	32°25'	48°20'	54°	67°45'	344°35'
5 exp.	40°30'	43°25'	12°40'	68°45'	316°20'
10 exp.	355°10'	65°40'	315°25'	70°	54°50'

The examination of the figures plotted according to the data contained in the tables referring to the neutrophil granulocytic series points out the following:

Out of figures containing data on the experimental groups, the control group shows a steadily increasing maturation rate in all 4 stages, followed by a sudden increase in the 5th stage, dominated by cytodiabesis (Fig. 3)

In the experimental group after 5 experiments, a great rate variation is observed from one experimental group to the other, this is plotted as a succession of increases and decreases, but for the last stage when cytodiabesis predominates and there occurs a decrease. A similar aspect is seen in the graph of the rate variation in the group with 10 experiments. It is, however, more pronounced and, therefore, the fifth and last evolution stage, dominated by cytodiabesis presents a much lower decrease. In our opinion, it is accounted for by the fact that jumps in the cytological evolution eventually result in insufficiently mature cells which cannot be released in the blood flow.

Figures with results per evolution stages show that (Fig. 4) =

- in the first evolute stage, there occurs a rate increase, followed by a sudden decrease, under the initial level;

- in the second stage, there occurs first or slow rate increase, followed by its fast increase;

- in the third stage there occurs a rate decrease and

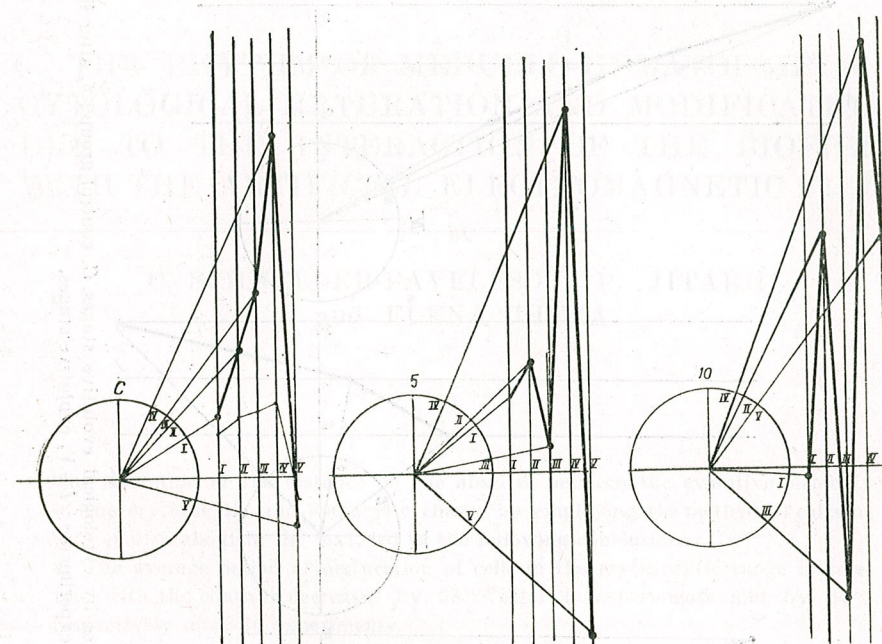


Fig. 3. — Maturation rate of neutrophilic granulocytes, by experimental groups. C, control animals; 5, after five experiments; 10, after ten experiments; I, II, III, IV, V, evolutive stages.

- in the fourth stage, there occurs an important compensatory rate increase;

- in the fifth stage, after decrease of the maturation rate there occurs its compensatory increase.

As observed so far, the granulocytic series is very sensitive to the action of electromagnetic radiations, reacting either by the increase of the maturation rate or by its decrease. These variations are rapid and pronounced, pointing out that the radiation sensitivity of the hematopoietic medullary tissue is high.

It is very difficult to put forward hypotheses concerning the mechanism of this important medullary reactivity but this very fact leads to the conclusion that body homeostasis does not allow great disturbances in the hematopoietic function and in the balance depending on it, in the case of excitations produced by the action and interaction between the biofield and the artificial electromagnetic field.

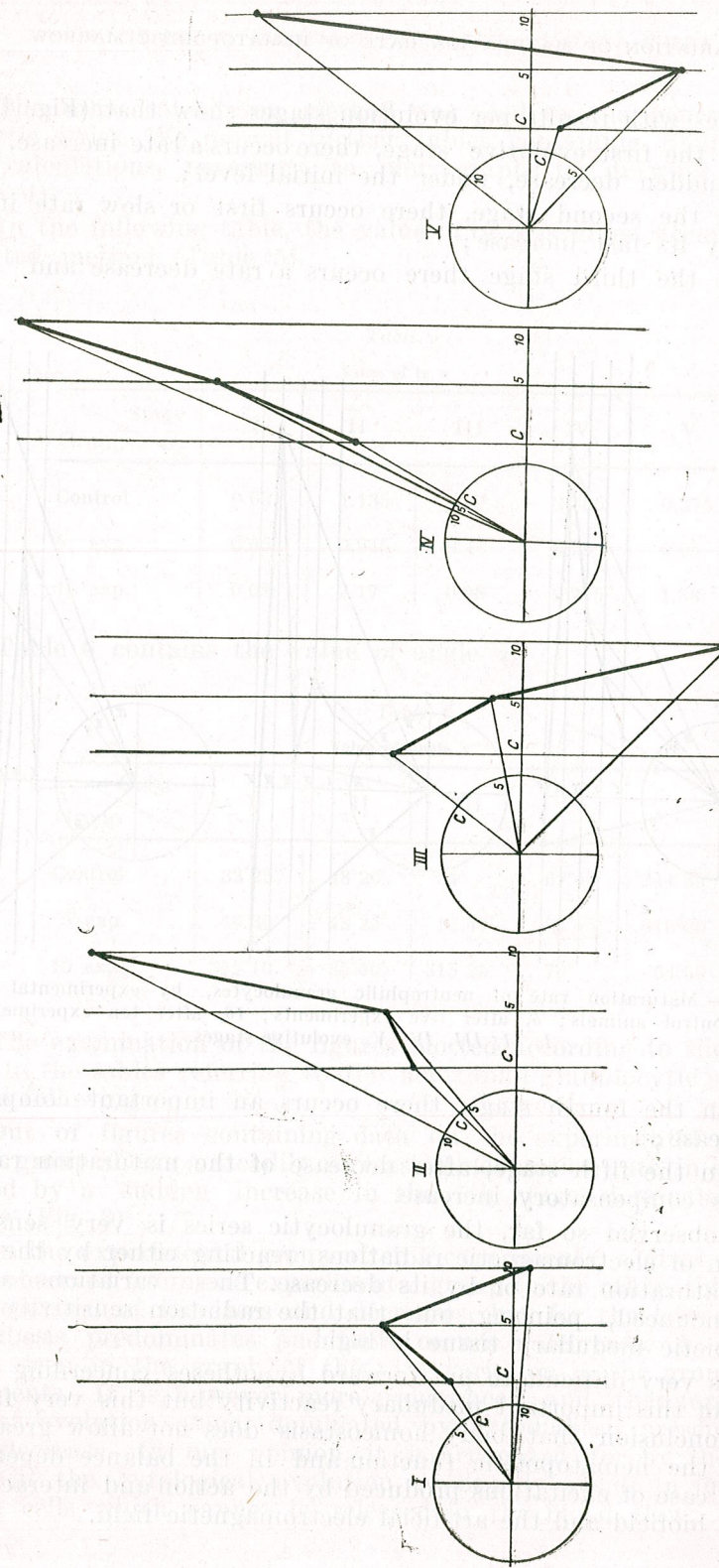


Fig. 4. — Maturation rate of the neutrophilic granulocytes by cytological evolutive stages. C, control group; 5, after five experiments; 10, after ten experiments; I — V, evolutive stages.

THE RHYTHM OF MEDULLO-HEMATOPOIETIC CYTOLOGICAL MATURATION AND MODIFICATIONS DUE TO THE INTERACTION OF THE BIOFIELD WITH THE ARTIFICIAL ELECTROMAGNETIC FIELD

BY

C. SCHNELLER-PAVELESCU, P. JITARIU
and ELENA CHERA

The alteration of the distance on the abscissa between the evolutive elements of the erythrocytic and leukocytic charge by employing the method of calculation spoken about in the text, led to the following conclusions :

1. The average period of maturation of cells in the erythrocytic range in relation with the controls decreases by 58% after 5 experiments and by 64% respectively after 10 experiments.
2. The average period of maturation of neutrophilic-leukocytic cells in relation with the controls decreases by 9% after 5 experiments, and increases by 225% after 10 experiments.

Part IV of our work has presented the dynamics of cytological maturation in the hematopoietic medullary tissue. On that occasion we used plane rectangular Cartesian nomograms, where the ordinate axis recorded percentage variation and the abscissa axis indicated the evolutive stage of the respective cell. The 0 value of the abscissa was represented by the youngest cell, from a morphological standpoint; the other stages were established at equal distances from one another, up to the adult or almost adult cell, on the point of being released into the circulating flow.

A more careful consideration of this way of nomographic presentation led us to the conclusion that this axis may actually be assimilated to the time axis. In fact, maturation, starting with the youngest

cell up to the adult one, always occurs in a certain lapse of time which is known so far only with great approximation, opinions and data varying with each author. On the other hand, equal stages between different morphological forms of the cell of the respective series cannot correspond to the actual course of the maturation process, as stages are unequal and, moreover, the number of intermediary elements included between the youngest cell and the adult one are not yet fully established. In our case, when the study was carried out on stained (hence fixed) slides, it goes without saying that the fact is self understood. In our opinion, in this case it is necessary to follow *in vivo* the medullary maturation, which could not be achieved so far.

The present work is an attempt at establishing it by a mathematical way and giving relative solutions as against the first evolutive stage, for erythroblastic and neutrophil leukocytic series. We give below our calculus principle. We must point out the fact that this calculation was based on myelograms obtained before and after exposure to the electromagnetic field. These myelograms are presented in previous notes.

In general, graphs had the following aspect, already described above (Fig. 1).

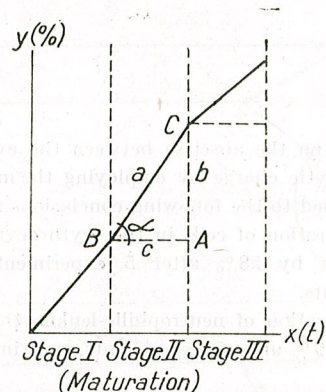


Fig. 1.

As may be seen, there is a broken line. By drawing parallels to the abscissa in the points of intersection with perpendicular lines on O_x , we notice a series of successive rectangular triangles, similar to evolutive stages. We have replaced this series of indifferently rectangular triangles by a series of isoscelic rectangular triangles, using the following way of calculus:

Let us assume ABC of figure 1, where: $\hat{A} = \pi/2$ (90°). Its area will be: $S = \frac{bc}{2}$ or $2S = bc$; if we consider angle α , then $b/c = \text{tg}\alpha$.

When $\alpha = 45^\circ$, then $\text{tg}\alpha = 1$ and $b = c$.

Replacing in this formula, we obtain:

$$b^2 = c^2 = 2S,$$

hence $b = c = \sqrt{2S}$

In our case c represents the time factor and b represents the % difference between two successive stages.

We have applied this calculation method only to the erythroblastic and neutrophil leukocytic series; we list below two tables with the obtained results (Tables 1 and 2).

These tables include: 1) a heading "cm" under which the new value of the abscissa pace is recorded and appears not to be constant and 2) a heading *Biol. U(t)*, where biological time units necessary to maturation were calculated. Those values were relative, since we considered that stage I has the value 1.

There are still other 2 headings: 3) the total of biological time units for the whole maturation and 4) the percentage variation of time, considering as origin the temporal value of the control.

Table 1
Time values of the erythroblastic series cytological maturation
E series

Group	cm			Biol. U(t)			Total Biol. U(t)	Variation %
Control	0.415	2.95	1.84	1	7.21	4.43	12.64	0
5 exp.	1.24	2.37	2.87	1	1.91	2.31	5.22	-58.71
10 exp.	1.36	2.33	2.39	1	1.71	1.75	4.46	-64.72

Table 2
Time values of maturation by cytological stages of the neutrophil leukocytic series
Ln series

Group	cm					Biol. U(t)					Total Biol. U(t)	Variation %
Control	2.037	2.38	2.61	3.45	1.17	1	1.16	1.28	1.69	0.57	5.90	0
5 exp.	2.06	2.21	1.01	3.53	2.17	1	1.07	0.49	1.71	1.05	5.32	-9.84
10 exp.	0.63	3.29	2.21	3.37	2.62	1	5.22	3.50	5.34	4.15	19.21	+225.59

For a clearer representation of the rhythm of these maturations we thought it is necessary to plot data included in the erythroblastic maturation table. From the examination of these graphs there appears that the interaction of the biofield with the artificial electromagnetic field results in the decrease of the maturation time in series submitted to 5 exposures and to 10 exposures, as compared to the control (Fig. 2).

The graph of the rhythm of neutrophil leukocytic maturation clearly shows that after 5 sittings time decreases insignificantly as compared to the control and after 10 exposures to the artificial electromagnetic field there occurs a prolongation by 225% (Fig. 3).

These two variations of the maturation time are plotted simultaneously in figure 4.

For the time being we cannot find an interpretation of our calculation modality.

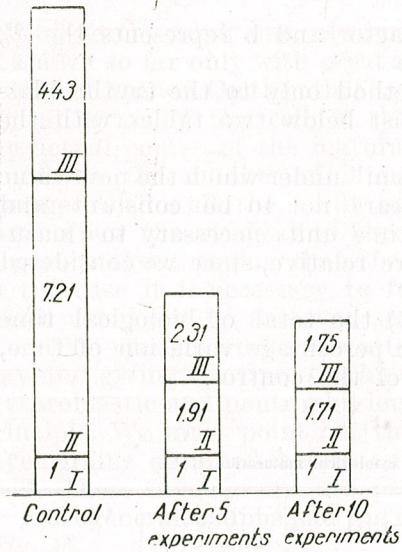


Fig. 2. — Rhythm of erythrocytic maturation. *I*, from the proerythroblast to the polychromatophil erythroblast; *II*, from the polychromatophil erythroblast to the orthochromatophil erythroblast; *III*, from the orthochromatophil erythroblast to the orthochromatophil nomoblast. 1 cm = Biol. U(t)

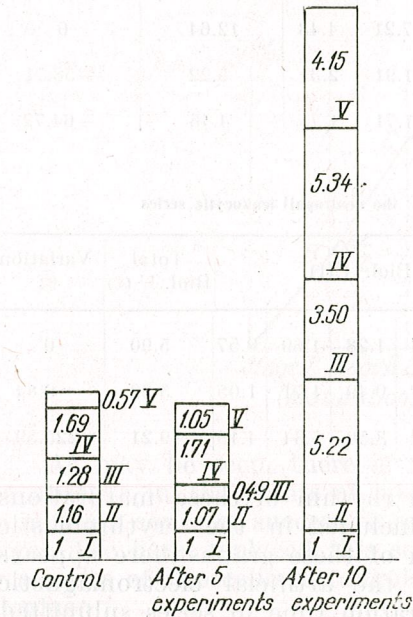


Fig. 3. — Rhythm of neutrophil leukocytic maturation. *I*, from the myeloblast to the promyelocyte; *II*, from the promyelocyte to the neutrophil myelocyte; *III*, from the neutrophil myelocyte to the neutrophil metamyelocyte; *IV*, from the neutrophil metamyelocyte to the neutrophil nonsegmented leukocyte; *V*, from the neutrophil nonsegmented leukocyte to the neutrophil segmented leukocyte.

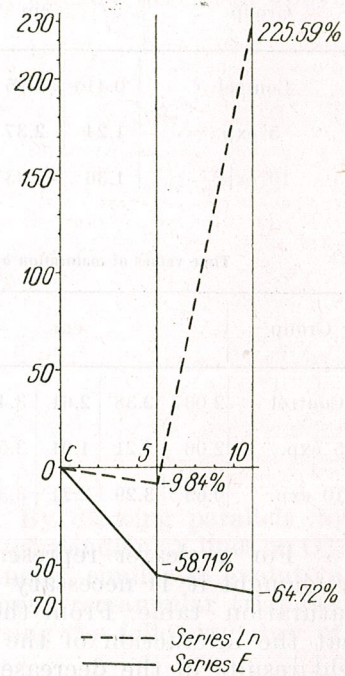


Fig. 4. — Variation of the maturation time of erythrocytic and leukocytic series.

INFLUENCE OF COLCHICINE AND THEOPHILIN COMBINED WITH AN ELECTROMAGNETIC FIELD ON THE FIRST DIVISIONS OF TUBIFEX EGGS

BY

R. BRANDSCH and P. JITARIU

With Tubifex eggs treated with colchicine and theophillin and an electromagnetic field of 300 Oe an increased percentage of divided eggs compared with eggs treated with the same concentrations of colchicine and theophillin but in the absence of the field is to be noticed. This is supposed to be due to a modified permeability of the egg membrane to these substances under the action of the electromagnetic field used.

In a previous paper [5] we reported a certain delay of the cleavage of Tubifex eggs exposed to an electromagnetic field of 300 Oe. In the attempt to find out on which processes the used electromagnetic field may act, we treated eggs with colchicine or theophiline, on the one hand, and with colchicine or theophiline together with an electromagnetic field, on the other. We proceeded in this way knowing that the permeability of the cell membrane of Tubifex eggs shows a progressive increase towards the second division [6], [8] with a main increase in the period before the first cleavage [8]. Both substances, colchicine and theophillin, act on the microtubules dissolving them, thus inhibiting cell division [1]. If we now suppose that the delay in the first divisions of eggs treated with an electromagnetic field is caused by modifications of biosynthetic processes induced by the field, then we would expect that treatment of eggs during the period before the first division shows the largest effect. If on the other hand we suppose that the action of the electromagnetic field is mainly one on the cortical structures involved in cleavage, this must result from the differences

in cleavage inhibition between eggs treated with chemical substances and an electromagnetic field and eggs treated with chemical substances alone.

From the results obtained by comparing the action of colchicine and theophiline alone and in combination with an electromagnetic field we reached the conclusion that the action of the field consists mainly in a modification of the cell membrane's permeability and not in a modification of the biochemical processes of synthesis.

MATERIALS AND METHODS

The electromagnetic field of 300 Oe was generated by an electromagnet described in [10]. Eggs freed of their cover under a stereomicroscope were grown in physiological solution after Lehmann [7]. They were divided into two groups, the first including eggs before the second reductional division, while the second group consisted of eggs after the second reductional division.

Treatment with colchicine and with colchicine combined with the electromagnetic field was performed after the second division of maturation, at various times corresponding to the beginning of the first mitotic division and lasted until the end of the first mitotic division. The period between the end of the second reductional division and the beginning of the first mitotic division was subdivided into 6 groups of treatment, the treatment being 30 minutes shorter for one group than for the previous. The concentration used was of a part colchicine to 1000 parts physiological solution.

Theophiline was used in a dilution of 5:1000, also in physiological solution. The two groups of eggs treated at various times before and after the second reductional division were subdivided each in 4 subgroups according to whether there was no mitotic division, one mitotic division, two mitotic divisions, or more than two mitotic divisions.

Exposure of eggs before and after the second reductional division to theophiline and the electromagnetic field lasted until the second mitotic division.

Eggs were kept at a temperature varying between 18°–19°C. Every 30 minutes the eggs were examined with a stereomicroscope for morphological modifications of division. 271 egg cells were examined in the case of colchicine and 163 in the case of theophilin.

RESULTS AND DISCUSSION

Lehmann and Woker [6] show that the sensibility of Tubifex eggs to colchicine increases towards the second mitotic division. They also could show that the increased sensibility is due to an increase of the permeability of the egg membrane to colchicine and that the cytostatic concentrations range between 1:500 to 1:3000. For the concentration of 1:1000 used by us, they found a 50% block of the first division if the eggs are put in the colchicine solution before the second reductional division and no block if they are treated after the second reductional division, the second mitotic division being blocked in all cases.

With our material we observed an almost total inhibition of the first mitotic division at this concentration, if the eggs were treated before the second reductional division and they were partly blocked if treated after the second reductional division.

Eggs treated with colchicine only divide to a lesser extent if treated immediately after the second reductional division, and show greater percentages of dividing with the approaching of the first mitotic division. Hence a role is played also by the length of exposure to colchicine previous to the first mitotic division. But the differences between the various groups of treatment remain insignificant, showing that the period of increased permeability before the first cleavage is decisive for the block of mitosis, a period all eggs have passed through (Table 1).

If exposed to colchicine and the electromagnetic field, the situation is similar to that of treatment with colchicine alone, thus a smaller number of cells divide after a longer treatment, number which increases towards the first mitotic division, with a significant rise between 150 and 120 minutes of treatment. The other differences between the percentages of dividing cells of different groups of treatment remain insignificant (Table 1).

Table 1

Time of treatment in minutes	Colchicine					
	180	150	120	90	60	30
% of cleaved egg cells treated with colchicine and EMF and statistical significance of their difference	8 unsig.	30 sig. p < 0.05	76	68 unsig.	83 unsig.	87 unsig.
% of cleaved egg cells treated with colchicine only and statistical significance of their difference	8 unsig.	25 unsig.	20	17 unsig.	46 unsig.	52 unsig.
Difference of % of cleaved eggs treated with colchicine + EMF and with colchicine only	unsig.	unsig.	sig. p < 0.01	sig. p < 0.01	sig. p < 0.05	sig. p < 0.05

But important is that the number of dividing eggs is significantly greater in the case of treatment with the electromagnetic field and colchicine than with colchicine alone. This holds true for each group of treatment, beginning with the group of 120 minutes.

On the one hand the course of mitotic division block of eggs treated with colchicine and electromagnetic field which parallels in essence that of eggs treated with colchicine only, and on the other hand the significant difference in the degree of block of mitosis between the two groups lead us to the assumption that a decrease of the permeability of the egg membrane for colchicine following the exposure to electromagnetic field is responsible for the reported difference. This supposition is also supported by the observation of others that magnetic and electromagnetic fields lead to modifications of permeability. So Mueller and Jitariu [10] could show that the permeability of the frog tegument

after Ussing, is modified following exposure to the action of a physical electromagnetic field, which is also true for a magnetic field as shown by Bianchi, Carraro and Gualti-Rotti [4].

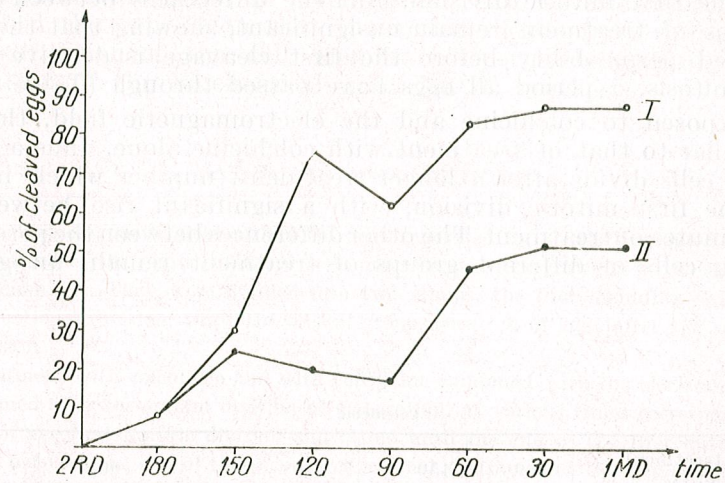


Fig. 1. — Variation of the number of cleaved egg cells with time of treatment, from the second reductional division (2RD) to the first mitotic division (1MD). I = egg cells treated with colchicine and EMF, II = egg cells treated with colchicine only.

For theophiline and electromagnetic field, the percentages of eggs of different groups and the significance of the differences between them, treated both with theophiline alone and with theophiline combined with an electromagnetic field, are given in table 2 and represented graphically in figure 2.

Table 2
Theophiline

No. of performed divisions	0 D		1 D		2 D		> 2 D	
	theoph.	theoph. + EMF	theoph.	theoph. + EMF	theoph.	theoph. + EMF	theoph.	theoph. + EMF
% of cleaved eggs if treated before 2RD and significance of their difference	12	0	82	66	5	9	0	28
	unsig.		unsig.		unsig.		sig. p < 0.001	
% of cleaved eggs if treated before 1MD and significance of their difference	8	3	58	49	34	26	0	21
	unsig.		unsig.		unsig.		sig. p < 0.001	
Difference of % of cleaved eggs treated before 2RD and before 1MD	unsig.	unsig.	sig.	unsig.	sig.	unsig.	unsig.	unsig.
			p < 0.02		p < 0.002			

From these it can be seen that eggs put in a solution of theophiline after the second reductional division show a shift towards the second mitotic division as compared with eggs put in theophiline solution before the second reductional division, without any case of more than two divisions. Thus we have a significant decrease of eggs dividing only once and a significant increase of eggs dividing twice if treated after

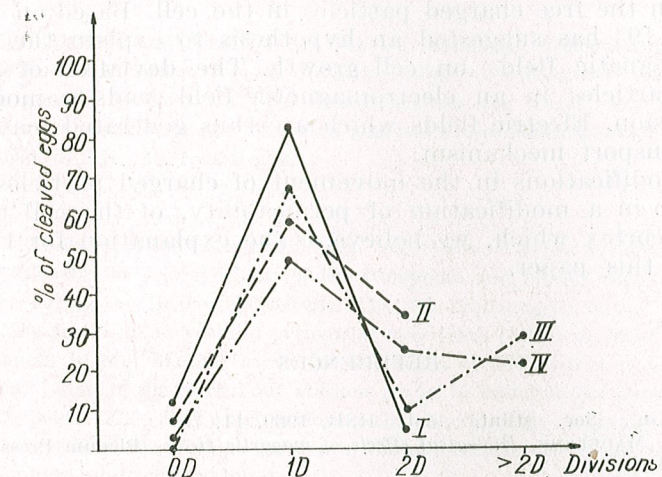


Fig. 2. — Number of egg cells cleaved and number of performed divisions as function of onset of treatment. I = treated with theophiline before the second reductional division (2RD), II = treated with theophiline before the first mitotic division (1MD), III = treated with theophiline and electromagnetic field (EMF) before the 2RD, IV = treated with theophiline and EMF before 1MD.

the second reductional division. In the case of theophiline it can also be seen that the shorter the time of treatment the greater the possibility of cleavage, which is evident if we admit a certain permeability of the egg membrane to theophiline, which will accumulate proportionally to the time of exposure of the egg, resulting in a greater or lesser block of the mitotic division.

In the case of treatment with theophiline and an electromagnetic field the shift of eggs to more rounds of divisions becomes more obvious. The number of eggs which divide only once is even smaller, but beside eggs dividing twice there appear also others which perform more than only two divisions leading to stages of 3, 4, 5 and even 10 cells.

With theophiline we can suppose the same mechanism of action of the electromagnetic field used, namely a reduction of the membrane permeability to theophiline, which would lead to a lesser inhibition of cleavage, expressed in more rounds of divisions.

There might also be another possibility of explaining these results by admitting a modifying action of the electromagnetic field used in the interaction of these substances with the substratum on which they act, in this case represented by microtubules. Changing, for instance,

the parameters of the reaction between these substances and the spindle proteins, the action of the field could lead to preserving a greater number of functional spindles and thus to a greater number of divisions. But taking into account the small energy of the electromagnetic field used compared to the energy required for changing a chemical reaction [2] [3] [11] [12], this latter possibility is much more unlikely. The effect of the electromagnetic field used may be more easily explained by an orienting action on the free charged particles in the cell. Based on this possibility Liboff [9] has suggested an hypothesis to explain the inhibiting action of magnetic fields on cell growth. The deviation of the paths of charged particles in an electromagnetic field leads to modifications of their diffusion. Electric fields which are thus generated may simulate an active transport mechanism.

Such modifications in the movement of charged particles must be expressed also in a modification of permeability, of the cell membrane and the egg cortex which, we believe, is the explanation for the results presented in this paper.

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Received February 17, 1971

Biological Research Centre, Jassy
Department of Animal Physiology

MIHAI BACESCU, GEZA MULLER și MARIAN T. GOMOIU, *Ecologie Marină. Cercetări de ecologie bentală în Marea Neagră. Analiza cantitativă, calitativă și comparată a faunei bentale pontice.* (Marine Ecology. Researches on the Black Sea benthal ecology. Quantitative, qualitative and compared analyses of the Pontic benthal fauna). Vol. IV, Ed. Acad. R.S.R., 1971, 352 pag.

The IVth volume of the "Marine Ecology" represents — as the authors themselves have stated in its introduction — a synthesis of the Romanian and foreign researches achieved in the Black Sea during the 1960—1970 decade. The rich bibliography of 267 titles is showing this as well. The results of 15 years of permanent researches (taken at fixed periods of time) on the Romanian littoral benthos as far as the depth of 200 metres, is forming the main part of the work; most of the carried out stations are to be found in the annex — an impressive table of 79 pages (272—352) which is closing the volume. This annex is particularly useful, it giving for everyone of the stations the depth, the zoobenthos, the temperature, the salinity as well as the phyto- and zooplankton quantities.

After a short characterization of the Black Sea environmental conditions — very different from the other seas ones — of the physiogeography of both the Romanian littoral and continental platform, the authors dedicated a special chapter to the benthal biocenology (p. 37—111). The bibliographical data they contain are critically presented. After having defined the five levels of the Black Sea: the super-, medio-, infra-, circa and periazotic littoral, and having characterized them in the Pontic basin conditions, they made a synthesis on the biocenoses and the associations of the sand and stone zones, of the silt infralittoral and circalittoral zones and of the periazotic level zones as well.

As concerns the infralittoral biocenoses, an extensive study is given for the fine sands biocenoses containing *Corbula mediterranea* — very important in the food of the fishes having economic value, and on the *Mytilus galloprovincialis* — *Actinia equina* biocenose, this last being characterized by its homogeneity and by being localized in the inferior strip of the rocky littoral.

Then the specific Black Sea biocenoses are analyzed, the benthal mussels biocenose dominated by *Mytilus galloprovincialis* var. *frequens* which according to its distribution area on the Romanian littoral finds itself on the 2nd place, while *Modiolus phaseolinus* biocenose is on the first place.

A compared analysis of the continental platform biocenoses of the western Black Sea littoral (Romanian and Bulgarian coasts) allow the authors to particularly insist on the halloccenose of the fields of *Phyllophora*, an algae with highly industrial importance.

The Mediterranean biocenose presentation of the prebosphoric circalittoral shelf is based, in its most part, on the dredges and on the personal authors' observations. The role

played by this sector as an „important preontic acclimation zones” for the Mediterranean immigrants penetrating the Black Sea through the Bosphorus, is also emphasized.

A special attention is paid also to the microbenthos study, some of the subcenoses being characterized by Foraminifera, Harpacticidae, a.s.o.

The largest chapter of the work (p. 112—257) is dedicated to the faunistic aspects inventory of the benthal invertebrates of the Pontic basin. The qualitative analysis of the benthal fauna reveals the presence in the Black Sea of the more than 1500 species (about 1800 when considering those from the Salmastrian annexes), of macro- and microbenthos. In the lists and in the synoptical tables there were enumerated 311 Protozoa, 38 Spongia, 40 Cœlenterata, 130 Turbellariata, 49 Nemerthians, 23 Gastrothricha, 10 Kinorhyncha, 33 Rotifera, 148 Nematodes, 192 Polychets, 7 Archiannelids, 35 Oligochets, 15 Hirudinea, 175 Molluscs, 1 Sipunculida, 186 Copepoda, 2 Cladocera, 77 Ostracoda, 5 Cirripeda, 107 Amphipoda, 6 Tanaisida, 34 Isopoda, 23 Cumaceans, 21 Mysids, 36 Decapods, 8 Tardigrades, 4 Hydrocariens, 25 Halacarids, 3 Pantopods, 8 Insects, 20 Bryozoa, 1 Entoprocta, 3 Phoronida, 14 Echinoderms, 11 Tunicates, 1 Cephalocordata.

The chapter ends with a zoogeographical analysis of the different groups of Invertebrates of the species, living in the Mediterranean, Adriatic and Black Seas, illustrated by two conspectuses.

The discovery in the Black Sea of the presence of 98 pontocaspian relict invertebrates which manifest preferences for the Salmastrian annexes of the Pontic basin, is very interesting indeed.

The work, as a whole, is very impressive by the vast documentation and by the richness of data, the more so valuable as they represent original results, most of them unpublished ones, from the researches achieved in over 1000 stations, all along the north-western littoral of the Black Sea, between Odessa and Bosphorus.

The IVth volume of the “Marine Ecology”, an eminently zoobenthic ecological work completed with faunistic lists and zoo- and paleozoogeographical considerations, succeeds in pointing out the great theoretical and economic importance of the benthal fauna and of the main Black Sea biocenoses. Very impressive are also the quantities of the benthal fauna found by the authors (i.e.: the average zoobenthic weight, including the microbenthos, in the *Corbula*-cenose, Mamaia zone, is oscillating around the value of 350 g/m², but the maximums can surpass 1500 g/m²) as well as their considerations on the important food resource represented by the Black Sea for the benthic fishes.

The distribution of the biocenoses throughout the western sector of the Black Sea and the distribution of the various animal groups are shown in the 20 original maps illustrating the text; of a singular importance is the coloured map of the north-western Black Sea biocenoses (fig. 3), as well as that of the zoobenthic biomasses distribution along the Romanian littoral (fig. 5), illustrated according to the main groups of animals, unique of its kind in the biological oceanologic literature.

The benthal fauna of the Pontic basin greatly surpasses the necessary quantity of food required by the benthal fishes that are fished in the Black Sea, the authors reaching the conclusion that the populating of this basin with benthofagous fishes brought from other seas is quite peremptory.

Valeria Mack-Firă

A V I S A U X A U T E U R S

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

Les auteurs sont priés d'envoyer leurs articles, notes et comptes rendus dactylographiés à double intervalle (31 lignes par page), en quatre exemplaires.

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PRINTED IN ROMANIA