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KATERYTHROPS S.GEN *AMATHIMYSIS BRASILIANA* N.SP. AND *MYSIDOPSIS SANKARANKUTTYI* N.SP. OF THE NE BRAZILIAN LITTORAL WATERS

BY
MIHAI BĂCESCU

Four species of Mysids collected at Potengu River mouth, near Natal (5°46′ S ; 35°12′6″W ; 3—0 m), were identified. Two of them (*Bowmaniella brasiliensis* Băc. 3/3 and 18. IV. 1979 and *Siriella chierchiae* — St. 19.IV.1979) are already known from the Brazilian waters. Two species *Katerythrops* (s. gen. *Amathimysis*) *brasiliana* n.sp. and *Mysidopsis sankarankuttyi* n. sp. are now described.
Six samples included only juveniles of *Siriella* and *Gastrossacini* which were not identifiable as species.

Fifteen species of Mysids from the Brazilian waters have been known so far, genus *Bowmaniella* including the largest number (4 species).

In a material collected not far from Natal, the easternmost point of Brazil, and sent to me for study by Prof. Dr. Sankarankutty, I have identified 4 species of Mysids, 2 of which have already been signalled in the Brazilian waters (*Bowmaniella brasiliensis* Băcescu and *Siriella chierchiae* Coifman) **(1,3,5)** and 2 new for science.

I am giving below the description of the new species :

1. KATERYTHROPS (AMATHIMYSIS) BRASILIANA N.SP.
(Fig. 1,A—M)

Diagnosis. Erythropini of small size (2.6—3 mm ♂♀), dumpy, with a strong difference in diameter between carapace and abdomen, with an antennal scale hardly three times longer than wider, with big eyes without papilla and visual portion wider than peduncle.

Telson of *K. (K.) resimora* O. Tattersall 1955 type, but hardly longer than wider ; it shows 2 lateral apical short spines which border the 2 long median phanera 7 times longer than the spines.

Material: 1 preadult ♂, 1 juv. ♀, 3 ovigerous ♀♀, 2 preadult ♀♀ and 3 juv. ♀♀ and ♂♂, all collected in the night plankton (5 stations), with and without light at 3 m deep (most of the specimens were collected on February 2, 1980, at 6 o'clock in the morning). The periodical captures (April 1979 — March 1980) were made from the same site in the easternmost point of Brazil, not far from Natal, at the mouth of the Potengy River (5°46′S ; 35°12′6″W).

Description. (♀♂). Tegument soft, glabrous, shiny. Cephalotorax clearly wider than abdomen (Fig. 1 A) without other prominences than

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those limited by the cervical sulcus which is well marked deep, with curved frons ending in 2 small antero-lateral corners; its posterior margin slightly excavated, smooth keeps free a whole thoracic segment. Eyes slightly pyriform, without dimorphism and devoid of papilla on peduncle; corneal portion brown-black, wider than peduncle, occupying 1/3 of the eye (Fig. 1 B). Only in the youngs of 1 mm, cornea is narrower and smaller than peduncle, reminding of *K. tattersalli* Illig 1906.

Antennula with vigorous basis, with a single distal inner seta without spines (Fig. 1 A and B). Antenna with thick peduncle, as long as the basis A_1, with a short and wide scale: 3.5 times longer than wider (in ♂ – Fig. 1 C), and 2.8 times in ♀ (Fig. 1 D) uncommon for a *Katerythrops;* however, by its long peduncle it exceeds the level of the basis of antennula and even the ♂ lobe (Fig. 1B).

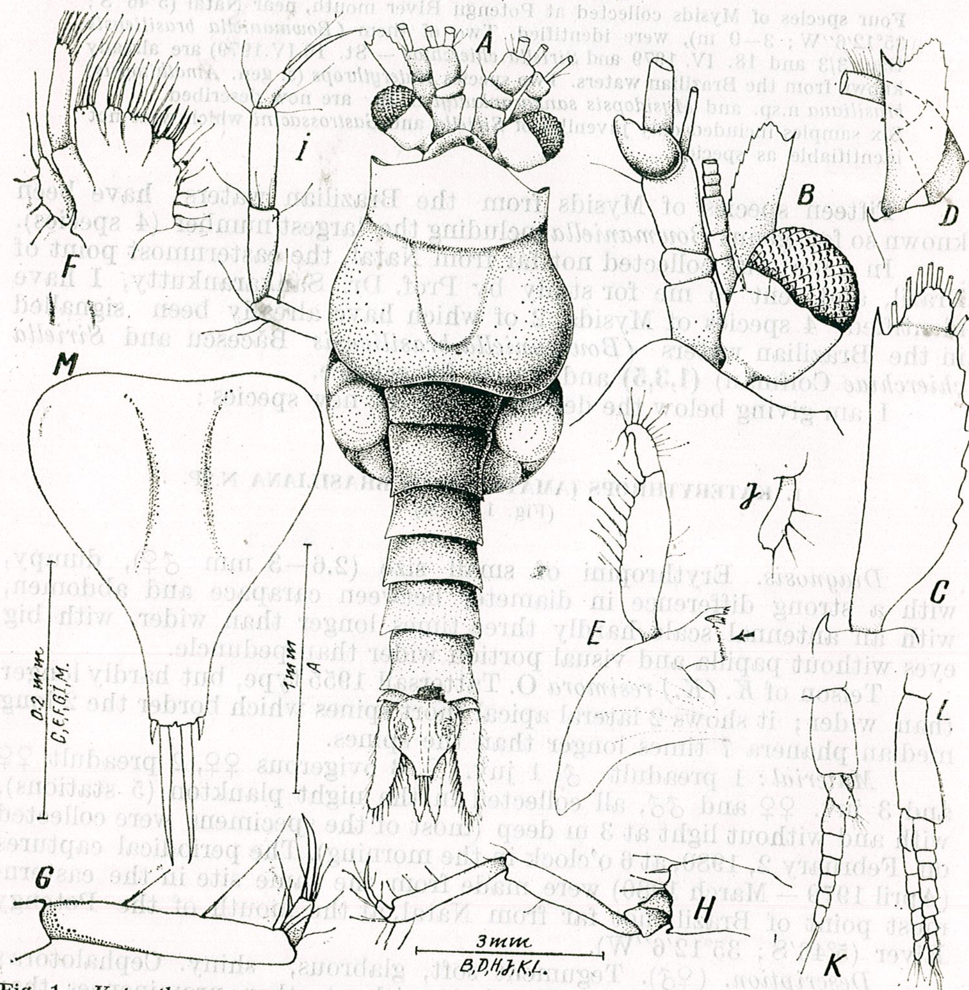

Fig. 1.—*Katerythrops (A) brasiliana* n.sp. A, ♀, tergal view; B, cephalic area, magnified; C, scale A_2 ♂; D, ditto, ♀; E, mandible; F, maxilla; G, maxillipede I; H, maxillipede II; I, peraeopod II (juv.); J, pleopod I ♀; K, pleopod I ♂; L, pleopod II ♂; M, telson.

Mandible, with ordinary 2-jointed palp, shows a pars masticatoria, which is characteristic of the genus; pars incisiva made up of two small tri-dentate practically non-chitinous plates (arrow) is continued by a straight edge with fine cilia (Fig. 1 E). This mandible reminds us of the similar organ in *Caesaromysis* (Illig. Fig. 131), but it is continued neither by lacinia mobilis nor by denticules, but by simple short cilia.

Maxillula common, with very narrow lobe. Maxilla as shown in Fig. 1 F. Maxillipede 1 differs a little from the second one (Fig. 1 G and H) with propodus as long as carpus.

Peraeopods lack in all adult specimens, which indicates besides the length, their great fragility; in juv. specimens (1–1.5 mm) they persist and show a 2-jointed propodus (Fig. 1 I). Females show 2 pairs of oostegites, the last one being enormously large. The ♀ pleopods are reduced to an oval little plate (Fig. 1 J); the ♂ pleopods with both rami long, pluri-jointed (Fig. 1 L) except for the first one which shows a rudimentary exopodite (Fig. 1 K).

Uropods short and wide (Fig. 1 A): exopodite hardly 4 times longer than wider, and endopodite only 3 times, [8], like *K. (K.) brattegardi* [3]: 4.2 respectively 3.6 [8] which is another morphological achievement as compared to the other species (in *K. resimora* O. Tattersall 1955, they are 7 and respectively 10 times longer than wider; in *K. (K.) triangulata* Pan. [6], 8 resp. 4.4 and in *K. oceanae* Holt. & Tatt. W. 1905, they are 6 and 11 times, respectively, longer). Endopodite, slightly longer than exopodite, with an immense statocyst, with reddish statolith with a golden aureola in the only specimens with which it was not dissolved by the (although neutralized) formalin in which it was preserved.

Telson (Fig. 1 M) triangular-cordiform, either of *K. (K.) resimora* or *K. (A.) brattegardi* type, hardly longer than wider (on the drawing made with the help of camera lucida it measured 95 mm up to the apex (130 mm with setae included) while towards basis it was 80 mm wide). Apex truncate, limited by 2 short thorns 7 times shorter than the 2 long median glabrous phanera. The strong proximal widening of telson covers exactly the area of immense statocysts.

Ovigerous ♀: 2.8–3 mm long; preadult ♂: 2.8 mm long.

Holotype: ovigerous ♀ under No. 580, in the Coll. of the "Grigore Antipa" Museum. Allotype, ♂ 1♀ paratype and 1 juv. paratype, ibid, no. 581; 2 ♀♀ paratypes in coll. Depto. de Oceanografia e Limnologia Univ. Federal de Rio Grande, Natal, Brazil.

Remarks. No sexual dimorphism in telson and uropods. Only ♂ lobe (glabrous in our incompletely adult ♂) and biramous pleopods represent dimorphic features of the external morphology of the Mysid. The enormous marsupial pouch (Fig. 1 A) contains 10–12 big golden spherical eggs. *K. (A.) brasiliana* singles out among the other 5 known species both by its external morphology and by its biology. It is by far the smallest signalled species (2.8–3 mm, compared to 5–12 mm in the others); it shows no trace of ocular papilla and particularly it has not a long narrow curved antennal scale, but a short wide scale with an almost straight outer edge although, due to the length of the basis of antenna it exceeds the basis of antennula. Consequently, the criterion of generic value of the scale is not valid.

As far as its ecology is concerned, considering the captures, it seems to be a littoral shallow water species (3—45 m deep) from coarse grey sand *(K. A. brattegardi)* and probably corallicolous, but with nycthemeral movements, while all the others are deep oceanic species (*K. resimora*, 850—1 100 m; *K. tattersali*, 1 500 m, *K. oceanae*, deeper than 1 500 m.

It is the first species of Erythropini cited for the Brazilian waters.

2. MYSIDOPSIS SANKARANKUTTYI N. SP.

(Fig. 2, A—D)

Diagnosis. Small-sized mysid (2.6 mm ad. ♀) characterized by a linguiform telson with only 4 big apical spines equal in length and 2 pairs of little subterminal spines. Carpopropod 2-segmented and uropodal endopodite with 4—5 curved sharp spines occupying its middle area.

Material. 1 ad. ♀ and 1 juv. collected at Nepal, from the fixed point described for the preceding species, between 3 and 5 m deep, at 18 o'clock, 3/3 : 80.

Description. Rostrum rounded, short hardly exceeds the basis of ocular peduncle, eyes large, with corneal part occupying half of their area (Fig. 2 A).

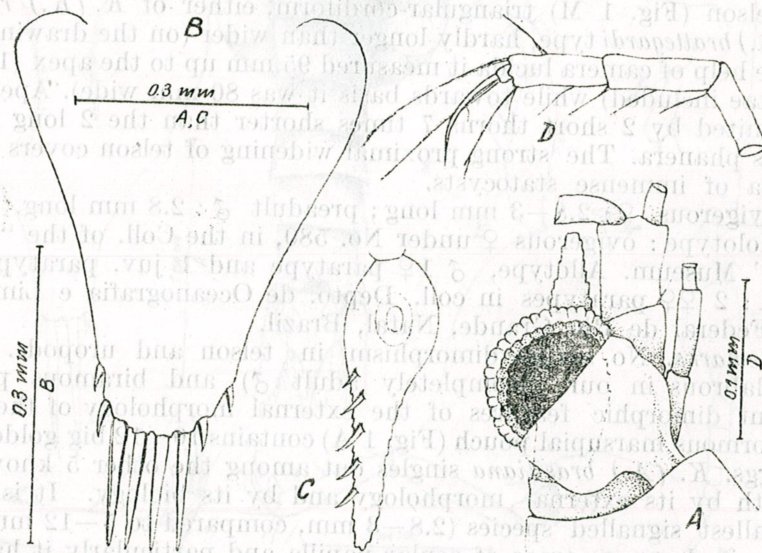

Fig. 2. — *Mysidopsis sankarankuttyi* n.sp. A, ♀, the anterior side of head; B, telson; C, the endopodite of uropod; D, the tarsus of peraeopod II (juvenile).

Telson (Fig. 2 B) linguiform, triangular, provided with spines only towards the tip: 4 apical, large, of the same length, but the middle pair more vigorous; 2 subapical, small, touching the level of the distal ones and a pair of very small ones, a bit far from one another.

Peraeopods with propodus and carpus devoid of secondary subdivisions, so that tarsus is 3-segmented in juv. (Fig. 2 D).

Uropodal endopodite with 5 sharp spines slightly curved towards the anterior side (Fig. 2 C).

Antennal scale lanceolate, as long as antennule.

Holotype (ad. ♀) in the coll. of "Gr. Antipa" Museum under no. 565.

Remarks. Telson is so typical in the world of the 40 species of *Mysidopsis* that saves us for the moment from an ampler description of *M. sankarankuttyi*. This type of telsonal armature reminds somehow only of *Mysidopsis suedafricana* O. Tatt. 1967.

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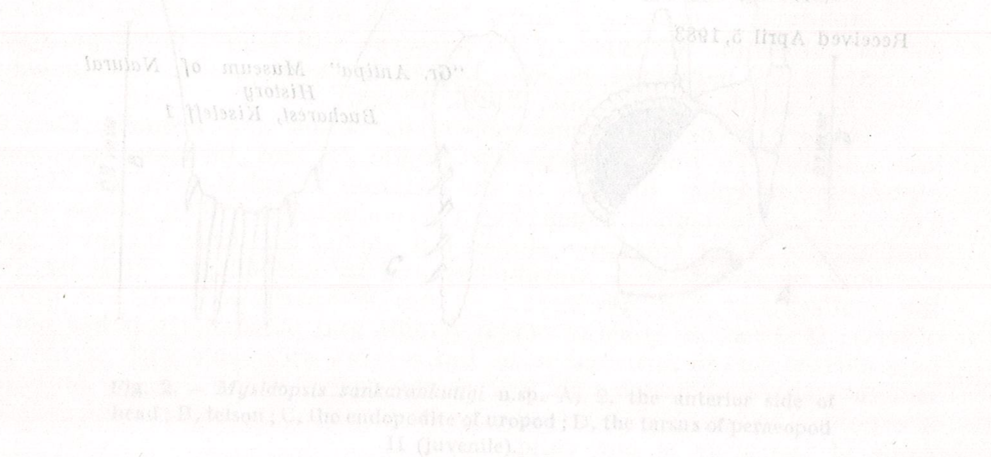

MATÉRIAUX POUR LA DÉFINITION DE L'ESPÈCE

PAR

EUGEN V. NICULESCU

In the present paper the author criticizes the biologists maintaining that "no species criterium is valid when taken separately". The author shows that the genital armature can clearly define the species and that a single sclerite taken separately is a valid criterium, as apparent from the presentation of the harp piece of 22 species of *Papilio* that are both clearly distinguished and identifiable only by their harps.

Pour l'identification et la délimitation des espèces on utilise un grand nombre de caractères, tant morphologiques que biologiques (l'isolement reproductif), écologiques, éthologiques, génétiques, etc.

La valeur de ces caractères est inégale sous l'aspect taxonomique. Certains caractères morphologiques constituent de bons indicateurs taxonomiques chez un groupe, mais n'ont aucune valeur chez un autre groupe. Il est donc nécessaire de choisir les caractères lorsque nous voulons établir la position taxonomique d'une forme et d'utiliser seulement ceux sur la valeur taxonomique desquels le lépidoptériste n'a aucun doute. Nous avons montré dans plusieurs travaux [7], [8], [9], [14], comment il faut choisir ces caractères et nous n'y revenons plus.

Dans ce travail nous voulons prouver la fausseté de l'affirmation selon laquelle aucun critère d'espèce *pris isolément* n'est valable, affirmation fréquemment rencontrée dans les traités de Biologie.

Les biologistes qui font de telles affirmations ignorent certains caractères morphologiques qui se sont avérés d'une grande valeur taxonomique, mais qui par divers motifs n'ont pas attiré l'attention des biologistes [10], [14].

Il s'agit des genitalia des Insectes, surtout des Lépidoptères, qui comprennent des caractères parfaitement valables pour l'identification et la délimitation des espèces, sans qu'il soit nécessaire de recourir à un autre caractère. Cela veut dire que l'armature génitale comprend de nombreuses pièces sclérifiées, de forme constante [1] et caractéristique de chaque espèce, qui ne se répètent plus, *sous la même forme*, chez aucune autre espèce de l'ordre.

[1] Chaque espèce présente une armature génitale constante intraspécifiquement et variée interspécifiquement. La constance intraspécifique n'est pas toutefois absolue. Les divers sclérites, surtout les harpes et le gnathos, ainsi que tous ceux munis de dents, épines, etc., présentent le plus souvent une variation individuelle concernant le nombre, la forme et les dimensions des dents.

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Dans le monde des Insectes on peut trouver des centaines de milliers d'exemples qui démontrent cette vérité ; nous allons présenter seulement quelques-uns de l'ordre Lepidoptera.

L'armature génitale d'un grand nombre de papillons a un sclérite nommé harpe (Pl. I, Pl. II) qui présente des formes variées chez les diverses espèces. Chez *Eques bromius* [2] la partie distale de ce sclérite a la forme d'une crête de coq (Pl. I, fig. 1,3), caractère qui n'appartient qu'à l'espèce *bromius*. Cette espèce peut être déterminée seulement d'après la harpe. Il suffit d'écarter les deux valves pour voir la harpe et de nous prononcer, en toute certitude, que le spécimen examiné appartient à l'espèce *E. bromius*. La harpe en forme de crête de coq ne se rencontre ni chez les autres espèces d'*Eques* ni chez n'importe quelle autre espèce de l'ordre Lepidoptera. Pour comprendre encore mieux la grande valeur taxonomique de l'armature génitale nous mentionnons que chez *E. nireus*, espèce qui ressemble beaucoup à l'espèce précédente quant à l'habitus, l'extrémité distale de la harpe a la forme d'une massue recouverte de nombreuses dents (Pl. I, fig. 2). Aucune confusion n'est possible entre la « crête de coq » de *bromius* et la « massue » de *nireus* [3].

Et maintenant la question : pourquoi ce caractère pris isolément n'est pas valable ? Aucun biologiste n'y pourra plus répondre maintenant que le phénomène a été présenté et qu'il est devenu clair pour tout le monde qu'un caractère comme la harpe d'*Eques bromius* ou d'*E. nireus* est parfaitement valable et n'a besoin d'aucun autre caractère auxiliaire. *Il définit parfaitement l'espèce puisqu'il se trouve chez une seule espèce et il est constant chez tous les individus de l'espèce dont il s'agit.* Mais cette dernière vérité est ignorée par certains lépidoptéristes. Ainsi, Guillaumin et Descimon [4] affirment : «Il est naïvement affirmé que le caractère spécifique est constant, qu'il est présent chez tous les individus de l'espèce ». On confond probablement le caractère spécifique avec les petites variations individuelles existant chez presque tous les sclérites. Chez *E. bromius* le caractère spécifique est la forme générale de la harpe comme une crête de coq ; ce caractère est absolument constant chez tous les individus de l'espèce. Ce qui varie d'un individu à l'autre ce sont le nombre, la forme et les dimensions des dents de la harpe (Pl. I, fig. 3–8). Ce sont des variations individuelles qui ne peuvent être confondues avec la forme même de la harpe — crête de coq — , qui est le caractère spécifique, constant, chez tous les individus.

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[2] Nous avons récemment réactualisé [11] le genre *Eques* Kirby.

[3] Il ne faut pas entendre que la harpe est le seul caractère spécifique d'*Eques bromius*. Le superuncus, l'uncus, la valve, le pseudosacculus diffèrent eux aussi de ceux d'*Eques nireus ;* il y a aussi des différences, à vrai dire minimes, en ce qui concerne l'habitus, mais le caractère spécifique du graphisme ne peut être valablement apprécié qu'après l'examen de l'armature génitale. La touffe frontale de poils et les palpes sont ressemblants chez les deux espèces, ce qui met en évidence à plus forte raison la valeur taxonomique de l'armature génitale, car quelque large que soit l'amplitude de la variation individuelle de la harpe, une « crête de coq » ne peut être jamais confondue avec une « massue ».

Une autre affirmation de certains biologistes avec laquelle nous sommes en désaccord est celle qui présente l'espèce comme une «communauté reproductive, écologique et génétique ». On a omis précisément la structure, c'est-à-dire le caractère morphologique qui définit le mieux l'espèce. La valeur du critère « communauté reproductive » n'est pas si grande que l'admettent certains zoologistes qui affirment que le critère de l'isolement reproductif est le seul critère d'espèce vraiment scientifique [1]. Nous avons montré dans trois travaux [7] [9] [15] comment nous devons interpréter le critère de l'isolement reproductif et quelle valeur nous devons lui accorder. Botnariuc dans son cours de Biologie générale [2] dit, lui aussi, que la définition de l'espèce biologique a certaines déficiences importantes et que la tendance des biologistes qui mettent le critère de l'isolement reproductif au premier plan et même tendent à le rendre absolu, est erronée. Nous avons exprimé cette idée plusieurs fois [7] [8] [9] [15] et nous nous réjouissons qu'enfin, un éminent biologiste roumain l'a reprise et l'a « popularisée » dans un cours de Biologie. Sur la valeur du critère écologique comme critère de l'espèce nous avons publié notre conception [12] et nous n'y revenons plus. Soulignons, encore une fois, que sa valeur est mineure en comparaison des autres critères et l'octroi du statut taxonomique spécifique à une forme seulement en considération des caractères écologiques est tout à fait illusoire et non scientifique. Enfin, si par « communauté génétique » nous entendons le nombre des chromosomes, « constant pour tous les individus de l'espèce », nous constatons que ce caractère est lui aussi déficient. A cette occasion il faut souligner l'erreur commise dans tous les traités de Génétique où l'on affirme que le nombre de chromosomes est constant pour tous les individus de l'espèce. Ainsi, Drăcea [3] affirme : « Le caryotype est reconnu comme un critère d'identification des espèces. Chez une seule et même espèce il est caractéristique et constitue un critère de détermination de sa position phylogénétique » [4]. D'après Popescu-Vifor [16], «le nombre des chromosomes varie beaucoup d'une espèce à l'autre tant chez les plantes que chez les animaux, mais il est constant chez tous les individus de l'espèce dont il s'agit et dans toutes les cellules somatiques de l'organisme ».

Toutes ces affirmations ne sont plus valables actuellement puisque les dernières décennies les cytologistes ont constaté une grande variation individuelle et raciale de la formule chromosomique. Cette variation a été constatée chez un grand nombre d'animaux. Dans le domaine des Lépidoptères s'est illustré H. de Lesse qui a étudié le phénomène chez quelques centaines d'espèces de Rhopalocères. Nous avons synthétisé et commenté les résultats de ses recherches dans deux travaux [5] [6] où nous avons souligné la valeur taxonomique très réduite de la formule chromosomique.

Vu la déficience des critères biologique, écologique et cytogénétique, ainsi que la valeur taxonomique réduite du graphisme, des palpes, des phanères, nous pouvons affirmer que certains caractères pris isolément

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[4] Plus exactement : taxonomique.

ne sont pas valables. Mais l'affirmation ne peut être généralisée pour tous les caractères ; quelques-uns (les genitalia chez les Insectes) font exception et peuvent être valables pris isolément, s'ils sont bien choisis et interprétés.

Vu que le critère morphologique est le plus important critère de l'espèce, nous sommes d'avis qu'il doit être inclus dans la définition de

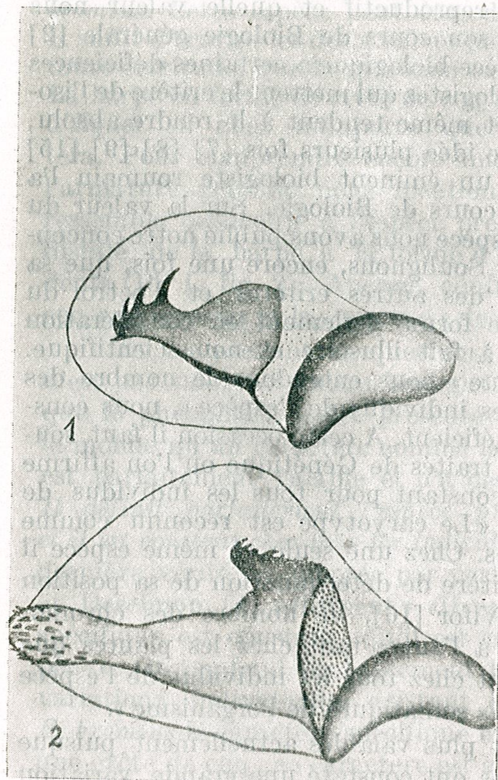

Planche I a. — 1, La valve gauche d'*Eques bromius*; 2, la valve gauche d'*Eques nireus*. Planche I b. — 3 — 9, Les variations individuelles de la harpe d'*Eques bromius* chez 7 individus.

l'espèce, ce qui nous a conduit à l'élaboration du concept morpho-biologique de l'espèce et à une nouvelle définition de l'espèce [9]. Elle ne peut être généralisée à tout le règne animal, mais elle est valable pour 80% des espèces, c'est-à-dire pour l'ensemble des Insectes. Pour le moment et nous référant seulement aux Insectes, nous mettons le critère des genitalia au premier plan et affirmons avec toute la conviction que l'espèce, chez les Insectes, est définie avant tout par des caractères morphologiques et deuxièmement par des caractères d'une autre nature.

Puisque l'espace ne nous permet pas de donner aussi d'autres exemples, nous présentons dans la planche II les harpes de 22 espèces de *Papilio* qui nous montrent clairement la grande variation interspécifique de ce sclérite, et partant sa grande valeur taxonomique.

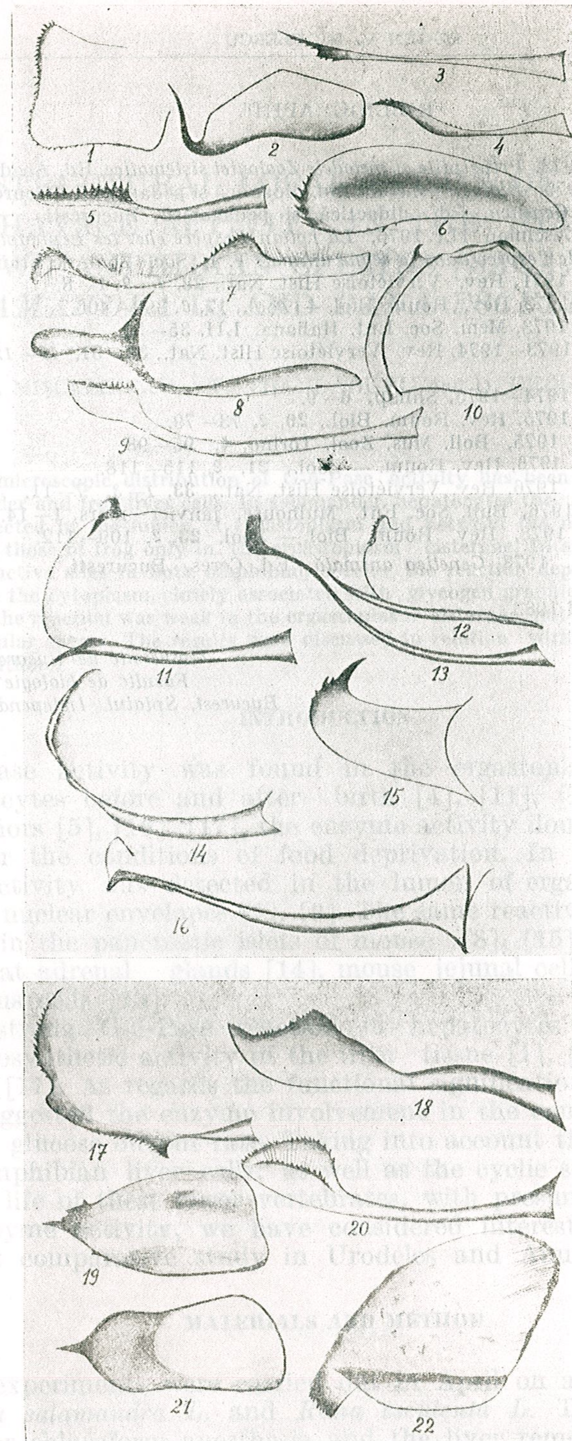

Planche II. — La variation interspécifique de la harpe chez le genre *Papilio*. 1, *P. alexanor*; 2, *P. ulysses*; 3, *P. cypraeofila*; 4, *P. cynorta*; 5, *P. polyxenus*; 6, *P. phorcas*; 7, *P. aegeus*; 8, *P. menestheus*; 9, *P. xuthus*; 10, *P. euchenor*; 11, *P. glaucus*; 12, *P. lycophron*; 13, *P. demodocus*; 14, *P. demoleus*; 15, *P. oribazus*; 16, *P. grosesmithi*; 17, *P. hesperus*; 18, *P. demetrius*; 19, *P. pharnaces*; 20, *P. macilentus*; 21, *P. chiansiades*; 22, *P. hypasson*.

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ULTRASTRUCTURAL LOCALIZATION OF GLUCOSE-6-PHOSPHATASE ACTIVITY IN THE HEPATOCYTES OF TWO AMPHIBIAN SPECIES (*SALAMANDRA SALAMANDRA* L., AND *RANA ESCULENTA* L.)

BY

D. MIŞCALENCU*, T. TRANDABURU and D. GEORGESCU

The submicroscopic distribution of G-6-Pase activity has been investigated in salamander and frog liver cells. In salamander hepatocytes the enzyme activity was detected in the lumen of ergastoplasm and between the nuclei envelopes, while in those of frog only in the ergastoplasm cisternae. In addition to these main reactive sites in both amphibian species the reaction deposits were also found in the cytoplasm, closely associated with glycogen granules. In frog hepatocytes the reaction was weak in the ergastoplasm cisternae, but abundant in the intercellular spaces. The results were discussed in relation with the findings of other authors.

INTRODUCTION

G-6-Pase activity was found in the ergastoplasm cisternae of rats hepatocytes before and after birth [4], [11], [12]. According to several authors [5], [16], [17], the enzyme activity doubles in rat hepatic tissue under the conditions of food deprivation. In mice hepatocytes G-6-Pase activity was detected in the lumen of ergastoplasm, as well as between nuclear envelopes [1], [9]. The same reactive structures were also found in the pancreatic islets of mouse [8], [15], salamander and frog [18], rat adrenal glands [14], mouse jejunal cells [6], [7] and in toad nervous cells [13].

The strong G-6-Pase reaction in hepatocytes was ascribed to the high biosynthetic activity of the liver tissue [1], [2], [4], [9], [11], [12], [16], [17]. As regards the functional signification, several authors [1], [3], suggested the enzyme involvement in the control of glycogenogenesis and glucose output rate. Taking into account the lack of information in amphibian liver cells, as well as the cyclic seasonal variations during the life of these lower vertebrates, with presumable implications on the enzyme activity, we have considered interesting to undertake the present comparative study in Urodeles and Anura.

MATERIALS AND METHOD

The experiments were carried out in April on adult specimens of *Salamandra salamandra L.* and *Rana esculenta L.* The animals were killed under chloroform anesthesia and the liver removed. Small tissue blocks were immersed for 30 min in a cold solution of 2 % glutaraldehyde

in 0.1 M cacodylate buffer (pH = 7.4) containing 7.5 % sucrose. After fixation the pieces were briefly rinsed several times (15 min in all) in the same buffer at 4°C, embedded in 7% agar and sectioned at 40 μm on Sorval TC—2 tissue chopper.

The incubation medium was prepared according to Hugon et al. [6]. As reaction substrate D-glucose-6-phosphate disodium salt was used. The incubation time was 30 min at 35°C, in a water bath under continuous shaking. In control experiments, the liver slices were incubated in a substrate free medium and, alternatively in a medium containing Na-β-glycerophosphate instead of glucose-6-phosphate.

Following incubation, the liver slices were quickly washed in cacodylate-sucrose buffer and refixed for 60 min in cold cacodylate-buffered 1% OsO_4(without sucrose). Thin sections, prepared on Reichert Om U_2 ultratome, were double stained with uranyl acetate and lead citrate. The grids were examined in a Siemens Elmiskop 101 electron microscope (80 kV).

RESULTS

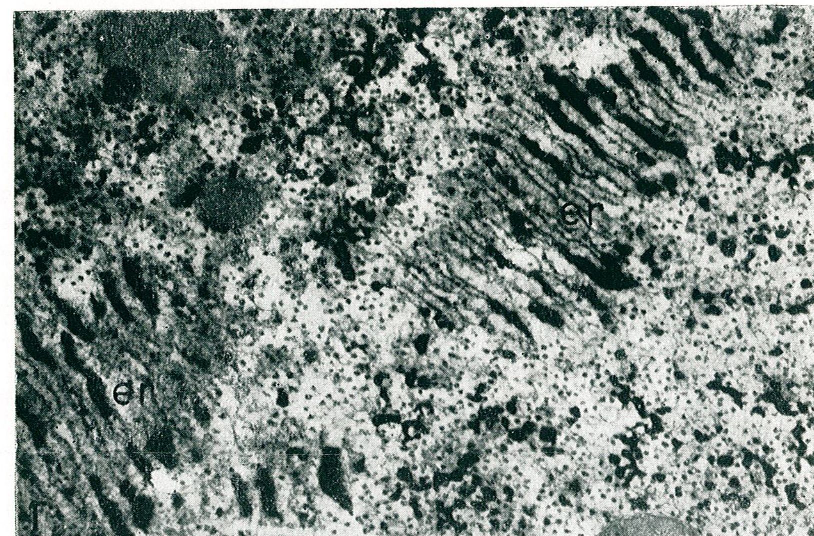
In salamander hepatic cells G-6-Pase reaction was constantly detected, as a more or less continuous precipitate, within the ergastoplasm cisternae, which frequently appear arranged as packages with ellipsoidal profiles in sections (Figs 1, 2). Like in other cell types, the reaction product was also observed within the perinuclear cisternae (Fig. 2). Besides these main localizations, heavy deposits of lead phosphate occurred randomly spread in the cytoplasm, in close association with glycogen granules (Fig. 1).

The reaction intensity, estimated according to the amount of lead phosphate deposits, was weaker in frog hepatocytes. Moreover, important variations of the staining intensity were recorded among the reactive cells. Small clusters of coarse granular precipitate were sometimes encountered within the lumen of the ergastoplasm, but never between the nuclear envelopes (Fig. 3). Unlike the salamander, in the frog liver the reaction product was frequently found in the intercellular spaces (Fig. 4).

When using glycerophosphate instead of glucose-6-phosphate in the incubation medium, no activity was recorded in the ergastoplasm, or in the double perinuclear space of both salamander and frog hepatocytes, in exchange, the Golgi complexes and lysosomes were highly positive. In control experiments, in which the substrate was omitted, no reaction occurred.

DISCUSSION

The localization of G-6-Pase activity in both amphibian species at the level of ergastoplasm was also reported in rat hepatocytes before birth [4], [11], [12]. If in rat embryos have been found only the incipient phases of enzyme elaboration (short time after birth the ergastoplasm cisternae were entirely full with reaction precipitate), in salamanders



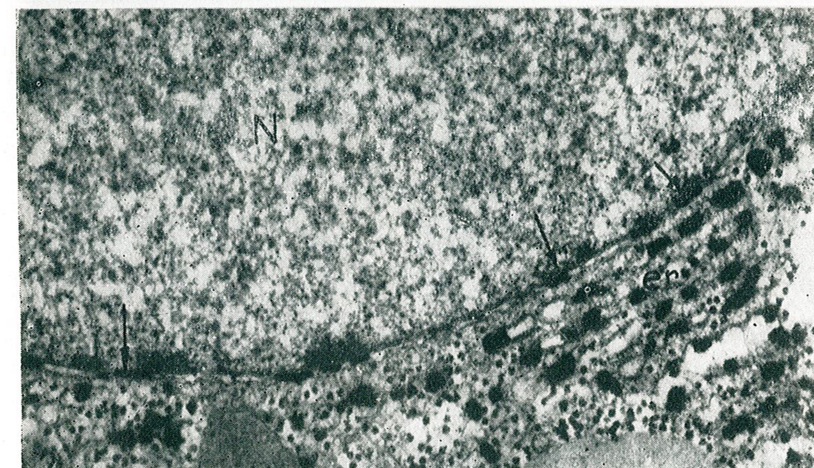


Fig. 1. — Portion of a salamander hepatocyte showing heavy deposits of lead phosphate in the lumen of the ergastoplasm(Er) and randomly spread in the cytoplasm. × 11.900.

Fig. 2. — Detail of a salamander hepatic cell displaying G-6-Pase activity within the nucleus (arrows) (N) and ergastoplasm cisternae. × 14.000.

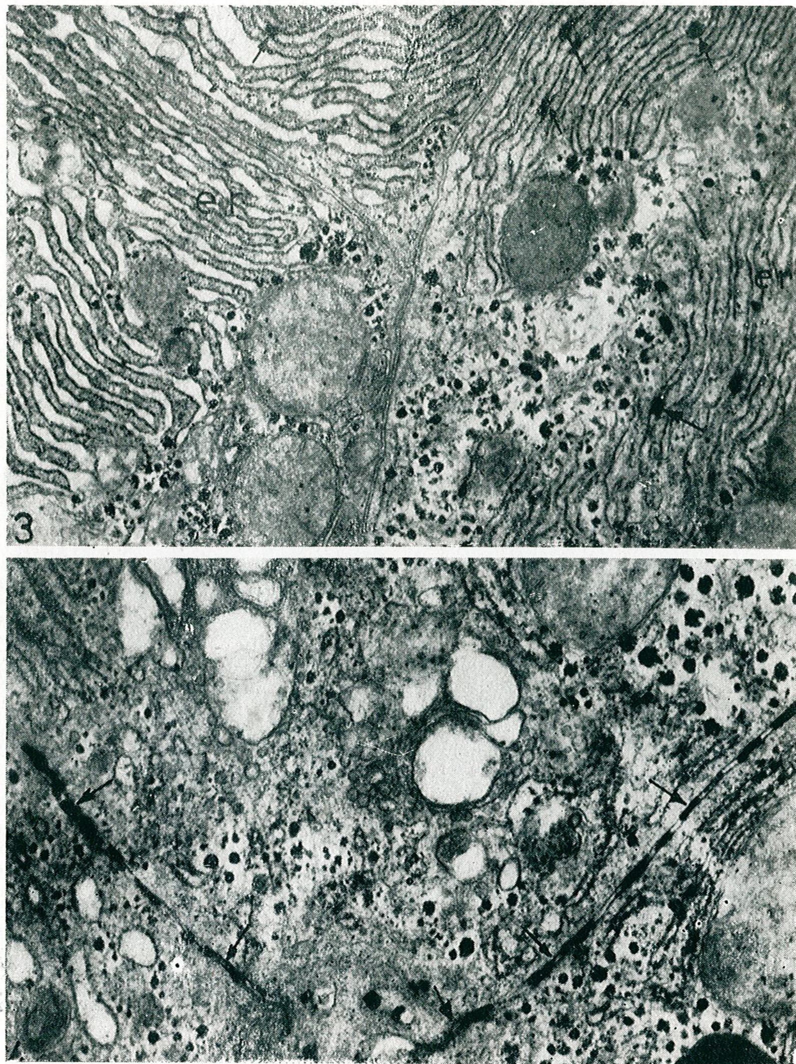


Fig. 3. — Small amounts of coarse granular reaction deposits occurring within the ergastoplasm of frog hepatocytes (arrows). × 10.000.
Fig. 4. — Frog hepatocytes exhibiting a strong reaction at the external surface of plasma membranes (arrows). × 13.500.

it seems to exist an intensive activity suggesting a high rate of enzyme turnover. The speed of turnover presumably leads to a synchronization of enzyme synthesis in all ergastoplasm cisternae.

Taking into account that salamanders have been sacrificed in April, when their own stores are intensively used for energetical activities, the release of glucose from G-6-phosphate was accelerated and G-6-Pase activity was higher. A similar phenomenon was also described in the hepatocytes of starved rats, in which the G-6-Pase level was double and directly correlated with the decreases of G-6-phosphate [5], [16], [17].

In frog hepatocytes the reaction does not exhibit the same features as in salamanders; the precipitate was very poor in the ergastoplasm cisternae and more obvious in the intercellular spaces. Its abundance in the proximity of ribosomes and glycogen granules suggests a correlation of the enzyme activity (or of other diffuse phosphatase [18]) with the hyaloplasm amount although, according to the current concept [1], the occurrence of G-6-Pase would be associated with the cell membranes.

As compared with the pancreatic islets [18], in the hepatocytes of the same amfibian species a reverse situation can be discerned; in salamander pancreatic B-cells the reaction was less intense than in frog, while in the hepatocytes it was stronger.

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L'ÉVOLUTION DES CONCEPTIONS SUR LE RÔLE PHYSIOLOGIQUE DU THYMUS

PAR

VIRGIL TOMA et MARIA-SENA CRIVII

The thymus was already described in antiquity as an organ protecting the heart and the great blood vessels, as the centre of courage and of love. Its study has followed a contradictory way, under the auspices of the following paradigms :
1) "The endocrine paradigm", based on the famous axiom of Brown-Séquard concerning the universality of the internal secretions. Experimental data obtained in this direction did not fully fit Gley's triad ; consequently, a crisis set in and it was necessary to revise the paradigm.
2) "The immunobiological paradigm", advanced in the 60's, has contributed to a great extent to demonstrate the role of the thymus as a central lymphatic organ coordinating all the immunocellular reactions, which, in their turn, are achieved through T lymphocytes. Both the theoretical and the practical achievements of this period allow to consider it as the "golden age of thymology".
3) "The immunoendocrine paradigm" is presently directing the research towards understanding the molecular mechanisms of immunocompetent instruction of T lymphocytes, based on the hormones of the glandular epithelium. In this light, the thymus must be regarded as a mixed — immune, producing T lymphocytes, and endocrine gland.

L'investigation du thymus a suivi une route sinueuse et contradictoire dont les étapes à résultats non significatifs ont prédominé, conduisant plutôt à des spéculations qu'à des conclusions certaines. Quelquefois ces étapes étaient bouleversées par des renversements spectaculaires, qui non seulement changeaient les conceptions de la thymologie, mais se reflétaient aussi, décisivement, dans la sphère d'activité théorique et pratique de nombreuses disciplines : l'immunologie en particulier, ensuite l'oncologie, l'endocrinologie, etc.

Décrite depuis l'antiquité, la glande thymique a été considérée comme étant « le matelas destiné à protéger les gros vaisseaux, et le centre régulateur du caractère et du comportement » [12]. Les premières données en ce qui concerne la structure macro- et microscopique, l'embryologie ou la composition chimique de la glande ont été obtenues graduellement, en fonction de l'étape du développement historique, des moyens d'investigation et des conceptions théoriques biomédicales. Sur le plan fonctionnel et surtout pathologique, à l'exception de quelques opinions d'après lesquelles le thymus contrôlerait la lymphopoïèse (1777) ou l'état thymo-lymphatique (1890), les connaissances concernant cette glande ont été extrêmement lacuneuses [2] [12] [14].

Au commencement du XXe siècle, l'étude du thymus devient systématique et bien organisée et nous considérons qu'elle s'est développée, conformément à la conception de Kuhn [21], d'après les paradigmes suivants :

1) LE PARADIGME ENDOCRINIEN, CLASSIQUE, DU THYMUS

Il s'est formé dans l'atmosphère de domination de la conception des sécrétions internes universelles, d'après le célèbre axiome de Brown-Séquard : « chaque organe, chaque tissu, chaque cellule a une sécrétion interne ».

Dans ce contexte, on a réussi à préparer un extrait polypeptidique [8] [9] qui présentait des propriétés opothérapiques substitutives et des effets endocrino-métaboliques spécifiques. Mais de nombreux autres essais d'intégrer le thymus, selon la triade de Gley (17), parmi les glandes endocrines ont donné des résultats équivoques à cause desquels les hypothèses l'emportèrent sur les conclusions indubitables [2] [14] [34].

C'est ainsi que, à la suite d'une accumulation d'« anomalies expérimentales sévères et prolongées », le premier paradigme endocrinien du thymus est arrivé à ne plus satisfaire les besoins de la recherche normale en thymologie, ayant comme conséquence une crise, c'est-à-dire la nécessité de changer ce paradigme.

Or, remplacer un paradigme par un autre qualitativement différent constitue une révolution dans le domaine de la recherche scientifique [21].

2) LE PARADIGME IMMUNOBIOLOGIQUE DU THYMUS, OU BIEN CELUI DES CELLULES T [16]

Il sort cette glande de l'inconnu et l'élève au rang de « l'âme de la personnalité biologique ou berceau des mécanismes les plus secrets de l'être » [14]. C'est maintenant que le thymus est considéré comme l'organe lymphatique central, responsable de la défense immunocellulaire de l'organisme. Des modèles expérimentaux d'une élégance et d'une ingéniosité remarquables ont restreint l'obscurité qui enveloppait les mécanismes complexes de l'immunité cellulaire, en les approfondissant jusqu'aux niveaux moléculaire, génétique, phyloontogénique, etc. [15] [18] [19] [20] — [22] [25] [40]. Dans ce climat se développe la conception de la sélection clonale [6], de la surveillance immunitaire [7], de l'expansion de l'immunité antitumorale [23] [32]. Ainsi, l'étape d'après les années 60 peut être considérée, sans exagérer, comme « l'âge d'or » dans l'histoire de la thymologie [26].

D'après les affirmations de Kuhn [21], « il n'existe pas d'exemples sans contre-exemples, et aucun paradigme qui pourrait offrir une base à la recherche scientifique ne peut résoudre tous les problèmes ». De même, dans le cas du thymus il est de plus en plus évident que ses fonctions endocriniennes [7] [27] ne peuvent être omises ni même dans son contexte immunobiologique [10] [11] [22] [28].

3) LE PARADIGME IMMUNOENDOCRINIEN DU THYMUS

En conséquence nous considérons que ce paradigme réunit harmonieusement toutes les directions de la recherche actuelle et de l'avenir dans le domaine de la thymologie. En même temps on doit souligner que les possibilités et les limites de l'endocrinologie actuelle sont bien différentes de celles du début du XXe siècle, jetant une nouvelle lumière sur l'hormonologie du thymus [30]. Cette manière d'envisager l'investigation est obligatoire, car les influences des hormones thymiques sur la potentialité fonctionnelle des lymphocytes T sont certaines.

Au commencement cette activité a été démontrée par le rétablissement de l'immunocompétence chez les animaux thymectomisés dans la période néonatale, à l'aide de greffons de thymus placés dans des chambres de diffusion « Millipore » [25]. Ensuite, « la recherche normale » du thymus immunoendocrinien a continué à solutionner les problèmes « puzzle » de nature théorique et méthodologique.

En synthétisant toutes ces données, nous soulignons les éléments suivants, qui ont été largement mentionnés dans la littérature : la préparation d'une vaste gamme d'hormones thymiques immunocompétitives de nature polypeptidique et stérolique et le fait que la microscopie électronique et les cultures de tissus ont réussi à préciser leur niveau de sécrétion dans l'épithélium glandulaire. En même temps on considère que l'action de l'hormone thymique sur le processus de différenciation lymphocytaire se manifeste sur place, dans la glande même, quoiqu'on ait pu mettre en évidence des facteurs thymiques sériques. Au moyen de tests d'une élégance, précision et originalité exceptionnelles on a étudié la structure chimique de nombreux extraits thymiques, approfondissant leurs mécanismes moléculaires de conversion lymphocytaire des précurseurs des cellules T [3] [15] [19] [20] [22] [23] [24]. D'un intérêt égal sont les résultats thérapeutiques dans le syndrome Di George, obtenus par la transplantation du thymus fœtal [22]. Enfin, l'inédit biologique du thymus est l'hormone qui produit la thymotoxicose et la myasthénie [19] ou la relation fréquente entre l'adénocarcinome thymique sécréteur d'hormone ACTH-like et le syndrome d'hypercorticisme paranéoplastique [12]. En conséquence, l'existence d'une activité hormonale thymique avec des répercussions immunobiologiques « sine qua non » constitue aujourd'hui une vérité objective.

D'autre part, un trait particulier de l'endocrinologie thymique est représenté par ses interrelations avec les glandes à sécrétion interne [1] [8] [9] [13] [28] [29]. En fait, la conclusion qui se dégage de ce phénomène est l'existence « d'un mécanisme coordinateur endocrinien des fonctions immunitaires » [11].

Parmi les corrélations endocriniennes du thymus, les mieux étudiées et qui ont la plus grande importance biologique sont celles établies avec les hormones sexuelles et glucostéroïdiennes.

Premièrement, les stéroïdes sexuels produisent à la puberté l'involution d'âge, normale, progressive et irréversible de la glande, qui est accompagnée de la diminution des capacités immunobiologiques, donc antitumorales de l'organisme [8]. Deuxièmement, le thymus représente pour les hormones glucocorticostéroïdiennes un organe visé. Ainsi, le stress

[31], y compris les agents offensants tumoraux [35] — [38], produit une involution « aiguë » de la glande. La signification biologique de ces réactions n'a pu être expliquée que lorsqu'on a découvert le lien d'entre les fractions immunocellulaires et l'activité du relais neuroendocrinien hypothalamo-hypophyso-cortico-surrénalien. Ainsi, par l'excès de glucocorticostéroïdes, dans la glande involuée sont éliminés les lymphocytes corticaux « sensibles à la cortisone », qui présentent des récepteurs spécifiques pour ces hormones [39], étant en même temps inertes du point de vue immunologique. Ce qui est très important c'est que par cette épuration endothymique, les lymphocytes de la zone médullaire «résistants à la cortisone » augmentent significativement leur potentialité immunobiologique [4] [5].

En corroborant toutes ces données nous arrivons à la conclusion que l'essence du paradigme immunoendocrinien de la thymologie actuelle est suggestivement exposée dans l'idée suivante : « le thymus est situé à l'interférence des fonctions de défense spécifique (l'immunité), non spécifique (stress) et endocrinienne » [33].

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AGE-DEPENDENT EFFECTS OF THYMUS ABLATION, PECTORAL MUSCLE DENERVATION AND INSULIN TREATMENT UPON INTRAVENOUS GLUCOSE TOLERANCE IN CHICKEN

BY

IOSIF MADAR, CAROL WITTENBERGER and ANA ILONCA

The dynamics of intravenous glucose tolerance was studied in fasted chickens aged 12, 24 and 40 days, in basal conditions, after pectoral muscle denervation, insulin treatment, thymectomy, or a combination of them. The major importance of age was shown on the influence of the above interventions upon the rate of glucose assimilation.

A recent work performed in our laboratory supports the assumption that there are several critical ages during posteclosional development of chicken [21]. This concerns not only the energetic metabolism of the normal muscle [22], but also the effects upon it of some experimental interventions, such as thymectomy [6], pectoral muscle denervation [23], and insulin treatment [21]. On the other hand, it has been well established that the skeletal muscle of birds is a major consumer of blood glucose [12], [18].

There are experimental and clinical data which show that glycemia homeostasis, glucose tolerance, and insulin secreting capacity of the endocrine pancreas are significantly affected by injuries of the thymus or of the neuromuscular system [2], [4], [5], [13] – [17], [19].

In the present paper we followed the dynamics of the rapid intravenous glucose tolerance as depending on the age, in fasted normal chickens, as well as after pectoral muscle denervation, thymus ablation and insulin treatment.

MATERIALS AND METHODS

Experiments were performed on ROBRO-69 chickens, a tetralinear Studler-Cornish hybrid. The animals were purchased in the day of eclosion from a commercial hatchery (Avicola, Cluj, Romania). They were reared in standard laboratory conditions and fed *ad libitum* with age-fitted concentrated fodder.

Three age-groups were used, e. of 12, 24 and 40 days from the experiment. Each group was divided in five variants as follows: intact controls (C); with unilateral denervation of the pectoral muscle (D); with

unilateral denervation of the pectoral muscle and daily insulin treatment (Di); with unilateral denervation of pectoral muscle and thymectomy (DT); with all the three above interventions combined (DTi).

Muscle denervation (by severing the right-side pectoral nerve) and thymus ablation were performed in conditions of sterility, 10 days before the experiment [20], [23].

Insulin (BIOFARM, Bucharest, Romania; dilution with physiological saline, 0.2 % w/v gelatine p.a. added) was administered in daily subcutaneous doses of 2×10^{-3} I.U. per 100 g body weight. The treatment lasted for 10 days, ending on the day before the experiment.

The experiments were carried out after a fasting of 14 – 18 hours (this time was somewhat shorter in the case of younger chickens and longer in older ones), under Nembutal anaesthesia (5 mg natrium pentobarbital SERVA per 100 g b.w., i. m. injected).

Blood samples of 50 microliters for testing glycemia before and after glucose loading were obtained by a unique incision of the tip of a finger previously soaked in 43°C water in order to produce hyperemia. Hemorrhage was stopped with rubber rings tighted with special clamps. This method allowed repeated sampling from the same incision. After the first blood sampling for fasting glycemia, the chicken was loaded with glucose by the rapid injection into the brachial vein of 80 mg per 100 g body weight (from a 40 % solution, w/v). Brachial vein hemorrhage was stopped with a fine clamp, then subsequent samples were obtained from the finger tip at 10, 20, 30 and 40 minutes.

Blood glucose concentrations were enzymatically assayed using GOD–Perid kit from Boehringer Mannheim GmbH. Optical densities were measured in a SPEKOL spectrophotometer (Carl Zeiss, Jena), at 610 nm. Results were expressed in mg glucose per 100 ml blood.

Glucose tolerance was mathematically evaluated calculating the glucose assimilation coefficients (K) by a procedure described in the literature [1], [3] and improved by one of us [8]–[11] after semilogarithmic representation of hyperglycemia curves (glycemia values being plotted on a natural logarithmic scale, and the time in minutes on a linear one).

All the results were statistically checked for the homogeneity of the means using Chauvenet's criterion. The mean values of K were compared according to Student's t test, $P = 0.05$ being accepted as the limit of significance.

RESULTS

The results of our observations are summarized in Table 1. It may be seen that in basal conditions (C groups) the values of the K assimilation coefficients were practically the same in the three age-groups. None of our experimental interventions affected significantly the glucose tolerance in the youngest group. In 14 + 10-day (D, Di and DT) chickens the tolerance was lowered, but again normalized if the interventions were

combined (DTi group). In 30 + 10-day-old chickens the only significant modification was the increase of K in the DTi group: + 48 % as compared to DT, and + 31 % as compared to C.

Table 1

Values of the glucose assimilation coefficients (obtained by intravenous glucose tolerance test) in fasted chickens, as depending on age and on neuroendocrine interventions. The results represent means ± standard errors. The number of experiments is given in parentheses

Groups	2 + 10 days	14 + 10 days	30 + 10 days
	K coefficient of glucose assimilation		
C	2.03±0.07 (10)	2.07±0.11 (6)	1.94±0.09 (6)
D ±%(C)	1.99±0.12 (6) −2.16	1.67±0.18 (6) −19.32	1.76±0.25 (6) −9.28
Di ±%(D)	2.01±0.07 (6) +1.35	1.44±0.08 (6) −13.77	1.86±0.13 (6) +5.68
DT ±%(D)	1.99±0.12 (6) 0	1.46±0.17 (6) −13.07	1.71±0.19 (7) −2.84
DTi ±%(DT)	2.02±0.07 (6) +1.5	2.04±0.19 (7) +39.73	2.54±0.24 (6) +48.54

C = intact control group; D = group with unilateral pectoral muscle denervation; Di = group with unilateral pectoral muscle denervation and insulin treatment; DT = group with unilateral pectoral muscle denervation and thymectomy; DTi = group with unilateral pectoral muscle denervation, thymectomy and insulin treatment. First line of the Table: age at the intervention plus 10 days until experimentation. Below the means percentage differences are given against the respective variants (these are given in parentheses in the first column of the Table. Underlined differences are statistically significant ($P < 0.05$).

DISCUSSIONS

We do not know any data in the literature on attempts to use the K coefficient in evaluating extraglucose assimilation during rapid intravenous glucose tolerance test in birds. Our data show that it is suitable, as the disappearance of extraglucose from the blood into the tissues proceeds in chickens in the same exponential manner as it was described in mammals [1], [3], [8], [9], [10], [11], [15].

Intravenous glucose tolerance in developing chickens reflects both the degree of maturation of insulin secreting capacity of endocrine pancreas, and the efficiency of hormone in provoked hyperglycemic state upon the entrance of glucose into the tissues [7]. As we found practically the same K values at the three ages investigated (C groups), it seems likely that both the insulin secretion and the insulin-dependent glucose transport systems are maturated in an early posteclosional stage.

As we have shown in this paper, denervation of the pectoral muscle affected the glucose tolerance only in 14-day-old chickens. This fact seems to support our previous view, that this is a critical stage in the ontogenesis of the chicken, concerning the maturation of muscular function [21], [22], [23] and, may be, of the neuro-endocrine regulatory systems of the carbohydrate metabolism. The lack of improvement after insulin administration suggests that muscle denervation led to an enhanced resistance against exogenou s insulin. Indeed, there are clinical data showing a lowered glucose tolerance and an increased resistance to endogenous insulin in myotonia or muscle atrophy affected patients [17], [19]. Even if nothing can be said yet on the mechanisms involved, a direct causal relation between the observed phenomena is strongly suggested.

Possible synergistic actions of the thymus and insulin have been repeatedly argued. E. A. Pora and M. S. Roşculeţ [15] found a decrease of glucose tolerance in thymectomized rats. However, as shown above, we found a potentiation of the insulin action in chickens deprived of thymus. We must conclude that, in our conditions, the thymus-insulin relation was not a synergistic but an antagonistic one, as it was sometimes described also in mammals [2], [4], [13].

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PROCAINE, DIETHYLAMINOETHANOL AND *PARA* AMINOBENZOIC ACID EFFECTS ON THE MEMBRANE POTENTIAL

BY

I. NEACȘU and N. OIȚĂ

The investigations were performed on the frog sartorius fibre membrane, using the glass intracellular microelectrode method. The procaine and diethylamino-ethanol cause similar effects as: membrane hyperpolarization, repolarization blocking of the depolarized membrane by high-potassium, abolition of the membrane hyperpolarization by high-calcium and remove of the deposited cholesterol from the membrane structure. Para aminobenzoic acid has different effects. These data show the possibility to use diethylaminoethanol in pharmaceutical products with effects similar with those containing procaine.

There are many researches about the procaine action relatively to the effects on the membrane potential, its properties of local anesthetic and membrane stabilizer [3], [25] or to the complex pharmacological action when is administered as Gerovital or Aslavital [5]–[8], [21], [22]. Some aspects of the procaine action on the vegetal organism have also been investigated [10], [15].

It is known that in the organism, after a relatively short time, procaine is splitted into *para* aminobenzoic acid and diethylaminoethanol [8], [9], [11], [22], [24]. In this process one supposes that to the observed eutrophic effects would participate also these compounds, especially the *para* aminobenzoic acid [7], [8], [21]. The action of these metabolites at the cell level and, especially, at the membrane level, comparatively with the procaine action was very little studied [10], [22]. A sufficiently clear connection between the direct effects of the procaine upon the membrane and its general pharmacodynamical action has been also not made and there is no unity of opinion about the mechanism of the studied phenomena.

In order to observe the direct action upon the cell membrane, we studied comparatively the procaine, diethylaminoethanol and *para* aminobenzoic acid effects on the membrane potential of the striated muscle fibre in various conditions.

MATERIAL AND METHODS

The experiments were performed on the membrane of frog (*Rana ridibunda*, Pall.) sartorius muscle fibres by the technique of intracellular glass microelectrodes.

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Normal Ringer solutions (NR) with pH = 7.2 (bicarbonate buffer) and pH = 6 (phosphate buffer) have been administered under continuous current. Procaine (PROC.), diethylaminoethanol (DEAE) or *para* aminobenzoic acid (PABA) equal concentration solutions (2.5 mM or 1 mM) were prepared by addition of the compounds to the Ringer solution. High-calcium (5 mM) and high-potassium (30 mM) solutions were obtained by substitution of an equimolar quantity of NaCl for $CaCl_2$ or KCl. Cholesterol (CHOLEST.) solutions 0.1 mM have been prepared by its dissolution in ethanol and the addition of the alcohol solution to the normal Ringer so that the alcohol concentration is 1 %, a concentration with no influence on the membrane potential [15]. In each experiment the measurements were performed at room temperature on five muscles of different animals. The statistical data treatment was made by Student's method.

RESULTS

In the anesthetic concentration [25] 2.5 mM procaine at pH = 7.2 causes a slight membrane hyperpolarization (4.69 mV) (Fig. 1—A), effect also described in other papers [3], [25]. In the same conditions, PABA determines only a very weak and unsignificant hyperpolarization (0.69 mV, $p > 0.25$) (Fig. 1 — B) and DEAE causes a significant ($p < 0.002$) and persistent hyperpolarization (2.85 mV) (Fig. 1—C).

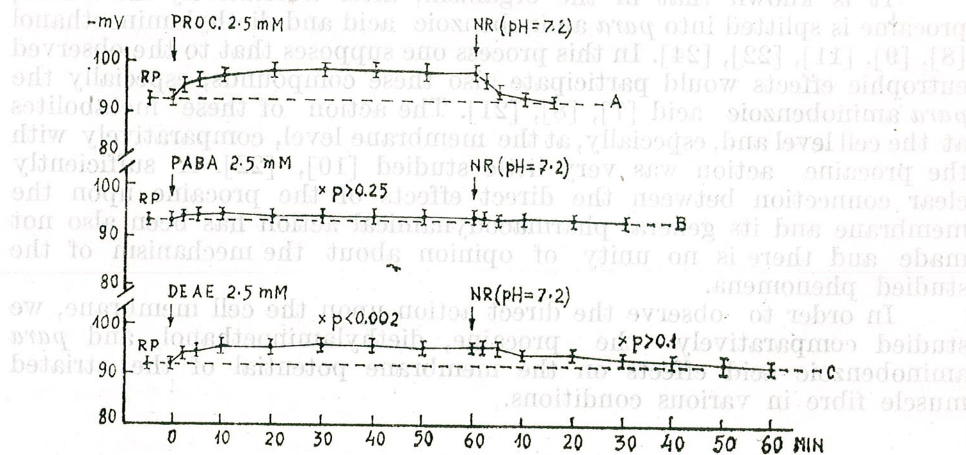

Fig. 1. — The effect of 2.5 mM procaine (A), 2.5 mM PABA (B) and 2.5 mM DEAE (C) on the membrane potential in normal Ringer, at pH = 7.2.

At pH = 6, 1 mM procaine determines a constant depolarization (Fig. 2—B) described in other paper [3]. In the same conditions, neither DEAE (Fig. 2 — A) nor PABA (Fig. 2 — C) modify the membrane potential.

In the 30 mM potassium medium, a prominent membrane depolarization occurs (Figs. 3 — C and 4 — B), also presented in other papers [16], [18]. Administration of 2.5 mM procaine to the depolarized membrane by high - K^+ causes, after an initial weak reversal to the normal value, a blocking depolarization (Fig. 3 — B). The 2.5 mM DEAE effect upon the depolarized membrane (Fig. 3—A) is more similar to that of

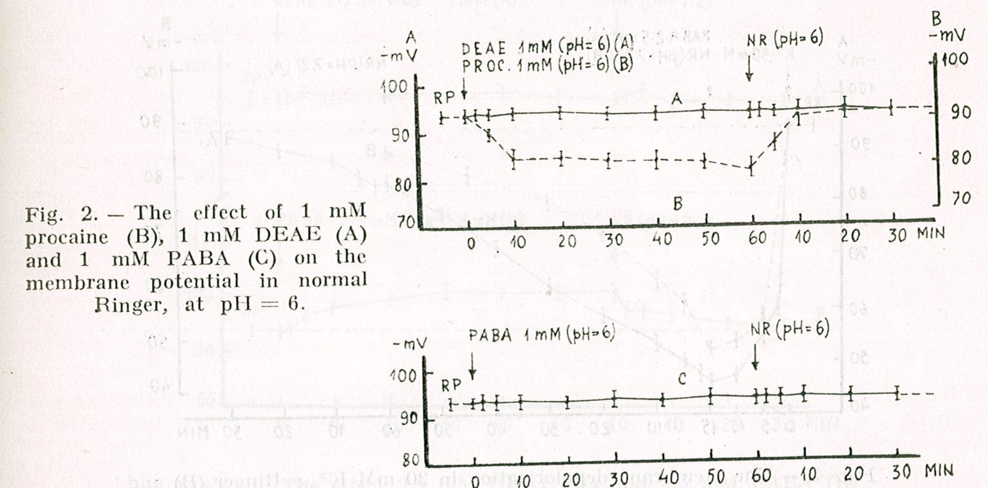

Fig. 2. — The effect of 1 mM procaine (B), 1 mM DEAE (A) and 1 mM PABA (C) on the membrane potential in normal Ringer, at pH = 6.

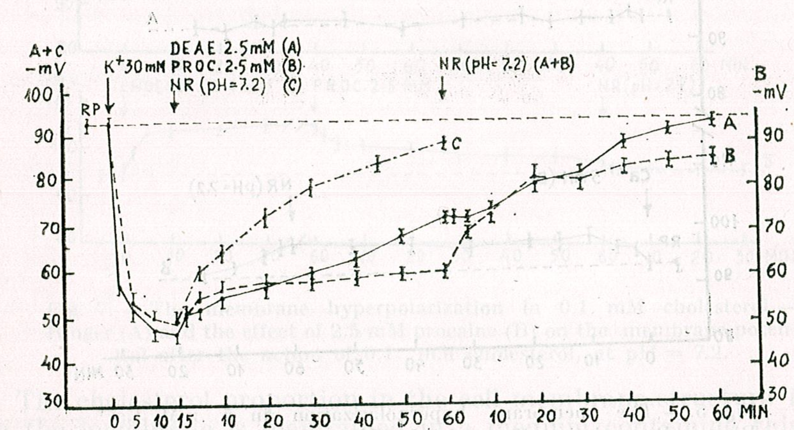

Fig. 3. — The membrane depolarization in 30 mM K^+— Ringer (C) and the effect of 2.5 mM procaine (B) and 2.5 mM DEAE (A) on the depolarized membrane, at pH = 7.2.

procaine (Fig. 3—B), especially during the initial 30 minutes, compared to that of normal Ringer (Fig. 3 — C). The re-establishment of the mem-

brane potential in the presence of 2.5 mM PABA (Fig. 4 — A) is much more similar to the effect occurring in normal conditions (Fig. 4 — B), with only a repolarization delay.

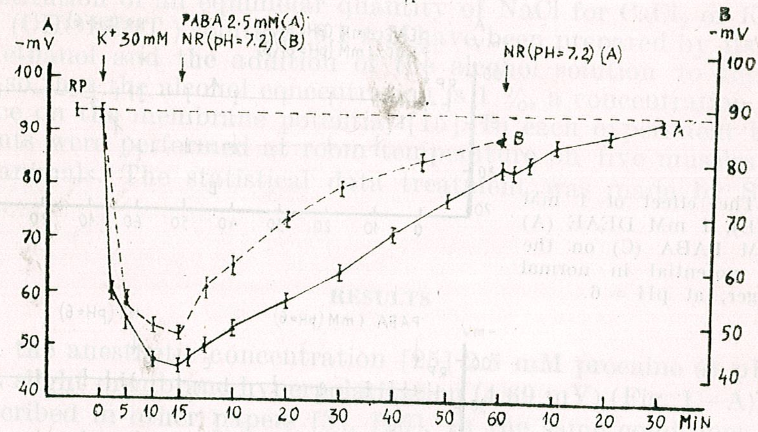

Fig. 4. — The membrane depolarization in 30 mM K^+ — Ringer (B) and the effect of 2.5 mM PABA (A) on the depolarized membrane, at PH = 7.2.

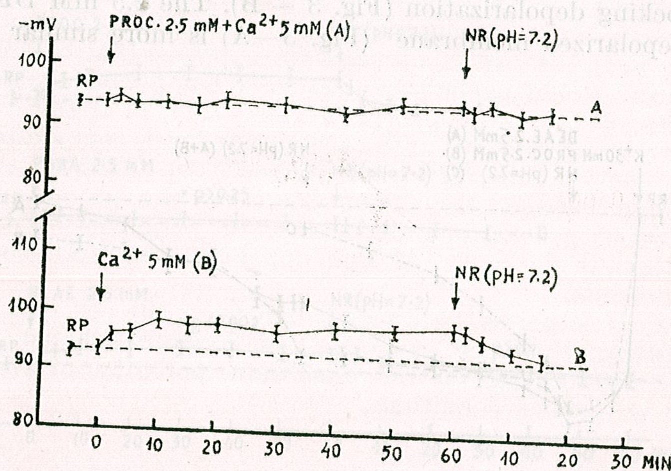

Fig. 5. — The membrane hyperpolarization in 5 mM Ca^{2+} — Ringer (B) and the effect of 2.5 mM procaine in 5 mM Ca^{2+} — Ringer (A) on the membrane potential, at pH = 7.2.

An increased calcium concentration (5 mM) determines a membrane hyperpolarization (Fig. 5 — B), effect described also in a previous paper [16]. This hyperpolarization is abolished by the simultaneous administration of 2.5 mM procaine (Fig. 5 — A) or 2.5 mM DEAE (Fig. 6 — A).

2.5 mM PABA causes but a hyperpolarization (Fig. 6 — B) with a slower onset and a larger amplitude in comparison with that caused by Ca^{2+} (5.95 mV compared to 4.41 mV).

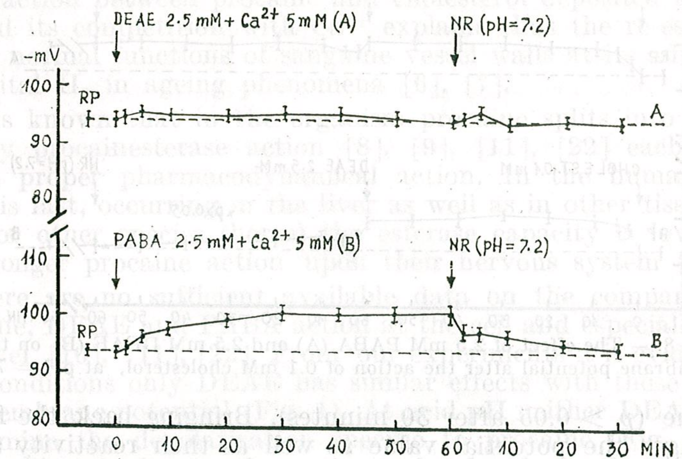

Fig. 6. — The effect 2.5 mM DEAE (A) and 2.5 mM PABA (B) in 5 mM Ca^{2+}— Ringer on the membrane potential, at pH = 7.2.

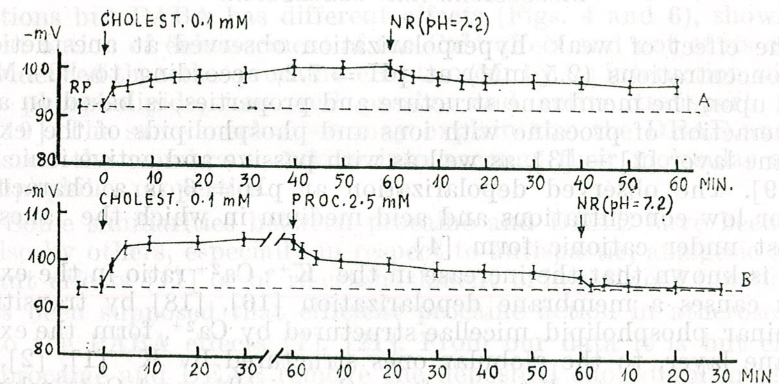

Fig. 7. — The membrane hyperpolarization in 0.1 mM cholesterol — Ringer (A) and the effect of 2.5 mM procaine (B) on the membrane potential after the action of 0.1 mM cholesterol, at pH = 7.2.

The cholesterol proportion in the cell membrane structure increases when the membrane is maintained in a medium containing this steroid [23]. In 0.1 mM cholesterol-Ringer a constant and irreversible hyperpolarization (7 mV) occurs in a normal medium (Fig. 7—A), indicating a tight cholesterol binding to the membrane. 2.5 mM procaine determines a decrease in the cholesterol effect (Fig. 7—B) up to the hyperpolarization characteristic for procaine. Bringing back the fibres into normal Ringer the potential re-establishment to its resting value is observed, indicating that procaine causes the remove of the cholesterol deposited in the mem-

brane. 2.5 mM PABA does not have such an effect (Fig. 8 — A), the membrane remaining hyperpolarized also in the normal medium. 2.5 mM DEAE determines but a rapid potential decrease (Fig. 8 — B) up to its

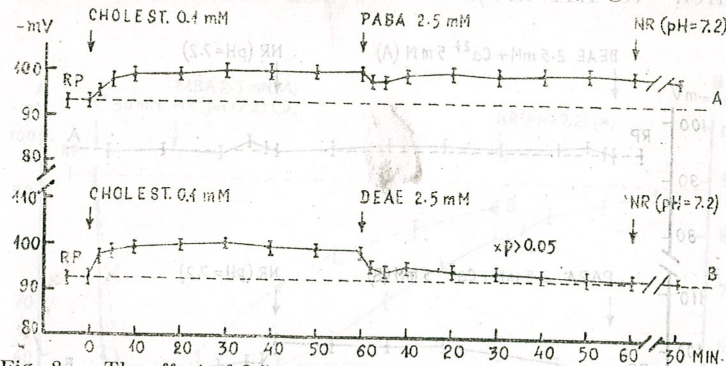

Fig. 8. — The effect of 2.5 mM PABA (A) and 2.5 mM DEAE (B) on the membrane potential after the action of 0.1 mM cholesterol, at pH = 7.2

resting value ($p > 0.05$ after 30 minutes). Bringing back the fibres into normal Ringer the potential value as well as their reactivity are maintained. The DEAE effect of membrane cholesterol removal is therefore stronger than that of procaine.

DISCUSSIONS AND CONCLUSIONS

The effect of weak hyperpolarization observed at anesthetic procaine concentrations (2.5 mM) at pH = 7.2, according to "2 — M.S.I" concept upon the membrane structure and properties, is based on a complex interaction of procaine with ions and phospholipids of the external membrane layer [1] — [3], as well as with passive and active ionic transport [19]. The observed depolarization at pH = 6 is a characteristic effect for low concentrations and acid medium in which the anesthetic is almost under cationic form [4].

It is known that the increase in the $K^+ : Ca^{2+}$ ratio in the external medium causes a membrane depolarization [16], [18] by transition of the laminar phospholipid micellae structured by Ca^{2+}, form the external membrane layer, to the globular ones structured by K^+ [1], [2], [18]. The procaine prevents K^+ issue from the micellae and Ca^{2+} rebinding, with structure and normal potential re-establishment [1]. The blocking of the membrane repolarization occurs also during the action potential [12]. This effect is characteristic of procaine as anesthetic agent and membrane stabilizer.

At the decrease in the $K^+ : Ca^{2+}$ ratio a membrane hyperpolarization occurs, owing to the laminar micellae prevailing [20]. Procaine and Ca^{2+} interact specifically [26] hindering one another in respect to their binding to the membrane phospholipids [16], [20], effect observed also in our experiments (Fig. 5 — A).

At the interaction between the fibres and cholesterol, the latter is strongly bound to the membrane structure [23]. This implies a change in the ratio of cholesterol with the other lipids and a tighter micellae

packing, expressed by an irreversible hyperpolarization and an altered membrane permeability. Procaine determines a gradual decrease in the hyperpolarization (Fig. 7 — B) by the labilization of the steroid binding in the membrane. This may be removed by washing in normal Ringer. The interaction between procaine and cholesterol deposited in the membrane and its competition with Ca^{2+} explains thus the re-establishment effects of normal functions of sanguine vessel walls at its administration as Gerovital H_3 in ageing phenomena [6], [7].

It is known that in the organism procaine splits into DEAE and PABA by procainesterase action [8], [9], [11], [22] each metabolite having a proper pharmacodynamical action. In the human body the splitting is fast, occurring in the liver as well as in other tissues and the blood. For other species (horse) the esterase capacity is lower explaining a stronger procaine action upon their nervous system [9].

There are no sufficient available data on the comparative study of procaine, DEAE and PABA action at the cell and especially cell membrane level [10], [11], [22]. From our experiments it is evident that in normal conditions only DEAE has similar effects with those of procaine on the membrane potential (Fig. 1). At acid pH neither DEAE or PABA do determine the depolarization specific to procaine (Fig. 2), showing that this effect is due to the entire molecule of anesthetic. DEAE has simliar actions with those of procaine both in respect to the repolarization blocking of the depolarized membrane by K^+ (Fig. 3) and the abolition of the membrane hyperpolarization by Ca^{2+} (Fig. 6). In the same conditions but PABA has different effects (Figs. 4 and 6), showing even a slight action of enhancement of the Ca^{2+} effect and not of its abolition. We evidenced that the procaine effects occur by its interaction with membrane phospholipids, ions and passive and active transport [1], [2], [3], [19]. The same mechanism may explain also the DEAE and PABA effects on the membrane, taking into account their molecular structure and lipophilic properties.

Some similiarities between procaine and DEAE have been pointed out also by others, especially in respect to antispastic, analgesic and antifibrilant effects [11] or of re-establishment of hepatic cell functions [24]. It has been supposed that efficient procaine action in atherosclerosis is due to the PABA effects [7], [21]. From our data it is but clear that only procaine and DEAE remove the deposited cholesterol in membrane (Figs 7 and 8). They re-establish the normal ratio between cholesterol and the other lipids as well as the structure and normal membrane functions. PABA has not such effects. The anticholesterolic action of DEAE is quicker and stronger compared to that of procaine, providing prominent hypocholesterolemiant effects.

It is known that the membrane phenomena are involved in the ageing processes [6], [8]. In the Gerovital H_3 treatment of ageing phenomena it is shown that the obtained results are a consequence of the procaine action as well as of its metabolites [8]. But our results prove that only DEAE has complex effects at the membrane level, similar with those determined by procaine.

The positive effects on the deposited cholesterol in membrane, the interaction with K^+ and Ca^{2+} ions and the re-establishment effects of the membrane structure and normal functions evidence the possibility of DEAE use in the pharmaceutical products with an action similar with that of procaine. These products would not have the side-effects characteristic of procaine and also undesired interactions with other drugs, owing mainly to PABA [27]. There are drugs in geriatrics for which dimethylaminoethanol [13] with a structure similar to DEAE is used.

New investigations are necessary to elucidate other aspects of these phenomena.

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CHANGE OF SOME KINETIC AND MOLECULAR PARAMETERS OF ARYLESTERASE FROM THE COLON AS A RESULT OF GUINEA PIG IMMUNIZATION WITH RIBOSOMAL VACCINES

DANA IORDĂCHESCU, I. F. DUMITRU, B. SOW, MARIANA FLOREA, V. DIMA*, DIANA DINU

When the distribution of arylesterase activity in different segments of the guinea pig digestive tract was studied, the highest catalytic capacity of β-naphthyl acetate hydrolysis was revealed in the colon. Following oral administration of ribosomes from different pathogenic microorganisms (*S. typhi*, *E. coli* and *Sh. flexneri*) an increase of the specific activity of arylesterase was found. By electrophoretic and chromatographic studies, three molecular forms of arylesterase from the colon were distinguished. The concentrations and the molecular weights of multiple forms of arylesterase are changed as a result of immunization. Kinetic studies on the A molecular form of arylesterase (with the highest molecular weight) demonstrated that the affinity of the enzyme to substrate increases as a result of immunization.

In the last ten years the immunization with ribosomal and RNA preparations has been a sustained concern of numerous researchers. Youmans and Youmans [18], [19] using strains of *Mycobacterium tuberculosis* H 37 Ra obtained a fraction containing a RNA-protein complex of about 15—16 S with immunogenic properties. Smith and Bigley [14] using the ribosomal vaccines from *S. typhimurium* showed that they induce an immunologic protection against the toxiinfection produced by this microorganism. Venneman et al [17] proves that ribosomal fractions and purified ribosomal RNA extracted from cultures of *A. typhimurium* induced in mice an immune response and a protection against experimental infection, comparable to that obtained with vaccines from attenuated living germs. Similar results were obtained by Thompson for *Diplococcus pneumoniae* [15], by Schalla and Johnson [13] for *Streptococcus pyrogenes*, by Actor et al. [1] for *Vibrio cholerae* etc. which have also shown the installation of an immunity against homologous and heterologous infection in laboratory animals immunized with ribosomal preparations of these germs. In all cases, the protection obtained was greater than that offered by vaccines prepared from heat-killed germs.

The problem of the precise nature of the immunogen of these fractions remains to be solved. The studies of Youmans and Youmans [20] and of Venneman et al. [16] show that immunity is due to rRNA while Johnson's researches [6] that the proteins linked to rRNA are the true immunogens.

In the speciality literature, we have not found studies concerning the modifications of the activities of some enzymes as a result of immunization of laboratory animals with ribosomal vaccines. With that end in view, we have initiated studies concerning the change of the activities of some enzymes at the level of the digestive tract: RN-ase [5], alkaline phosphatase [3] and acid phosphatase [4] — as a result of the oral vaccination of guinea pigs with the ribosomal fractions isolated from different pathogenic microorganisms.

In this paper there are presented the results of the investigation of the immunization effects with ribosomal vaccines on the arylesterase from the guinea pig digestive tract. The role of arylesterase in the lipid metabolism is debated [11], but it seems that slightly specific carboxylesterases are implied in the transport of esterified fatty acids from the structure of lipoproteins and in the protection of the organism against some bacterial endotoxins [10].

MATERIAL AND METHODS

The crude ribosomal fractions were prepared according to the procedure described by Kita and Kashiba [7]. Groups of ten mature guinea pig females, gestant for approximately 45—48 days, were subcutaneously immunized at seven day intervals, with three doses from the crude ribosomal fraction obtained from *S. typhi*, *E. coli* and *Shigella flexneri* (2 mg proteins/dose). Two weeks after birth, the young guinea pigs were orally immunized with one single dose of ribosomal vaccine and after 7—10 days they were sacrificed.

From the sacrificed animals the five segments of the digestive tract (stomach, jejunum, ileum, cecum and colon) were separated and after washing in pysiological serum, the biological material was frozen at $-15°C$.

The enzyme extraction was realized by grinding the tissues in quartz sand and Triton $\times$ 100 0.1 % solution (v/v), the extraction ratio being 1 g: 10 ml. After extraction for one hour at 4° C, the resulted homogenate was centrifuged at 5 000 r.p.m., for 10 minutes and the obtained supernatant represented the total protein extract.

The dosing of the enzyme activity was realized by using the method of Nachlas and Seligman [9] adapted by us. The reaction mixture contains 400 nmoles β-naphthyl acetate and the enzyme preparation (20—70 μg pretein) in a final volume of 1.1 ml buffered at pH 9.5 with a buffer solution glycine-NaOH 0.2 M. The naphthol released was complexed with Fast Blue B and the product formed was colorimetrically determined at 540 nm. The optical densities were transformed in terms of concentration by means of a standard curve. The specific activity of arylesterase was expressed in nmoles β-naphthol released/mg protein/min/37°C.

The protein concentration in the medium was assayed with the method of Lowry et al [8] using bovine serum albumin as standard.

The chromatographic study of arylesterase from the colon was realized on a Sephadex G-200 column (1.8×30 cm) in which the gel was equilibrated with a 0.9 % NaCl solution. The elution has been performed with the same saline solution at a rate of 20 ml/hr, fractions of 5 ml each being collected and analysed as concerns the protein concentration and enzyme activity.

Electrophoresis in polyacrylamide gel was carried out according to the method described by Davis [2], applying samples of 200 μg protein on gel. For making evident the protein bands with arylesterase activity, the gels were incubated one hour at 37°C in a solution which contains 50 mg β-naphthyl acetate, 100 mg Fast Blue RR dissolved in 50 ml 0.1 M phosphate buffer, at pH 7.4

RESULTS AND DISCUSSION

In Table 1 there are presented the specific activities of arylesterase from the protein preparations obtained from the stomach, the jejunum, the ileum, the cecum and the colon of guinea pigs. One establishes that the highest arylesterase activity is found in the colon and the smallest in the jejunum (53.59 % from the activity found in the colon). Large

Tabel 1

The distribution of the specific activity of arylesterase from the different segments of the guinea pig digestive tract

Digestive tract segment	nmoles β-naphthol/mg protein min/37°C	%
Stomach	362.0	91.30
Jejunum	212.5	53.59
Ileum	293.0	73.89
Cecum	379.3	95.66
Colon	396.5	100.00

specific activities were also determined in the protein preparations obtained from the cecum (95.66%) and from the stomach (91.30%). The preferential localization of arylesterase in the large intestine may be correlated with the role of these enzymes in the degradation of bacterial endotoxins, by splitting the lipopolysaccharides from their structure.

The specific activities of arylesterase from the five segments of the digestive tract, isolated from the control guinea pigs and from the animals immunized with the crude ribosomal fraction prepared from the cultures of *S. typhi*, *E. coli* and *Shigella flexneri*, are presented in Table 2. One establishes that the arylesterase from the stomach is little affected by the treatment with the ribosomal vaccines, observing only weak activatory effects. The arylesterase from the jejunum-ileum is activated as a result of immunization with the ribosomal vaccines, to the highest degree in the case of the experiment with the crude ribosomal fraction isolated from *S. typhi*, microorganism which provoked the typhoid fever affecting especially the small intestine. The immunization with the ribosomal preparations isolated from *Shigella flexneri* (produces dysentery) induces the strong processes of activation of arylesterase in the large intestine — region upon which this microorganism acts.

Because at the colon level, the highest specific activities of arylesterase and high percentages of activation were revealed, as a result of immunization with ribosomal vaccines, a series of investigations on the enzyme from the colon were performed.

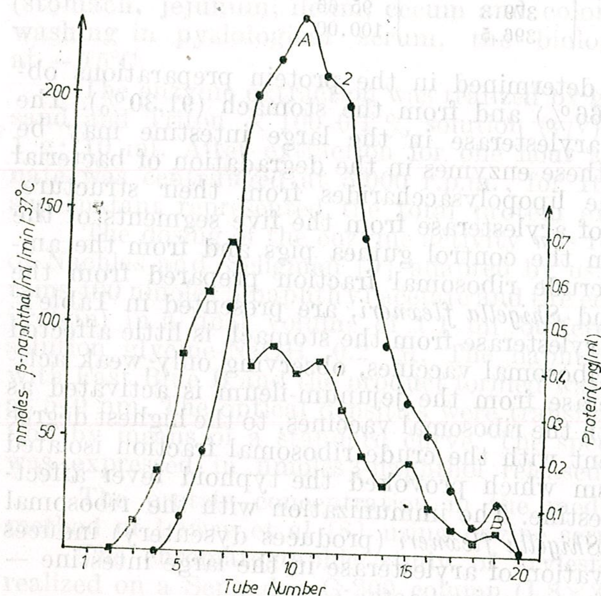
Table 2

The modification of the specific activity of the arylesterase from the different segments of the guinea pig digestive tract as a result of the immunization with ribosomal vaccines*

nmoles β-naphthol mg/min/37°C segment	i	ii	iii	iv
Stomach	362.0	364.0	366.2	363.5
Jejunum	212.5	301.4	236.2	226.9
Ileum	293.0	331.0	325.3	298.9
Cecum	379.3	409.0	405.7	423.4
Colon	396.5	421.2	410.6	453.5

* The enzyme preparations obtained from the digestive tract of the control guinea pigs (i) and of the animals immunized with the ribosomal vaccines from *S. typhi* (ii), *E. coli* (iii) and *Shigella flexneri* (iv).

For the enzyme partial purification, the fractional precipitation of the total protein extract obtained from the colon was realized. The arylesterase activity is concentrated in the protein fraction precipitated within 30–90% saturation range in ammonium sulfate. This enzyme preparation was submitted to chromatography on a Sephadex G–200 column. In figure 1 it is presented the chromatogram obtained in the case



Fig– 1. Elution curve of proteins of an enzyme preparation obtained from the colon of the guinea pig control on a Sephadex G–200 column (1-protein concentration ; 2-enzyme activity).

of the separation of the enzyme preparation isolated from the colon of the control guinea pigs. Two protein peaks with arylesterase activity are evinced, the first with an elution volume of 30 ml and an asymmetrical aspect and the second with a small activity, eluted with 57 ml 0.9% NaCl solution.

The chromatogram obtained in the case of the separation of proteins from the enzyme preparation isolated from the colon of the guinea pigs immunized with the ribosomal vaccines from *S. typhi* is presented in figure 2. One observes an increase of the molecular polymorphism

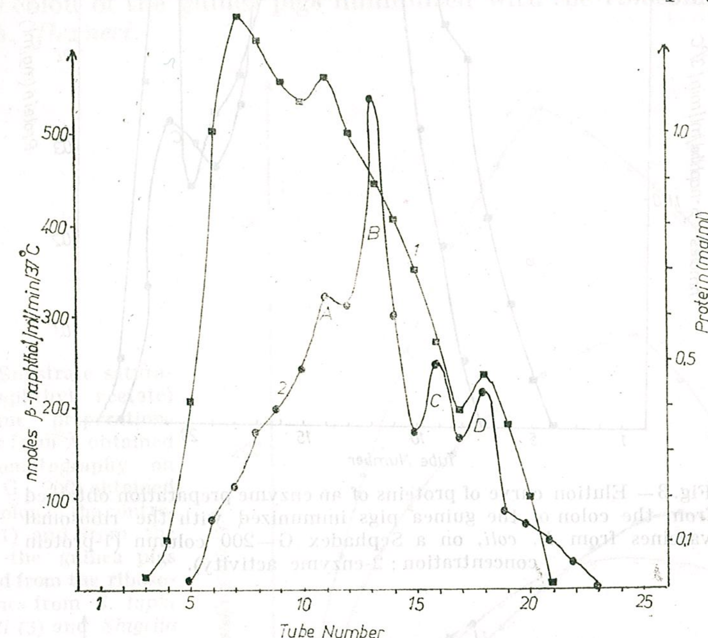


Fig. 2.— Elution curve of proteins of an enzyme preparation obtained from the colon of the guinea pigs immunized with the ribosomal vaccines from *S. typhi*, on a Sephadex G – 200 column (1-protein concentration ; 2- enzyme activity)

of arylesterase from the colon, eluting four protein peaks with enzyme activity with 0.9% NaCl volumes of 33 ml – 39 ml – 48 ml and 54 ml. The aspect of the chromatogram is totally different from that obtained in the control experiment, although the separations were realized exactly in the same conditions.

As a result of the immunization with the ribosomal vaccines from *E. coli*, one obtains the preparations, which submitted to chromatography on Sephadex G–200 (figure 3), separate in three protein peaks with enzyme activity, characterized by the elution volumes of 36 ml – 45 ml and 63 ml. One observes that in this case the immunization also produces an increase of the molecular polymorphism of the arylesterase from the colon and allows us to reveal some forms with molecular weights different from those characteristic of unimmunized guinea pigs.

In figure 4 it is presented the chromatogram of the proteins separated on Sephadex G–200 from the enzyme preparation isolated from the colon of the animals immunized with the ribosomal vaccines from

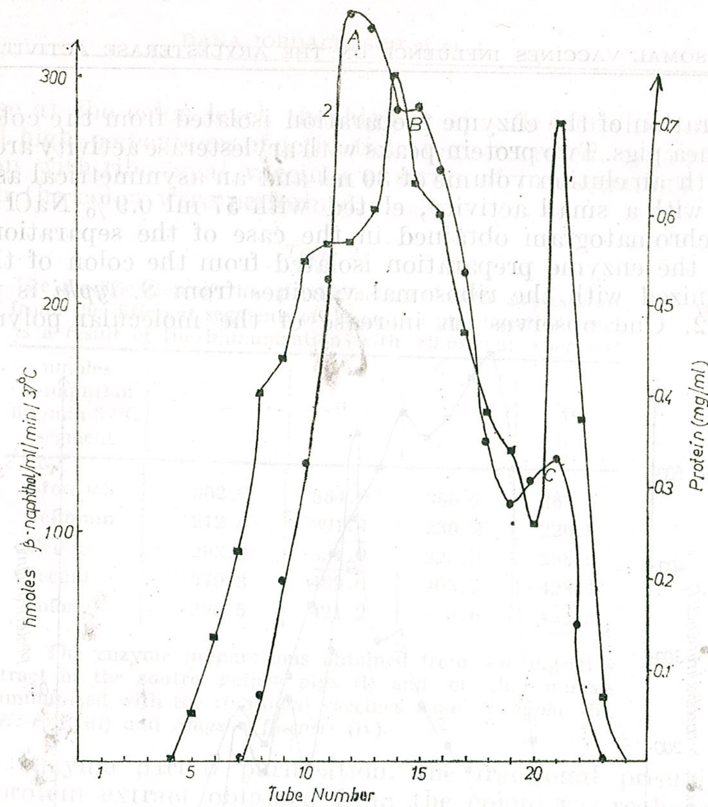


Fig. 3 — Elution curve of proteins of an enzyme preparation obtained from the colon of the guinea pigs immunized with the ribosomal vaccines from *E. coli*, on a Sephadex G—200 column (1-protein concentration; 2-enzyme activity).

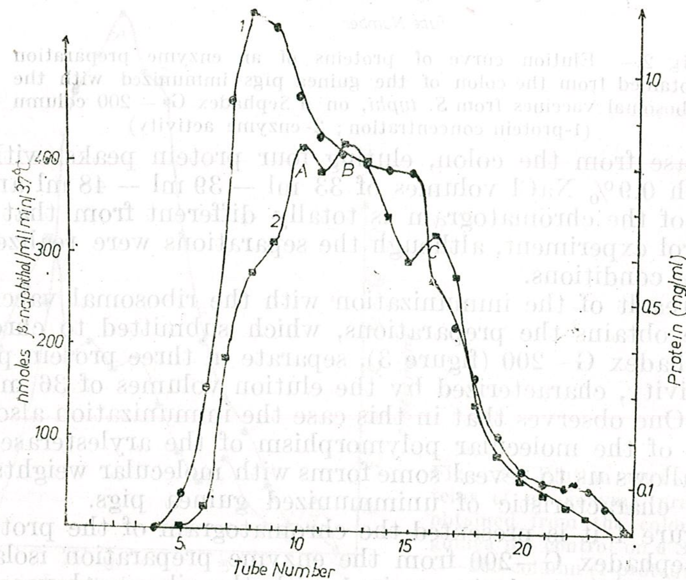


Fig. 4. — Elution curve of proteins of an enzyme preparation obtained from the colon of the guinea pigs immunized with the ribosomal vaccines from *Shigella flexneri* on a Sephadex G—200 column (1-protein concentration; 2-enzyme activity).

Shigella flexneri. Three peaks with enzyme activity, eluted with 30 ml—36 ml and respectively 48 ml NaCl 0.9 %, were less revealed. As compared to the control, in the case of the immunization with the ribosomal vaccines from *Shigella flexneri*, the first peak eluted with 30 ml remains, but with a smaller specific activity. On the other hand, another two molecular forms appear — with smaller weights — which present increased catalytic capacities, reflecting the strong activation of total arylesterase from the colon of the guinea pigs immunized with the ribosomal vaccines from *Sh. flexneri*.

Fig. 5.— Substrate saturation (β-naphthyl acetate) for enzyme preparations (molecular form A obtained after chromatography on Sephadex G—200) obtained from the colon of the control animals (1) and from the colon of the guinea pigs immunized from the ribosomal vaccines from *S. typhi* (2), *E. coli* (3) and *Shigella flexneri* (4).

Studying RN-ase from the cecum [5], we have shown that the immunization with ribosomal vaccines produces an activation of the enzyme and induces an increase of its molecular polymorphism.

Using the first protein fraction with an enzyme activity eluted from the Sephadex G—200 column, studies of the change of the arylesterase affinity from the colon for β-naphthyl acetate as a result of immunization were realized. In figure 5 are presented the saturation curves in β-naphthyl acetate. It has been found that the enzyme preparation obtain-

ed from the control experiment (eluate No. 30 from Sephadex G – 200) is saturated in substrate at 481.5 nmoles β-naphthyl acetate in the medium, while the preparations obtained from the experiments of the immunization with ribosomes from *E. coli* (eluate No. 36) and *S. typhi* (eluate No. 33) reach the maximum catalytic capacity at 428 nmoles substrate, after which inhibition phenomena appear. The preparation obtained from the colon of the guinea pigs immunized with the ribosomes from *Shigella flexneri* is saturated in substrate at the lowest concentrations of β-naphthyl acetate – at 321 nmoles. These saturation curves show that immunization induces an increase of the enzyme affinity for the substrate.

For the estimation of the constants K_M of the enzyme preparations partially purified from the colon of the control and immunized guinea pigs, the experimental data obtained in the study of the reaction rate variation with the substrate concentration were worked out according to Lineweaver-Burk (figure 6). One observes the decrease of the Michaelis

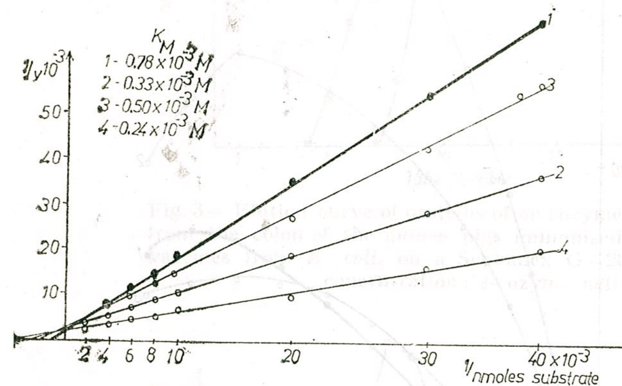


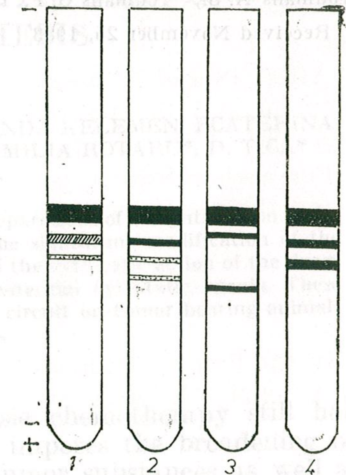
Fig. 6. – The Lineweaver-Burk plots of the substrate saturation curve for enzyme preparations obtained from the colon of the control guinea pigs (1) and of the animals immunized with the ribosomes from *S. typhi* (2), *E. coli* (3) and *Sh. flexneri* (4).

constant value of the enzymes from the colon, as a result of the immunization with ribosomal vaccines, especially in the case of the treatment with ribosomes from *Shigella flexneri* when the enzyme affinity to β-naphthyl acetate increases 3.25 times. This increase of the enzyme affinity for the substrate may represent an adaptation of the organism to the immunization with the crude ribosomal fractions from the three pathogenic microorganisms. The enzyme induction may be due to the lipopolysaccharide from the structure of the crude ribosomal fraction, which may constitute a substrate, *in vivo*, for the arylesterase from the colon. A similar behaviour was emphasized in the study of the RN-ase from the cecum of the guinea pigs immunized with ribosomal vaccines.

By electrophoresis in polyacrylamide gel, three protein bands with arylesterase activity were separated from the guinea pig colon (figure 7). The three protein bands are localized in the α-globulins region, two more intense with a slower migration (2 and 3) and one weaker (1) with a faster migration. The electrophoretic studies confirmed the results of the chro-

matographic separation, showing that immunization with ribosomal vaccines changes the concentrations of the three molecular forms of the arylesterase from the colon, easily affects the electrophoretic mobilities of

Fig. 7. – Enzymogram obtained as a result of electrophoretic separation (2 mA/gel, 220 V) of proteins of the enzyme preparations isolated from the colon of the control guinea pigs (1) and of the animals immunized with the ribosomal vaccines from *S. typhi* (2). *E. coli* (3) and *Shigella flexneri* (4).



these forms and produces the increase of the molecular polymorphism. Quantitatively, the highest modifications were produced as a result of the treatment with the ribosomal fraction from *Shigella flexneri* and qualitatively by immunization with ribosomes from *S. typhi*.

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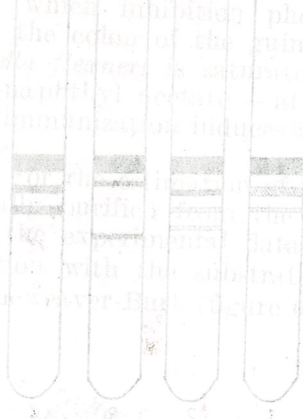

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SELECTION OF SOME POTENTIAL CYTOSTATIC AGENTS FROM NEW ANTIBIOTIC PREPARATIONS OF BIOSYNTHESIS

BY

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The "in vitro" action of some new antibiotic preparations of biosynthesis on HeLa cell culture development was investigated. The significant modification of the protein dynamics considered as an expression of the cytostatic action of the drugs tested by us allowed the selection of some potential cytostatic agents. These should be included in the "in vivo" screening circuit on tumor bearing animals in order to characterize them as cancerostatics.

At present, the human antineoplasic chemotherapy still holds a priority, but its relatively low efficiency imposes the broadening of the investigations both for finding new antitumor substances as well as for discovering new therapeutic ways of action on the malignant process.

These major objectives justify the great financial support the world all over for the identification of new agents with antitumor action.

The introduction of a new drug in the category of pharmacological cancerostatic agents is the result of a long, complex and careful process of investigation.

The chemotherapeutic screening programme meant for the identification of new antitumor preparations requires a step-like investigation of the drugs with possible antitumor activity, "in vitro" and "in vivo", at various levels of organization characterized by different reactive potencies [9], [13], [14], [15].

This paper presents the results of the "in vitro" tests of the cytostatic action of some new antibiotic preparations of biosynthesis, isolated at the Center for Antibiotic Research – Iași, carried out on HeLa cell cultures and valuable by their effect on the cell culture development.

MATERIAL AND METHODS

"In vitro" testing of the cytostatic action on HeLa cell cultures included a series of secondary metabolites of biosynthesis with antibiotic activity, hydrosoluble, coded A 52.26, A 54.24, A 52.41, A 54.10, A 46.41, A 46.45, A 40.25, A 48.18, A 42.126, U 369, U 82, U 145, U 278, U 292.

The evaluation of the cytostatic action was based on the comparative total protein dynamics during the evolution of control and treated cell cultures.

The culture tubes were innoculated with 1×10^5 cells in IC—65 medium. After 24 hours when the monolayer stage was attained and the cultures were in the logarithmic phase of development, the initial medium was replaced with a medium containing the drugs in a concentration of 1.5 mg/ml.

After 48 and 72 hours of culture development the medium in both the control and the treated tubes was discarded. The cell layer was washed with TFS and the total proteins were determined [10], [12].

For each culture type and time interval five culture tubes were used and the results were evaluated statistically by Student's "t" test.

RESULTS

In the first experiment, illustrated in Fig. 1, the antibiotic preparations A 52.26, A 54.24 and A 52.41 were tested.

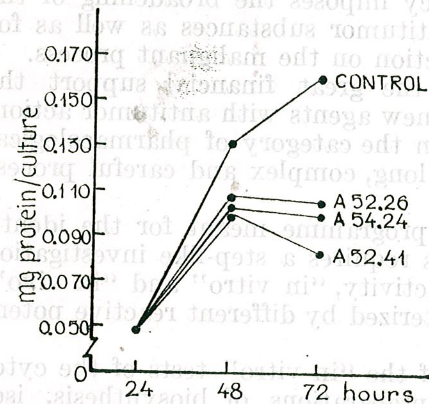

Fig. 1. — Protein dynamics of HeLa cell cultures incubated with the antibiotic isolates A 52.26, A 54.24, A 52.41 (1.5 mg/ml).

The cultures incubated with the isolate A 52.26, as compared to the controls, were characterized by a progressive decrease of the protein concentration, non-significant at 48 hours (17 %) but significant ($p < 0.001$) at 72 hours (34 %).

Protein dynamics in the cultures treated with A 54.24 showed significant decreases ($p < 0.05$ and $p < 0.01$) as compared to the controls; of 23 % and 38 %, respectively, at the time intervals analysed.

A similar result was obtained with the cultures incubated with the preparation A 52.41. Thus, the protein values determined at 48 and 72 hours, registered significant decreases ($p < 0.01$ and $p < 0.001$). respectively) of 24 % and 48 % as compared to the controls.

Another set of preparations tested included A 54.10, A 46.41, and A 46.45 and the results are shown in Fig. 2.

It is observed that the protein dynamics registered for the cultures incubated with A 54.10 and A 46.41, does not differ significantly from the control, highlighting the lacking cytostatic activity of cell culture development inhibition.

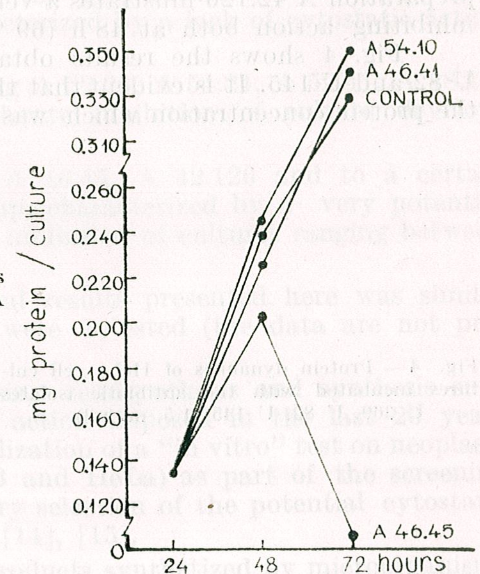

Fig. 2 — Protein content of HeLa cell cultures treated with the antibiotic isolates A 54.10, A 46.41, A 46.45 (1.5 mg/ml).

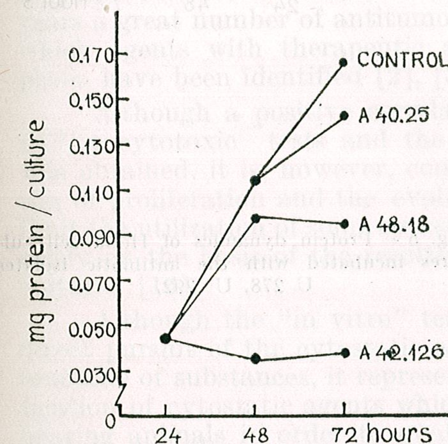

Fig. 3 — Protein dynamics of HeLa cell cultures incubated with the antibiotic isolates A 40.25, A 48.18, A 42.126 (1.5 mg/ml).

Conversely, the preparation A 46.45 induced a significant reduction ($p < 0.001$) of the protein concentration during the whole experiment, illustrating an inhibition of culture development up to 88 %.

The screening tests also included the antibiotic preparations A 40.25, A 48.18 and A 42.126 (Fig. 3).

The isolate A 40.25 proved to be inactive, since the cultures treated with this drug had a development similar to the control ones. A moderate

cytostatic activity characterized the preparation A 48.18, the incubated cultures presenting a significant decrease ($p < 0.001$) of 43 % in the protein concentration only at 72 hours.

The protein dynamics registered for the cultures treated with the preparation A 42.126 illustrates a very strong and significant ($p < 0.001$) inhibiting action both at 48 h (69 %) and 72 h (77 %).

Fig. 4 shows the results obtained with the preparations U 369, U 82 and U 145. It is evident that the first two induced a slight decrease in the protein concentration which was significant ($p < 0.02$) at 72 h (25 %).

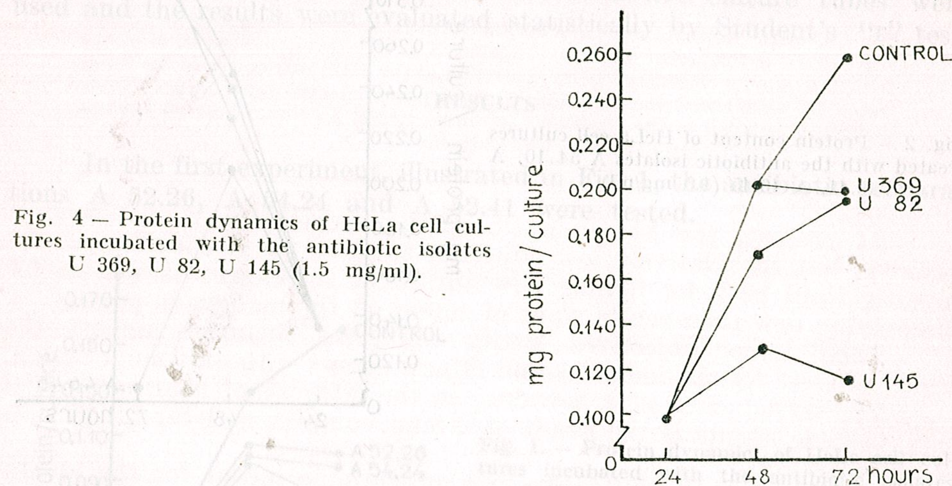

Fig. 4 — Protein dynamics of HeLa cell cultures incubated with the antibiotic isolates U 369, U 82, U 145 (1.5 mg/ml).

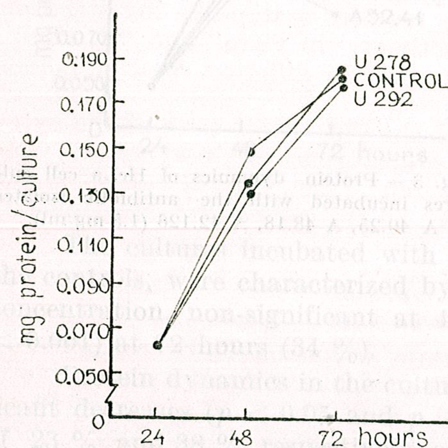

Fig. 5 — Protein dynamics of HeLa cell cultures incubated with the antibiotic isolates U 278, U 292.

On the contrary, the preparation U 145 revealed a clear cytostatic activity, the protein values significantly decreased ($p < 0.001$) during the whole experimental interval, reflecting an inhibition of the culture development of 40 % and 56 %, respectively, as compared to the controls.

The last test, presented in Fig. 5, included the preparations U 278 and U 292 which revealed no cytostatic activity.

DISCUSSION

The "in vitro" screening tests of the cytostatic activity of the new antibiotic preparations of biosynthesis allowed their classification into three groups, according to the intensity of the effect induced:

— the first group, including the preparations A 54.10, A 46.41, A 40.25, U 278, U 292, was characterized by a lack of cytostatic action on cell cultures;

— the second group, including A 52.26, A 54.24, A 52.41, A 48.18, U 369 and U 82, induced a moderate inhibition of culture development and

— finally, the preparations A 46.45, A 42.126 and to a certain degree U 145, form the third group, characterized by a very potential cytostatic activity determining an inhibition of cultures ranging between 56 % and 88 %.

The bulk of the experimental results presented here was similar when the antibiotic preparations were retested (the data are not presented).

The multitude of biosynthesis, semisynthesis and synthesis substances with supposed antitumor action imposed in the last 20 years the introduction and intensive utilization of a "in vitro" test on neoplasic cell cultures of human origin (KB and HeLa) as part of the screening programms meant for a preliminary selection of the potential cytostatic and/or cytotoxic drugs [9], [13], [14], [15].

In the class of the natural products synthetized by microorganisms a special interest is devoted to antibiotics all the more so as in the last years a great number of antitumor antibiotics have been discovered, from which agents with therapeutic action on some types of human neoplasia have been identified [2], [3], [4], [6], [8], [11], [17].

Although a positive correlation between the "in vitro" cytostatic and/or cytotoxic tests and the clinical antitumor effect [5], [7], [18] was obtained, it is, however, considered that the differences in the kinetics of proliferation and the evolutive cycle of tumoral cells considerably limit the utilization of some cytostatics in human neoplastic chemotherapy solely on the basis of the results obtained exclusively "in vitro" on cell cultures [16].

Although the "in vitro" testing system offers the possibility of a direct pursuit of the cytostatic action in a short time and requires small amounts of substances, it represents only a preliminary step in the identification of cytostatic agents which need to be tested "in vivo" on tumor bearing animals in order to qualify as cancerostatic agents.

The reproductibility, stability, statistic significance of the cell culture inhibition as well as the high intensity of cytostatic action and its rapidity allow the characterization of the antibiotic preparations A 46.45, A 42.126 and U 145 as true and potent cytostatic agents. From this point of view they represent a category of products of maximum interest in the perspective of future investigations on their "in vivo" antitumor activity.

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RADIOSENSITIZATION BY QUINONES

BY

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Some of the electron-affinic compounds, such as quinones, can be activated through microsomal metabolism, giving rise to free radical species which are able to interact specifically with DNA or to generate hydrogen peroxide in the immediate vicinity of cellular targets. The intervention of the enzymatic systems involved in H_2O_2 decomposition (catalase) leads to local generation of oxygen, thus enhancing radiosensitivity.

INTRODUCTION

The molecular mechanisms of radiosensitization have been subject of intense investigations in recent years. It is to be expected that future results in the field will contribute to the elucidation of the factors governing radiosensitization at the cell level.

Knowledge of the sensitization mechanism, beside theoretical relevance, can be of practical importance, both in the field of clinical practice and the design of new compounds with higher selective sensitizing action on tumors.

A large class of radiosensitizers, especially electron-affinic compounds, are efficient free-radical scavengers, particularly towards hydrated electrons (e^-_{aq}), leading to much stable structures with higher biological activity.

Some of the electron-affinic compounds, such as quinones, can be activated inside the cell (during microsomal metabolism) to free radical species with site specificity for DNA or lead to the production of H_2O_2 as a toxic product in the cell [1, 2, 3]. The decomposition of hydrogen peroxide by catalase causes eventually an increase in the oxygen concentration and thus an enhancement in the radiosensitivity of the cellular systems involved. We referred to this radiosensitization mechanism as "biophysical radiosensitization", in contrast to the more biochemical approach to radiosensitization, which is to use compounds having a depressing action on DNA [11].

In the present paper we report a series of *in vitro* results in order to evaluate the role of hydroquinone as a potential radiosensitizer of the biophysical type mentioned above.

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MATERIAL AND METHODS

Normal and H 18 R tumor-bearing rats (Wistar, male, with tumor implants of 13 days), weighing 120—150 g, were killed by cervical distribution and samples of liver and tumor were immediately collected on ice. Each tissue sample (200 mg) was homogenized with cold phosphate buffer (0.01 M, pH = 7) in a Potter homogenizer, at a final volume of 4 ml. The homogenates were centrifuged 10 min at 750 g and 20 min at 10 000 g and the supernatant fraction retained for catalase activity measurements. Catalase activity was determined using the method of Lottsfeld [12], modified by us.

The reaction mixture contained 0.1 ml supernatant + 1.9 ml 50 mM H_2O_2 solution in 0.01 M phosphate buffer (pH = 7). Hydroquinone was added in the reaction mixture in concentrations ranging from 1 μg/ml to 4 μg/ml, immediatly before determining catalase activity.

Catalase activity was monitored on a Beckman model 26 spectrophotometer, studying the decrease rate of the optical density of the reaction mixture at 230 nm.

RESULTS

In Fig. 1 are presented the absorption spectra of hydroquinone in distilled water, in the absence and in the presence of normal liver homogenate supernatant fraction. The disappearance of the peak region at 198 nm can be regarded as an indication that hydroquinone is oxidized in the presence of normal liver homogenate supernatant fraction.

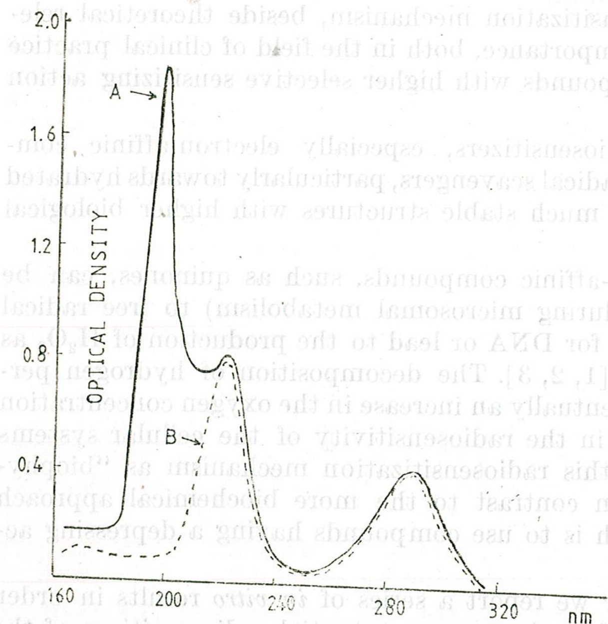

Fig. 1. — Absorption spectra of hydroquinone: A-in the absence and B-in the presence of normal liver homogenate supernatant fraction. The reaction mixture contained: A — 1.9 ml hydroquinone solution (20 mg/l) + 0.1 ml distilled water (reference: distilled water). B — 1.9 ml hydroquinone solution (20 mg/l) + 0.1 ml liver homogenate supernatant fraction (reference: distilled water (1.9 ml)+ + 0.1 ml liver homogenate supernatant fraction).

In Fig. 2, catalase activity in normal liver is represented as a function of hydroquinone concentration.

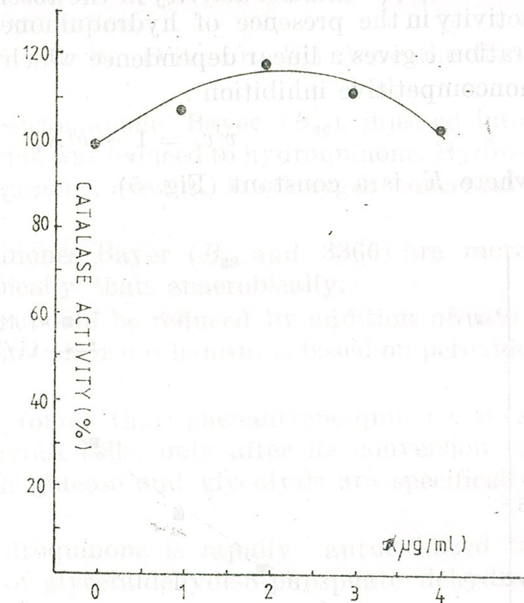

Fig. 2. — Catalase activity in normal rat liver as a function of hydroquinone concentration (c).

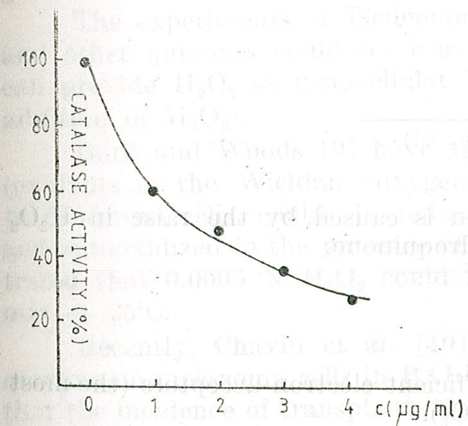

Fig. 3. — Catalase activity in H 18 R tumor as a function of hydroquinone concentration (c).

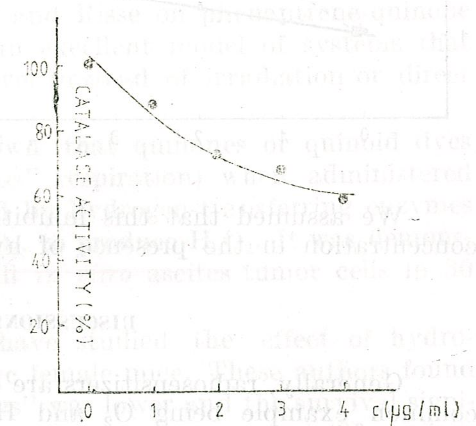

Fig. 4. Catalase activity in the liver of H 18R tumor-bearing rats as a function of hydroquinone concentration (c).

It can be seen that up to 2 μg/ml hydroquinone, catalase activity increases, suggesting an increase in substrate (H_2O_2) concentration, as stated by the simple Michaelis formulation.

At hydroquinone concentrations beyond 2 μg/ml, catalase activity decreases, indicating a mechanism of substrate inhibition.

In the case of homogenate supernatant fractions from tumor and liver of tumor-bearing rats, hydroquinone causes a decrease of catalase activity, which is more marked in the case of tumor homogenates (Figs. 3 and 4). A plot of v/v_i (v-catalase activity in the absence of hydroquinone, v_i—catalase activity in the presence of hydroquinone) against hydroquinone concentration c gives a linear dependence which can be described in terms of noncompetitive inhibition :

$$v/v_i = 1 + c/K_i$$

where K_i is a constant (Fig. 5).

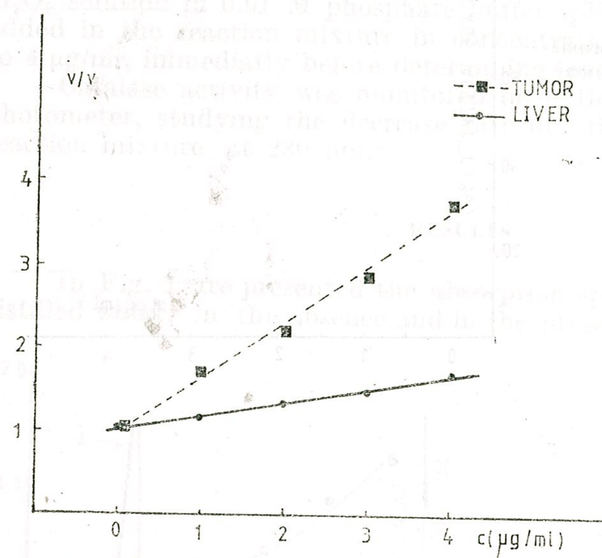

Fig. 5. — Plot of v/v_i (v-catalase activity in the absence of hydroquinone, v_i-catalase activity in the presence of hydroquinone) versus hydroquinone concentration (c), in the liver and tumoral tissue of H18R tumor-bearing rats.

We assumed that this inhibition is caused by the raise in H_2O_2 concentration in the presence of hydroquinone.

DISCUSSIONS

Generally, radiosensitizers are efficient electron acceptors (the most common example being O_2 and H_2O_2).

If radiobiological sensitization is highly attributable to the reactivity of the hydrated electron (e^-_{aq}), then this is a consequence of the hypothesis that the radiosensitizer molecule somehow enhances the probability of the electron reacting at the relevant biological site [4].

The sensitizer may in this case be regarded as an electron carrier which combines great electronic affinity with a structure apt for delocalization, leading eventually to a stabilization of the transient radical ion.

It is known that substances which contain C = O bond, such as quinones, are efficient scavengers for hydrated electrons.

Quinones can represent resonant structures which are able to enhance the stability of the radical-ion formed following irradiation, and thus enhance the probability of the biological damage.

It is noteworthy that quinones represent cytotoxic antitumor agents, even in the absence of irradiation.

The works of Mitchell on Synkavite (Vitamin K_3) were the first in this field [5].

Frimmer [6] showed that benzoquinone Bayer (B_{39}), injected into intraperitoneal ascites tumor material, was reduced to hydroquinone. Hydroquinone was rapidly oxidized to quinone, even at low oxygen concentrations.

Pütter [7] showed that quinones Bayer (B_{39} and 3366) are more active against tumor cells aerobically than anaerobically.

The action of these quinones could be reduced by addition of catalase, which led to the conclusion that their mechanism is based on peroxide production.

Tiedemann and Risse [8] found that phenantrene-quinone is a strong toxic agent against cancerous cells, only after its conversion to reduced hydroquinone, for which glucose and glycolysis are specifically required.

In the presence of O_2, hydroquinone is rapidly autooxidized to H_2O_2, leading to the inhibition of glyceraldehyde-3-phosphate dehydrogenases.

The experiments of Tiedemann and Risse on phenantrene-quinone and other quinones could serve as an excellent model of systems that can provide H_2O_2 at intracellular level (instead of irradiation or direct addition of H_2O_2).

Burk and Woods [9] have shown that quinones or quinoid dyes (oxidants in the Wieldan "oxygenless" respiration) when administered to cancerous cells, could be reduced by hydrogen-transferring enzymes and autooxidized in the presence of O_2, to produce H_2O_2. It was demonstrated that 0.0005 N H_2O_2 could kill *in vitro* ascites tumor cells in 30 min at 25°C.

Recently, Chavin et al. [10] have studied the effect of hydroquinone on melanoma cells in BALB/c female mice. These authors found that the incidence of transplant "takes" was lower and the survival significantly higher in mice who received hydroquinone treatment (80 mg/kg). The significant good response to hydroquinone treatment *in vivo* was considered encouraging for chemical manipulation of melanoma.

As for the mechanism of action of hydroquinone, the authors suggest that this is achieved via DNA.

Hydroquinone depressed *in vitro* DNA synthesis in Harding-Passey melanoma in 5 minutes, after 4 hours DNA synthesis being only 4% of the control value.

Bachur et al. [1] have studied the mechanism of action of some quinones used as antitumoral drugs (from the class of benzanthrenequinones, naphthoquinones and *n*-heterocyclic quinones). It has been demonstrated from EPR experiments that those quinones are converted by microsomes to free radical forms which are sufficiently stable to enter the nucleus and bind specifically to nuclear DNA, or may generate reactive oxygen radicals, such as peroxide, in the vicinity of DNA, and thereby produce the specific destructive effects on DNA that have been reported.

Our results point out that hydroquinone can generate H_2O_2 inside the cells and that this process is enhanced in the case of tumoral cells. These findings support the idea that hydroquinone can be a promising alternative in cancer therapy, both as an antitumoral agent per se and as a radiosensitizer.

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SEX PHEROMONE IN THE FRESHWATER PRAWN, *MACROBRACHIUM KISTNENSIS*

BY

R. SAROJINI, M. S. MIRAJKAR and R. NAGABHUSHANAM

The freshwater female prawn, *Macrobrachium kistnensis*, undergoes a moult before mating which is called parturial moult. These parturial moulted females release sex pheromone into water. This sex pheromone attracts only the intermoult mature males and successful copulation takes place between a postmoult female and intermoult stage male in the presence of the sex pheromone. The sex attractant pheromone is effective only for a short duration.

INTRODUCTION

Invertebrates show a wide variety of behaviour which is influenced by hormones. Sexual recognition and attraction which precedes the mating behaviour is stimulated by a hormone called sex pheromone [1]. A pheromone is defined as a chemical communicant released by an organism that influences the behaviour of the other organism of the same species [2].

Little information is available on the sex pheromones in freshwater crustaceans. Templeman [3], Templeman and Tibbo [4] suggested that successful mating in the American lobster, *Homarus americanus* is a moult related phenomenon guided by sex pheromone. Clutter [5] observed that a postmoult female shrimp, *Metamysidosis elongnate*, might release a sex pheromone which attracts the male. Similar observations have been made on other species of shrimps [6], lobsters [7] crabs [8] and crayfishes [9]. Regarding the freshwater prawns only one citation was available that of Kamiguchi [10] on *Palaemon paucidens*. The present study was undertaken to study the sex pheromones present in the freshwater prawn, *Macrobrachium kistnensis*.

MATERIAL AND METHODS

The prawns, *Macrobrachium kistnensis*, were collected from Kham river near Aurangabad. The prawns were kept in aerated aquaria and were fed daily on branned wheat and pieces of dead prawns. The water was changed daily. Mating behaviour of prawns was studied by keeping females and males in pairs, in glass aquaria. The prawns having a carapace length of 18–22 mm were chosen for the experiments.

OBSERVATION AND RESULTS

Macrobrachium kistnensis breeds twice in a year. (1) January to April (2) September to October. Prior to mating and subsequent egg laying the female undergoes a moult known as the parturial moult. This moult results in the enlargement of pleura formation of ovigerous hairs and the development of brooding chamber in the abdominal region. The female does not moult until the hatching of the eggs and all the changes that take place at the time of parturial moulting are lost in the next moult.

The results are given in the following pattern.

(1) Mating behaviour of male prawn
(2) Behaviour response of male prawn
(3) Behaviour assay of males during copulation
(4) Copulation response of males to different types of moult water and
(5) Effectiveness of sex pheromone in relation to time.

(1) MATING BEHAVIOUR

When a male prawn which is mature spots a partturial moulted female, it starts vigorous swimming on substratum, stretches the chelae forward and the antennules are moved very actively. This kind of behaviour is clearly distinct from the feeding behaviour (Plate 1). When the male prawn comes across the parturial female mounts on her back. The male prawn turns his mounting posture and slowly migrates towards the ventral side and attaches itself to the females thoracic sternum in a

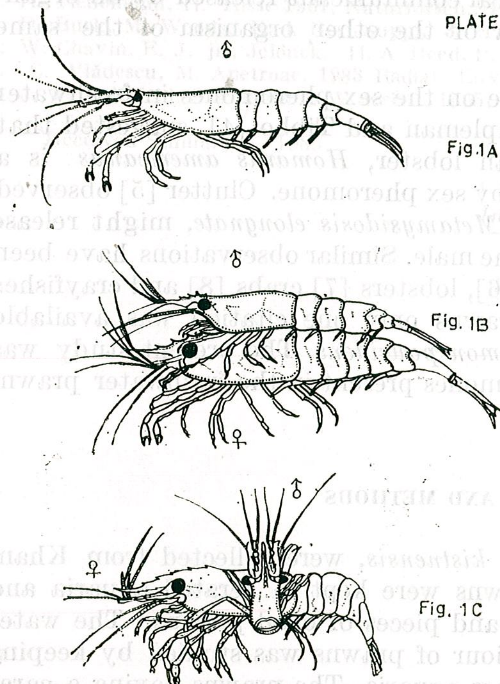

PLATE I. The diagrammatic representation of the different phases during the mating behaviour of freshwater prawn, *M. kistnensis*.
Fig. 1_A — The male in the searching phase.
Fig. 1_B — The male mounted on the female's back, phase mounting.
Fig. 1_C — The male clasped the female ventrally, phase copulation.

horizontal position and deposits the spermatophores. The time required for the spermatophore deposition hardly takes five to ten seconds. After sperm deposition both male and female prawns separate and swim differently (Plate 1, Fig. 1 A, Fig. 1 B and Fig. 1 C).

(2) BEHAVIOURAL RESPONSE OF MALE PRAWN

After observing the mating behaviour, the behavioural response of male prawns was studied by the following experiment. Ten mature males of intermoult stage were maintained in four separate aquaria. Into the first aquarium a single premoult female was introduced. Similarly a single intermoult female, a single postmoult female and a single parturial moult female were introduced into the second, third and fourth aquaria respectively. No copulatory response was observed in the males that were in the first and second aquaria. In the third aquarium out of ten males only three males approached the female and showed searching and mounting behaviour white in the fourth aquarium eight males showed searching, mounting and copulation behaviour. The results are presented in Fig. 1. From the above observations it can be seen that the males copulated successfully with the females which have undergone parturial moult.

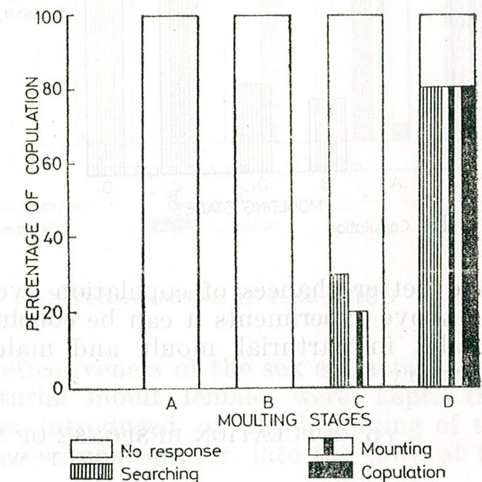

Fig. 1. — *Macrobrachium kistnensis*. Behavioural response of male prawns, at different moulting stages of female prawn.

(3) BEHAVIOURAL ASSAY OF MALES DURING COPULATION

Male prawns are polygamous and if a copulated female is replaced from the company of a male prawn by a newly parturial moulted female then the male is attracted toward female prawn and immediately copulates with the female. The above experiment revealed that in the case of female prawns copulation takes place successfully in the parturial

moult stage. The following experiment was designed in such a way to see which moulting stage is more successful for copulation in the male prawns.

Five troughs were taken and into each trough ten mature males belonging to the moult stages A, B, D_{1-2} C and D_0 were placed along with a single parturial moulted female. The results are presented in Fig. 2. From the figure it can be seen that the moulting stages C and D_0

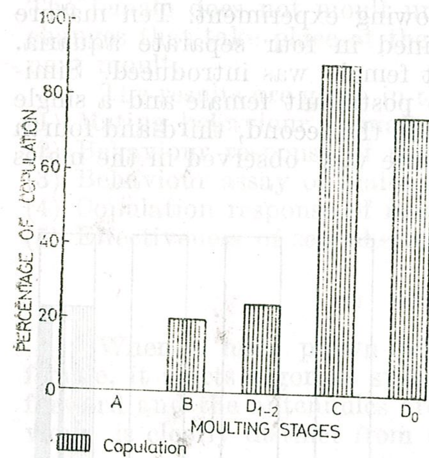

Fig. 2. — *Macrobrachium kistnensis*. Behavioural assay of males in copulation at different moulting stages.

have better chances of copulation over that of the other stages. From the above experiments it can be concluded that for successful copulation females in parturial moult and males from the intermoult stage are necessary.

(4) COPULATION RESPONSE OF MALES TO DIFFERENT TYPES OF MOULT WATER

From the above two experiments it can beseen that some sex attractant from parturial moult female is released into the water and this substance is stimulating the intermoult stage males. This observation is confirmed further by the following experiment.

Female prawns whose gonads were fully grown and were in premoult stage were kept in aquaria and allowed to undergo parturial moult. The water from this aquaria is called as parturial moult water (P. M. water). Male prawns were similarly kept in aquaria and allowed to undergo moulting. The water from these aquaria is called as male moult water (M. M. water). Female prawns from different stages were kept in P. M. water and M. M. water and they were divided into the following groups.

(1) MM — water + postmoult female
(2) MM — water + parturial moult female
(3) PM — water + intermoult female
(4) PM — water + premoult female
(5) PM — water + postmoult female
(6) PM — water + parturial moult female.

Into each group five intermoult stage males were introduced. The results are summarized in Fig. 3. No copulatory response was observed in the first four groups. In the fifth group 5 % of copulation took place. In the last group 90 % copulation occurred. From these observations it is confirmed that soft exoskeleton of the female and sex attractant are necessary factors for inciting males to copulation.

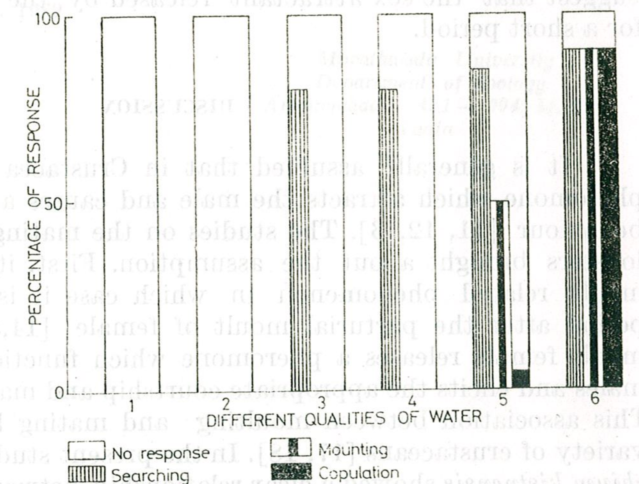

Fig. 3. — *Macrobrachium kistnensis*. Copulation response of males to different qualities of moult water.

(5) EFFECTIVENESS OF THE SEX PHEROMONE IN RELATION TO TIME

To measure the time of the effectiveness of the sex attractant, five troughs each containing a parturial moult female were kept. Into the first trough five males were introduced at the beginning of the experiment, into the second at the end of one hour, into the third at the

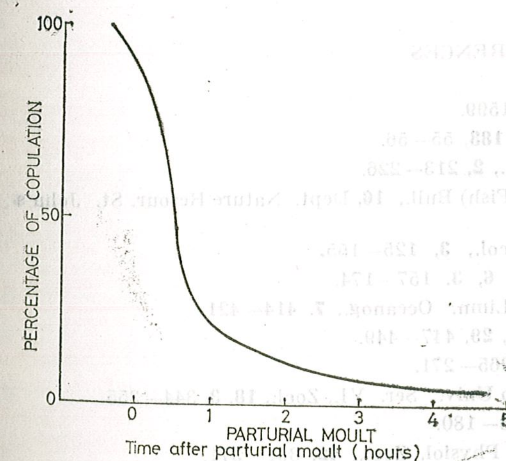

Fig. 4. — *Macrobrachium kistnensis*. Effectiveness of sex pheromone related with lapse of time after parturial moult.

end of two hours, into the fourth at the end of three hours and into the fifth at the end of four hours and their mating behaviour was observed. The results are presented in Fig. 4. From the figure it is clear that 90% of the males copulated with the females immediately after the parturial moult. The rate of mating behaviour decreased rapidly after one hour of parturial moult. No male prawn copulated after four hours. These data suggest that the sex attractant released by the female is effective only for a short period.

DISCUSSION

It is generally assumed that in Crustacea the female releases a pheromone which attracts the male and causes an appropriate courtship behaviour [11, 12,13]. The studies on the mating behaviour of American lobsters brought about the assumption. First, it was assumed to be a moult related phenomenon in which case it is restricted to a short period after the parturial moult of female [14,3]. Secondly, the post-moult female releases a pheromone which functions as an attractant to males and elicits the appropriate courtship and mating behaviour [15, 16]. This association between moulting and mating has been observed in a variety of crustaceans [17, 18]. In the present study the prawn, *Macrobrachium kistnensis* showed a clear relationship between moulting and mating. The sex pheromone is released into the water only after parturial moult and the mating phenomenon is elicited. Probably the softness of the exoskeleton and sex pheromone are important for the courtship behaviour. These results agree with the observation made earlier in other freshwater prawns, *Palaemon paucidens* [10] and *Caridina weberi* [19].

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THE CORRELATION BETWEEN THE FILTERING ZOOPLANKTON AND PHYTOPLANKTON

IV. EFFECTS OF VARIOUS FOOD CONCENTRATIONS AND OF PARTIAL OXYGEN PRESSURE UPON THE ENERGY FLOW

BY

ANGHELUȚĂ VĂDINEANU, RALUCA MUNTEAN

The cumulated energy budget of an individual during its life cycle has been determined, in the case of *D. magna*, *D. pulex*, *Simocephalus vetulus* (*Cladocera*) and *Eudiaptomus gracilis* (*Copepoda*) species. On the basis of experimental results, the effects of various food concentrations and of PO_2 are estimated upon the energy budget of natural populations of the species studied.

The role of a natural population within its community could be defined on the basis of the energy flow rates and nutrients recycling [5, 9, 7].

The parameters of the energy flow represented by the energy intake (C), energy expense (R), accumulated energy (P) and nonassimilated energy (FU) modify in time according with changes that occur in the structure of the population and with fluctuations in the quality and quantity of food, temperature, PO_2, etc.

The effects of excessive concentrations of algae (above 20 mg dry weight/l) upon the filtration rate and food uptake rate and of the concentration of algae and of partial oxygen pressure on the energy expenditure of filtering species *Daphnia magna*, *D. pulex*, *Simocephalus vetulus* (*Cladocera*) and *Eudiaptomus gracilis* (*Copepoda*) have been presented in other works [8].

It was considered important to present in this paper how and to what extent the concentration of algae and the reduced partial pressure of oxygen influence the energy flow and the efficiency of energy transformation for the same species.

MATERIALS AND METHODS

Since the responses of the populations of *Cladocera* species, i.e. *D. magna*, *D. pulex* and *S. vetulus*, do not differ significantly [8] it was decided to characterize the response of *Cladocera* populations on the basis of the results obtained with *D. pulex* and for filtering Copepodae based on the results obtained with *Eudiaptomus gracilis*. Thus the cumulated energy budget of an individual during its life cycle was worked out.

The energy expenditure (R_c) was calculated as a function of size (W), food concentration (H) and partial oxygen pressure (PO_2) [8]. The

energy consumption (C_c) was established on the basis of the filtration rate and food concentration [8] and production (P_c) by summing up the daily increase in the biomass (P_g) and egg number (P_r).

For both species the cumulated energy budget has been calculated for optimal feeding (1 mg dry wt/l) and oxygen concentration (PO_2 > 120 mm Hg).

For *D. pulex* the energy budget was also determined for two other states, characterized by optimal food levels and oxygen deficit (PO_2 = 20 ± mm Hg) and by food in excess (H = 40 ± 5 mg dry wt/l) and oxygen deficit respectively. The cumulated energy budget was also estimated for *E. gracilis* under food excess (40 ± 5 mg dry wt/l) conditions. In all experiments the temperature was maintained at 20 ± 1°C.

RESULTS AND DISCUSSION

From the analysis of the results presented in table 1 and figures 1,2 and 3, the following modifications of the energy budget values and of the coefficients characterizing the efficiency of energy transformations occur :

– The production or energy accumulation within the population (P_c) decreases by 30 % under low oxygen level and by 40 % under food excess conditions as compared with the production under optimal food and oxygen concentrations. The reduced productivity reflects both a growth limitation and a reduced prolificity.

Table 1

The cumulative energy budget and efficiency of energy transformation for the whole individual life cycle of *D. pulex*

No.	Environment conditions	C_c	A_c	P_c (cal)	R_{1c}	R_{2c}	R_c	U^{-1}	K_1 %	K_2
I	H = 1 mg dry matter/l PO_2 > 100 mmHg T = 20 ± 1°C	2.75	2.2	1.1	1.1	—	1.1	80	40	51
II	H = 1 mg dry matter/l PO_2 = 20 ± 3 mm Hg T = 20 ± 1°C	2.36	1.79	0.77	0.706	0.31	1.02	76	33	42
III	H = 40 ± 50 mg dry matter/l PO_2 = 20 ± 3 mm Hg T = 20 ± 1°C	25.7	2.18	0.67	0.61	0.89	1.5	9	3	31

– The assimilability (U^{-1}) is reduced by more than 80% under food excess conditions, as compared with the values obtained under optimal conditions.

– The efficiency of storing the assimilated energy $\left(K_2 = \frac{P}{A}\right)$ decreases as the animals are confronted with increasingly adverse conditions which induce an increase in the energy expenditure by considerably reducing the efficiency of the catabolic processes.

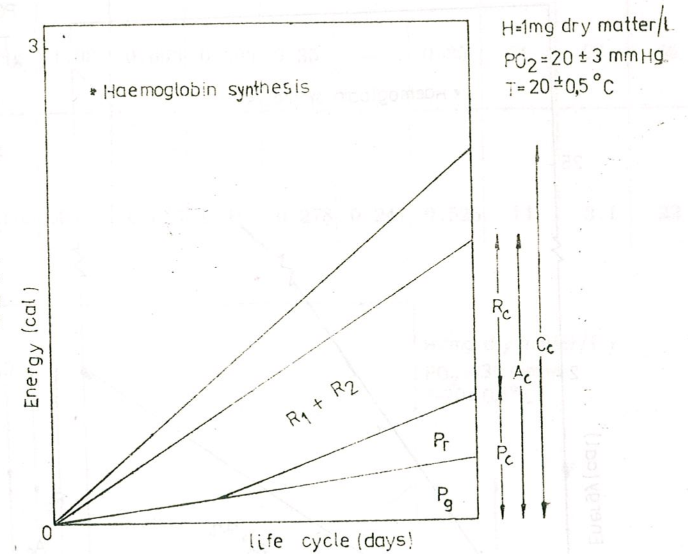

Fig. 1. – The cumulated energy budget of an individual of *Daphnia pulex* during its life cycle (29 days).

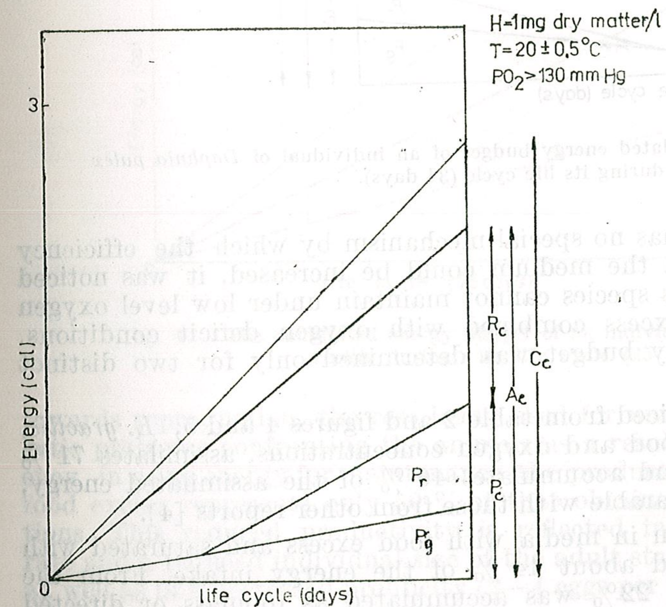

Fig. 2. – The cumulated energy budget of an individual of *Daphnia pulex* during its life cycle (33 days).

— Due to an increased energy consumption and to a reduced assimilability as food concentrations in the medium reaches values above 20 mg dry wt/l, the efficiency of accumulation of energy intake (K_1) decreases from 40 % to only 3 %.

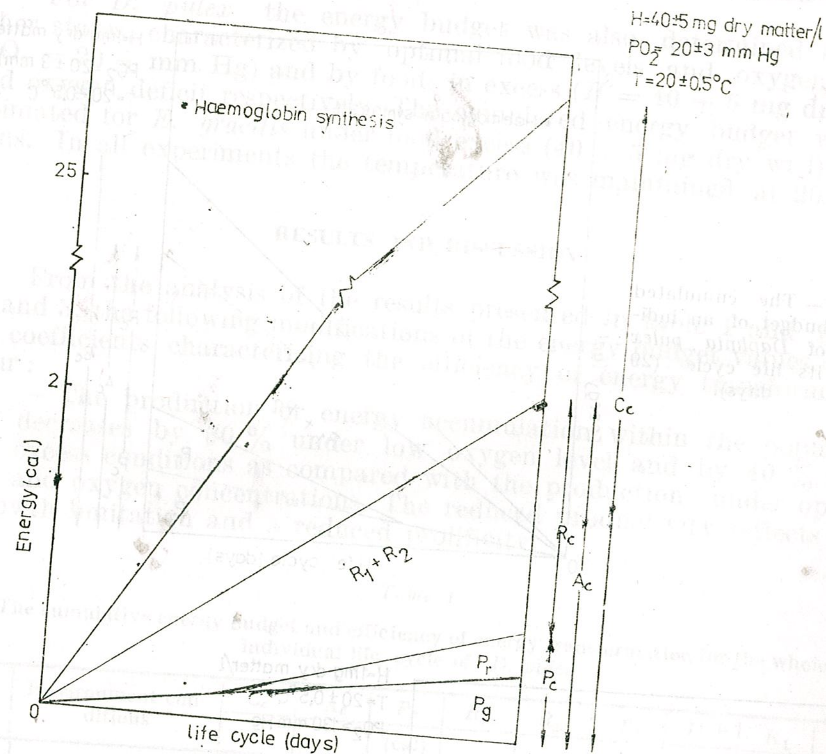

Fig. 3. — The cumulated energy budget of an individual of *Daphnia pulex* during its life cycle (34 days).

Since *E. gracilis* has no special mechanism by which the efficiency of oxygen uptake from the medium could be increased, it was noticed that populations of this species cannot maintain under low level oxygen waters or under food excess combined with oxygen deficit conditions. Consequently, its energy budget was determined only for two distinct states.

As it could be noticed from table 2 and figures 4 and 5, *E. gracilis*, grown under optimal food and oxygen concentrations, assimilates 71 % of the energy intake and accumulates 46 % of the assimilated energy, these values being comparable with those from other reports [4].

The animals grown in media with food excess and saturated with oxygen have assimilated about 14 % of the energy intake. From the assimilated energy only 22% was accumulated as biomass or directed

Table 2

The cumulative energy budget and efficiency of energy transformation for the whole individual life cycle of *Eudiaptomus gracilis*

No	Environment conditions	C_c	A_c	P_c (cal)	R_{1c}	R_{2c}	R_c	U^{-1}	K_1 %	K_2
I	$H = 1$ mg dry matter/l, $PO_2 > 100$ mmHg, $T = 20 \pm 1$°C	1.08	0.653	0.303	0.35	—	0.35	71	28	46
II	$H = 40 \pm 5$ mg dry matter/l, $PO_2 > 100$ mmHg, $T = 20 \pm 1$°C	4.8	0.675	0.15	0.278	0.247	0.525	14	3.1	22

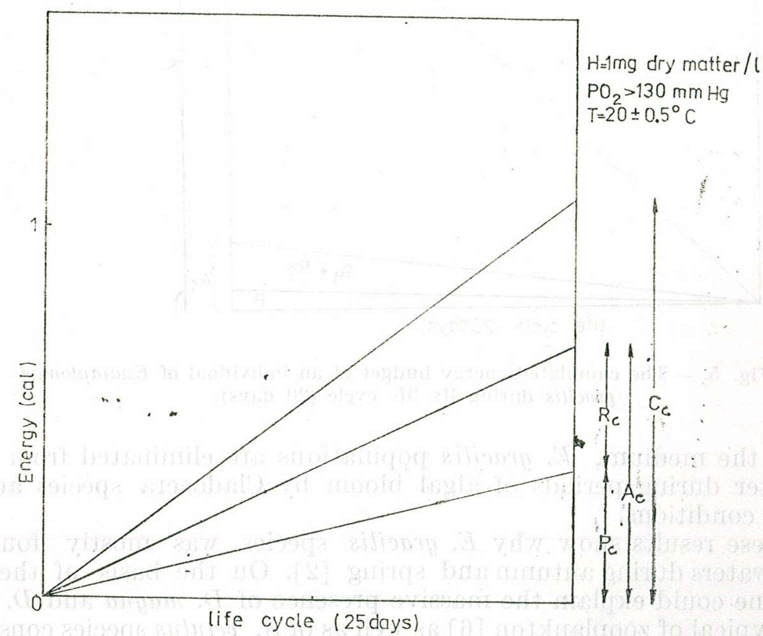

Fig. 4. — The cumulated energy budget of an individual of *Eudiaptomus gracilis* during its life cycle (25 days).

towards reproduction, the rest being used for maintenance. Due to energetic problems confronting the animals as a result of the reduced efficiency in using energy for maintenance the production under conditions of food excess represents only 50 % of that obtained under optimal conditions. This reduced productivity is reflected in a diminishing growth rate and a reduced individual size of the adult stages ($W \sim 17$ µg dry wt) as well as in a decreased prolificity (2—4 eggs per brood).

Our results on the influence of food concentration and of partial oxygen pressure on the energy flow for the populations of Cladocera and Copepoda studied show the inability of *E. gracilis* populations to maintain under conditions of waters with low oxygen levels and a high organic matter content. Due to energetic difficulties at high concentrations of

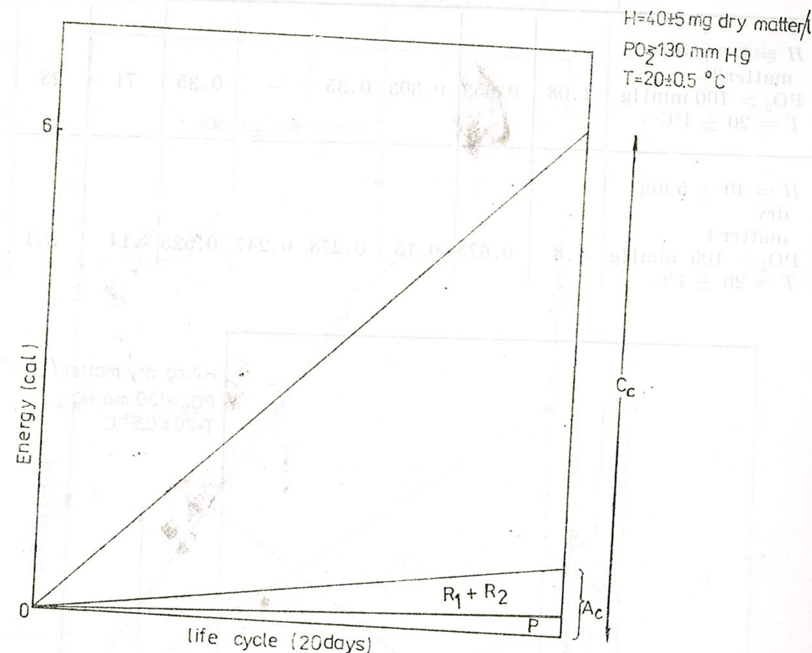

Fig. 5. — The cumulated energy budget of an individual of *Eudiaptomus gracilis* during its life cycle (20 days).

algae in the medium, *E. gracilis* populations are eliminated from eutrophic water during periods of algal bloom by Cladocera species adapted to such conditions.

These results show why *E. gracilis* species was mostly found in natural waters during autumn and spring [2]. On the basis of the same results one could explain the massive presence of *D. magna* and *D. pulex* species typical of zooplankton [6] as well as of *S. vetulus* species considered macrophytophitic [6] in waters in the Danube flood plains during the massive development of algae or in waters with a high and continuous influx of organic matter [1,3].

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ZOOGEOGRAPHIC ANALYSIS OF THE FAUNA OF TERRESTRIAL *HETEROPTERA* FROM ROMANIA

BY

I. ROŞCA

A correlation was established between the similarity and the geographic location or environmental conditions of the *Heteroptera* fauna occurring in Romania and in four other areas, with a view to determining the relationships between the terrestrial Heteroptera from Romania and the similar fauna from Egypt, Israel, Turkey and Tien Shan.

The data on the *Heteroptera* fauna from Romania are relatively scarce and, generally, included in faunistic studies carried out in restricted areas [3], [4] [15]. Most authors published either lists of all *Heteroptera* caught in a given area or only of the species of one family [1], [9], [14], [15] or even of one genus [10].

Heteroptera species are characterized zoogeographically [3], [4], [12], [13] only in a few studies and in others the totality of the examined fauna is characterized briefly [9], [17].

As throughout the world new data have been published on the systematics, ecology and distribution of *Heteroptera* [2], [5], [6], [7], [8], [11] and as only the species of the *Pentatomidae* superfamily were analyzed zoogeographically [16], we consider that a first attempt at grouping and characterizing as a whole from the zoogeographic viewpoint the entire terrestrial Heteroptera fauna of Romania is opportune.

MATERIALS AND METHODS

The zoogeographic classification of the Heteroptera species, made as mentioned previously [12], [13], has as basis the species level, while for the zoogeographic analysis of the entire fauna the distribution based on the present range of the species was used as single criterion.

The 693 species of terrestrial *Heteroptera* recorded in Romania [12] which belong to 25 families were grouped into 9 categories of zoogeographic elements (Cosmopolitan, Holarctic, Palaearctic, Euro-Siberian, European, Mediterranean, Ponto-Mediterranean, Turanic and Endemic) which are unanimously agreed on by the various authors interested in this subject.

The whole fauna of terrestrial *Heteroptera* from Romania was compared zoogeographically to that from Egypt, Israel, Turkey and Tien Shan.

RESULTS AND DISCUSSIONS

As shown in table 1, 6 (0.87 %) out of the 693 species of terrestrial *Heteroptera* recorded in Romania are Cosmopolitan, 53 (7.65%) are Holarctic, 61 (8.80 %) Palaearctic, 128 (14.87 %) Euro-Siberian, 169 (24.39 %) European, 191 (27.55 %) Mediterranean, 53 (7.65%) Ponto-Mediterranean, 23 (3.32 %) Turanic and 9 (1.30 %) are Endemic.

Table 1

Grouping by zoogeographic elements of the fauna of terrestrial *Heteroptera* from Romania

No.	Faunistic elements	Significance	Number	Percentage %
1	Cosmopolitan	known in all large zoogeographic areas	6	0.87
1	Holarctic	found in North America, Europe, northern Africa, Asia Minor, Central Asia, Siberia and the Far East	53	7.65
3	Paleoarctic	known in Europe, northern Africa, Asia Minor, Central Asia, Siberia, the Far East	61	8.80
4	Euro-Siberian	distributed in the forest, forest-steppe and steppe areas of Europe and Siberia	297	42.86
5	European	known in Europe	169	24.39
6	Mediterranean	distributed in the areas surrounding the Mediterranean Sea	191	27.55
7	Ponto-Mediterranean	distributed in the Balkan Peninsula, the Greek archipelago, Crete, Asia Minor, Iran and Iraq	53	7.65
8	Turanian	adapted to cold winters, as in the vicinity of the Caspian Sea, in Central Asia, the steppes of South-western Siberia and Kazakhstan	23	3.32
9	Endemic	recorded only in Romania	9	1.30

By bringing together the nine zoogeographic elements into larger groups and taking into consideration the groupings advanced by Popov [11], it appears that in Romania there are 120 (17.32 %) largely distributed species (Cosmopolitan, Holarctic and Palaearctic), 297 (42.86 %) Euro-Siberian (Euro-Siberian and European), 244 (35.20 %) Mediterranean (Mediterranean and Ponto-Mediterranean), 23 (3.22 %) previously Asiatic (in Romania, only the Turanic elements belong to this group) and 9 (1.30 %) endemic species.

A comparison of the data on the species of *Heteroptera* from Romania and those from Egypt [8], Israel [5], [6], [7], Turkey [2] and Tien Shan [11], as summarized in table 2, shows that in all mentioned areas the species with Mediterranean distribution are prevalent, i.e. from 30.6 % at Tien-Shan to 55.5 % in Turkey (35.3 % in Romania). This aspect can be ascribed to the fact that Romania is located in the area which has harboured, after the last glaciation, the species which escaped in the

Table 2

Proportions of the principal faunistic elements of the species of terrestrial *Heteroptera* occurring in Egypt, Israel, Turkey, Romania and Central Asia

Faunistic elements	Egypt	Israel	Turkey	Romania	Tien Shan
Widely distributed	3.4%	2.8%	4.1%	17.3%	19.5%
Euro-Siberian	1.4%	5.5%	11.8%	18.5%	11.0%
European	0.7%	2.0%	2.8%	24.3%	6.8%
Mediterranean	34.8%	41.6%	55.5%	35.3%	30.6%
Endemic	9.9%	17.2%	12.7%	1.3%	19.5%
Other categories	49.8%	30.9%	13.1%	3.3%	13.4%

area of the Mediterranean Sea and extended their range northwards; Egypt, Israel and Turkey are circum-Mediterranean areas, but Tien Shan is located far off the Mediterranean Sea and, therefore, the lowest percentage of Mediterranean elements is present there.

The variation of the European elements in terms of the distance from the central area of Europe and the latitude is obvious. Thus they represent 24.3 % of the species from Romania, while in the other areas the precentages are lower and range between 0.7 % (Egypt) and 6.0 % (Tien Shan). The percentage of the Euro-Siberian elements increases from the south (1.4 % in Egypt) northwards (18.5 % in Romania). In the northern area, the widely distributed faunistic elements are more numerous.

It can be seen that the fauna of terrestrial *Heteroptera* of Romania contains less endemic species than the other areas surveyed (i.e. 1.3 %), this being due to the fact that Romania is not separated from the surrounding countries by effective geographic barriers, and that similar environmental conditions occur in a wide area.

CONCLUSIONS

1. Among the 693 species of terrestrial *Heteroptera* recorded in Romania, 6 are Cosmopolitan, 53 Holarctic, 61 Paleoarctic, 12 Euro-Siberian, 169 European, 191 Mediterranean, 53 Ponto-Mediterranean, 23 Turanian and 9 are Endemic species.

2. The comparison of the data on the *Heteroptera fauna* from Romania to that from Egypt, Israel, Turkey and Tien-Shan shows that the similitudes and differences are related particularly to the geographic position and the climate conditions.

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QUELQUES DONNÉES BIOLOGIQUES SUR L'ESPÈCE *EXORISTA XANTHASPIS* WIED (*DIPTERA — LARVAEVORIDAE*)

PAR

M.C. MATEIAŞ

Exorista xanthaspis Wied. (*Diptera — Larvaevoridae*) is a species parasite in the larvae of *Heliothis viriplaca* Hfn. (*Noctuidae*), which in Romania is a new host for parasites. The larvae of the parasite feed both inside and outside the host larvae, in the last days of the larval stage. This could be a hypothetical type of exoparasitism.

Exorista xanthaspis Wied. (*E. fallax* Meig.) est un Diptère parasite des Lépidoptères répandu dans la Région pontique et ayant une importance parasitaire dans les Balkans pour le Noctuide *Hyphantria cunea* Drury [1]. Parmi d'autre hôtes, le même auteur mentionne les Lépidoptères *Stilpnotia salcis* L. (*Lymantriidae*), *Laphygma exigua* Hb. (*Noctuidae*), *Dendrolimus pini* L. (*Lasiocampidae*), l'efficience parasitaire s'élevant à 25 %.

Nous avons identifié l'espèce dans la zone de Fundulea, où elle parasitait les larves d'*Heliothis viriplaca* Hfn. (*Noctuidae*), l'hôte étant nouveau pour la Roumanie [2]. L'identification de l'espèce a été faite par le Dr. A.Z. Lehrer, du Centre de Recherches Biologiques, Jassy.

MATÉRIEL ET MÉTHODE

Le matériel biologique a été récolté dans les luzernières par des fauchages à l'aide du filet entomologique. Les larves de Lépidoptères ont été élevées chacune isolément, dans des conditions contrôlées (lumière — 12 heures à la température de 25°C; obscurité — 12 heures à 21°C et à une humidité relative de l'air de 65—75%). La luzerne a été utilisée comme nourriture.

RÉSULTATS

L'infestation a été faite au mois d'août, par voie naturelle, la ponte du parasite (espèce ovipare) étant déposée sur la partie dorsale des deux premiers segments de la larve d'*Heliothis viriplaca* (Fig. 1). On n'a pas pu détacher les œufs parce qu'en les déposant, la femelle du parasite les a collés au tégument de la larve à l'aide d'une substance adhésive.

Dans les conditions indiquées ci-dessus, l'éclosion a eu lieu après une periode d'incubation de 2 jours. Il est pourtant possible que l'incubation dure plus longtemps.

Les larves du parasite pénètrent dans le corps de l'hôte juste au-dessous du lieu où est déposée la ponte et non pas antérieurement par rapport à ce lieu, comme au cas d'autres espèces d'*Exorista* [1]. Après une période d'endoparasitisme, les larves du parasite apparaissent à la surface de l'hôte (Fig. 2). Le déplacement du lieu de la pénétration jusqu'à celui de l'apparition à l'extérieur a lieu pendant la nutrition endoparasitaire.

Nos observations ont montré que la période larvaire a duré 9 jours, dont les 3 derniers ont été d'exoparasitisme. Nous considérons ainsi que le Diptère *E. xanthaspis* n'est pas une espèce typiquement endoparasitaire. Cette hypothèse est soutenue par le fait que les larves du parasite se nourrissent pendant quelque temps à l'extérieur du corps de la larve hôte, comme on le voit dans la figure 3. Des deux larves, une seule (la plus développée) a atteint sa maturité parfaite (Fig. 4), réussissant à constituer son puparium (Fig. 5). L'adulte (Fig. 6) a apparu six jours après la nymphose de la larve par une ouverture apicale du puparium (Fig. 7).

Selon Herting [1], *E. xanthaspis* a plusieurs générations annuelles. Au printemps c'est le *Stilpnotia salicis* qui lui sert d'hôte, tandis qu'à partir du mois de juillet, il se reproduit sur des espèces du genre *Hyphantria*. L'hibernation a lieu sous forme de pupe dans le sol, ou de larve dans la pupe de l'hôte.

CONCLUSIONS

L'espèce *Exorista xanthaspis* est le parasite des larves du Noctuide *Heliothis viriplaca* Hfn. La période d'incubation compte deux jours, ou peut même dépasser cette durée; la période larvaire compte 9 jours et la nymphose en compte 6.

Le développement larvaire a lieu au commencement à l'intérieur de l'hôte et ensuite à l'extérieur, une phase d'exoparasitisme n'étant donc pas exclue à la fin de la vie larvaire du parasite.

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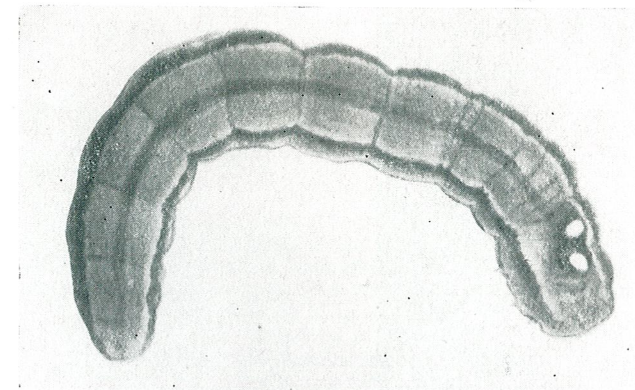

Fig. 1. — La larve hôte, avec les œufs du parasite.

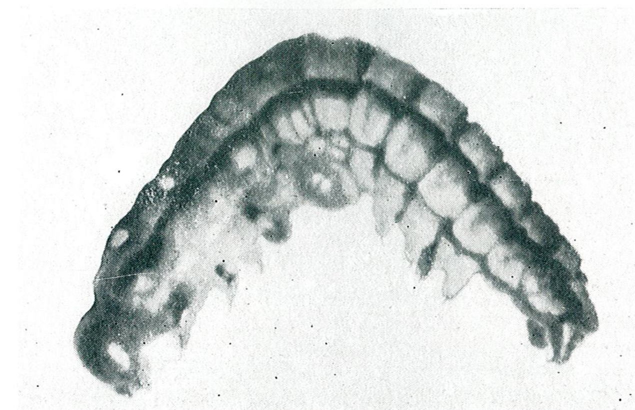

Fig. 2. — Les larves du parasite apparues à la surface de la larve hôte.

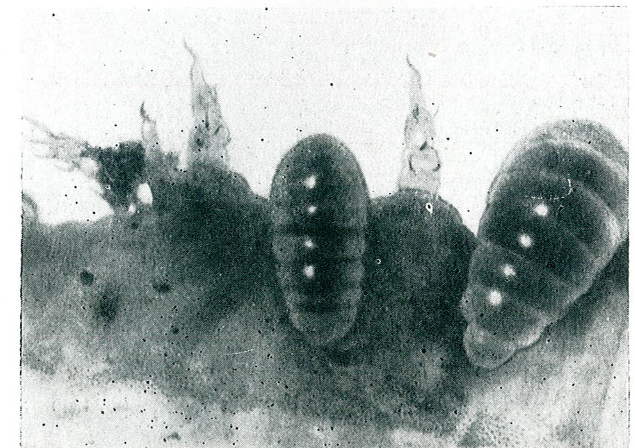

Fig. 3. — Deux larves d'*Exorista xanthaspis* qui se nourrissent sur le corps de la larve d'*Heliothis viriplaca*.

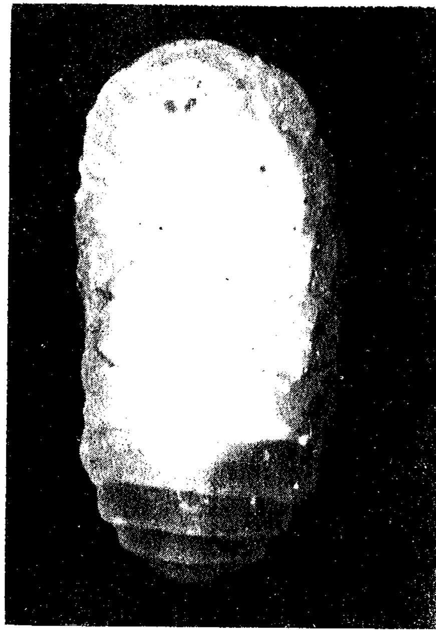

Fig. 4. — Une larve mûre d'*Exorista xanthaspis*.

Fig. 5. — Le puparium du parasite.

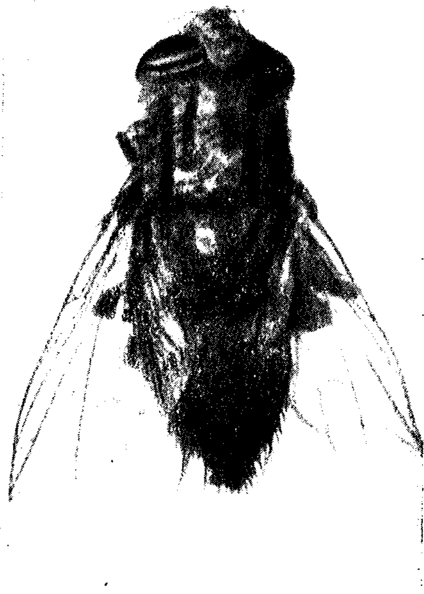

Fig. 6. — L'adulte de l'espèce *Exorista xanthaspis*.

Fig. 7. — Le puparium après l'apparition de l'adulte.

ST. NEGREA, *Cladocera*, in: *Fauna R. S. România*, vol. IV, issue 12, Edit. Academiei, 1983, 399 p., 150 figs.

This is the eleventh issue in the series "Fauna of Romania" dealing with a major group of crustaceans; it represents the results of the studies on the waterflows carried out over more than 30 years throughout most of Romania. Like all issues in the same series, Negrea's book includes a general part (pp. 11—65) and a special part in which the genera and species are described. In the few pages dealing with the history of the studies on *Cladocera*, the author makes an interesting remark: he considers that the present and actually the phyletical period begins with Frey's paper of 1959 on the significance of the cephalic pores in *Chydoridae*, this criterion being subsequently used for other families of waterflows too, including fossils. The general part also includes original data about the period of gamogenetical and parthenogenetical reproduction of 97 species in Romania, about polymorphismus in six genera as well as an original biogeographical analysis of all species living in Romania mention being made of the general distribution of each of them (only seven are true cosmopolitan), the biotops in which they live in Romania and the presence or absence in the species in the four areas ("Gebiete") accepted by Illies in "Limnofauna Europaea" for southeastern Europe.

The special part of the book is much more extensive. 114 species and 49 subspecies live in Romania; they belong to ten families and 47 genera; 17 of them have not been recorded yet within the country but their occurrence is considered sure. Nine other species recorded in Romania by earlier authors are considered invalid or dubious; it can not be asserted to which valid species the Romanian specimens recorded under these names actually belong.

The book is based above all on the specimens collected by the author during his own field investigations (1951—1982); all are preserved in his own collections; he also examined the specimens of Daday's collection in the University of Cluj-Napoca. With few exceptions, the descriptions are based exclusively on Romanian specimens; most of the illustrations are original, based again on Romanian specimens. Specimens from other countries were used for comparison. The description of each species includes, besides illustration: the scientific name, main synonymies, size, colour, short description of ♀♀ and ♂♂, bionomy, general range, distribution in Romania. All localities in which the species was recorded are listed, those in which the species was found by the author or recorded by earlier students being mentioned separately. The distributions of most species in Romania are mapped, the maps using the international Universal Transveese Mercator system. All localities from which waterflows were recorded in Romania are listed at the end of the volume, their position being mentioned on a separate map. This fact makes possible the location of the localities for each species for which no special maps are given, or without looking at these maps. Several localities are former shallow lakes from the Floodplain of the Danube which were damed in the late fifties and early sixties and do no more exist now, others being, on the contrary, recently built damlakes, as for example the Iron Gates damlake. How detailed the distribution records of the various species are can be realized from the fact that for many of them one mentions the occurrence not only in this large damlake, but also in its various golfs.

The book includes the description of a new species, *Diaphanosoma orghidani*; its diagnosis also in an international language would have been welcome. One genus (*Wlassicsia*), eight species and 24 subspecies are recorded for the first time in Romania; *Moina salina* is redescribed and emmended; there is a new combination (*Latonopsis australis hospitus*) and nine new synonyms.

A special remark deserves the subspecies problem. Smirnov (1976) distinguished subspecies in various species of Chydoridae, Moinidae and Macrothricidae; actually, most of these have largely overlapping ranges, hence being not subspecies in the true, geographical acceptation, but either individual variants, morphs or sibling species. Negrea lists them in the paragraph dealing with "Variation", keys most of them and mentions whether these are actually

subspecies or not, adding to which of them do the Romanian specimens belong. By mentioning that most of them have no allopatric ranges and are not true subspecies, their status being uncertain, he does not accept them as valid subspecies. However, a specialist that does not read Romanian can conclude that these forms are actually accepted as subspecies.

The book ends with a rich (still selective) bibliography, index of localities and of scientific names. It is one of the best local faunae of waterflows published during the last decades and one of the best issues in the "Fauna of Romania" series.

Petru Bănărescu

AVIS AUX AUTEURS

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

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La responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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