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KALLIAPSEUDES GIANUCAI, A NEW TANAIDACEA FROM THE BRAZILIAN WATERS

BY

MIHAI BĂCESCU

After a review of Tanaidacea species reported from the Brasil waters, the author described a new species of *Kalliapseudes* from Lagoa dos Patos near Rio Grande, found in a material sent by Prof. Mattos Gianuca.

Diagnosis. Species allied with *Kalliapseudes schubarti* Mané-Garzon from which it differs, in the first place, by the lock of maxillary palp and of exopodite at cheliped ♂.

Description. Although one of the earliest references about Tanaidacea concerned the Brazilian waters (*Leptochelia brasiliensis* Dana, 1852), this fauna is far from being well known along the large coast of the American continent. Despite all contributions of the authors who, later on, dealt with these crustaceans, only 14 species are surely known in the Brazilian waters, namely :

Leptochelia brasiliensis (Dana 1852) (syn. *Tanais brasiliensis* Dana)
Tanais stanfordi Richardson 1901 (syn. *T. sylviae* Mello-Leitao 1941 ; syn. *T. fluviatilis* and *T. herminiae* Mané-Garzon 1943)
Kalliapseudes schubarti Mané-Garzon 1922 ;
Psammokalliapseudes granulosus Brum 1973 ;
„ *mirabilis* Lang 1956 ;
Telotanais gerlachi Lang 1956 ;
Tanais cavolini Brum 1969 ;
Apseudes paulensis Brum 1971 ;
„ *inermis* Brum 1973 ;
Parapagurapseudopsis carinatus Brum 1973 ;
Acanthotanais oculatus (Vanhöf. 1914) Brum 1973 ;
Leptochelia savigny (Kr. 1842) Brum 1973 ;
Anatanais ohlini (Stebb. 1914) Brum 1973 ;
Neotanais tricarinatus Gardiner 1975.

Therefore, we have been very glad to receive for examination thanks to the kindness of Prof. Mattos Gianuca, Director of the Atlantic Oceanographic Base of South Brasilian Rio Grande area, a small collection of Monokonophora from Lagoa dos Patos brackish waters (mezohaline).

Two of these stations contained a large number of *Kalliapseudes schubarti*, alone or associated with *K. gianucai* described below (45 specimens in St. Gr. 18, n° 12 (♂ ♀) ;

35 specimens ♂ ♀ of the former and 5 specimens of the latter in St.2nd t n° 11) ; in other two stations there were *Tanais stanfordi* Rich (Cr. 10,

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n° 15, 3 specimens) and *Tanais* sp. (dozens of specimens in St. Cr. 7 n° 17 — among them a *K. schubarti* juv. and other 23 ♂ ♀ in St. Cr. 10 Bara Falsa). We shall deal below with :

KALLIAPSEUDES GIANUCAI [1] n.sp.

Materials and locality: 4 ♂♂ and 1 ♀ preadult collected by prof. Mattos Gianuca [1] in station 2T, n° 11, from the mixohaline area of Lagoa dos Patos, not far from the town of Rio Grande (South of Brasil) 2—4 m deep, 1973, S‰ = 10—14 g (31°48′02″S ; 52°08′00″V).

Male: big size (7—9 mm). Body prolonged, not quite flat (twice as large as high), with pleon obviously wider than pereon ; the latter's segments are ± rectangular, well spaced out, except for the first free somite closely moulded on the posterior region of cephalothorax. Carapace (Fig. 1 A) is slightly longer than wide ; traversed by slight depressions it has short curved rostrum prolongation.

Tegument white, bright, soft and so transparent that the opaque, yellow-brown cylinder of the alimentary canal, filled with silt, can be seen throughout the length of the animal. Only two or three simple hairs in the postero-dorsal area of each pereonite ; as for the rest, they are glabrous. In exchange, pleonites are plentifully provided with lateral plumose setae protecting pleopods ; in the last pleonite, plumose setae were found as well on the curvature partially covering telson (Fig. 1 B). Pleotelson, as big as the last two pleomeres, is perfectly round, slightly larger than long and surrounded by a dense series of cilia ; not special apical or plumose setae.

There is not any trace of hyposphenia either on thoracomers or on pleonites. Only the genital tubercle is prominent in the posterior area of the last thoracomer (Fig. 1 M). The copulatory organ is like an eminence with two craters genital orifices — directed caudaly (m, Fig. 1).

Antennule's first basal segment is three or four times as long as large, representing half of the whole length of appendage and bears externally 12 shorty plumose setae, a little shorter than the 7 ones of the second segment. Its exopodite is two-segmented and endopodite is nine-segmented (Fig. 1 C) out of which the three terminals are much smaller ; four of the big segments bear one or two short aesthetasks. *Antenna* (Fig. 1 D) provided with 12 plumose hairs on the big basal segment and short plumose flagellated hairs on the following three segments ; scale provided with 3 plumose hairs. *Labrum* armed with three areas of apical hairs ; no basal hairs like in *K. schubarti* (Lang, 4, pl. 34). *Mandible* (Fig. 1 E) shows an eminence near the insertion of one-segmented palpus which is provided with 20 plumose hairs. *Maxillula* (Fig. 1 H) without palp ; it is slightly toothed at the curvature of its basis ; F, terminal portion of its outer endite. *Maxilla* has particular phanera on the inner endite. Labium as in figure 1 G.

Maxilliped I of *K. schubarti* type ; basis with two short, soft curved coupling hooks.

[1] Dedicated to Prof. Mattos Gianuca who sent us this material for study.

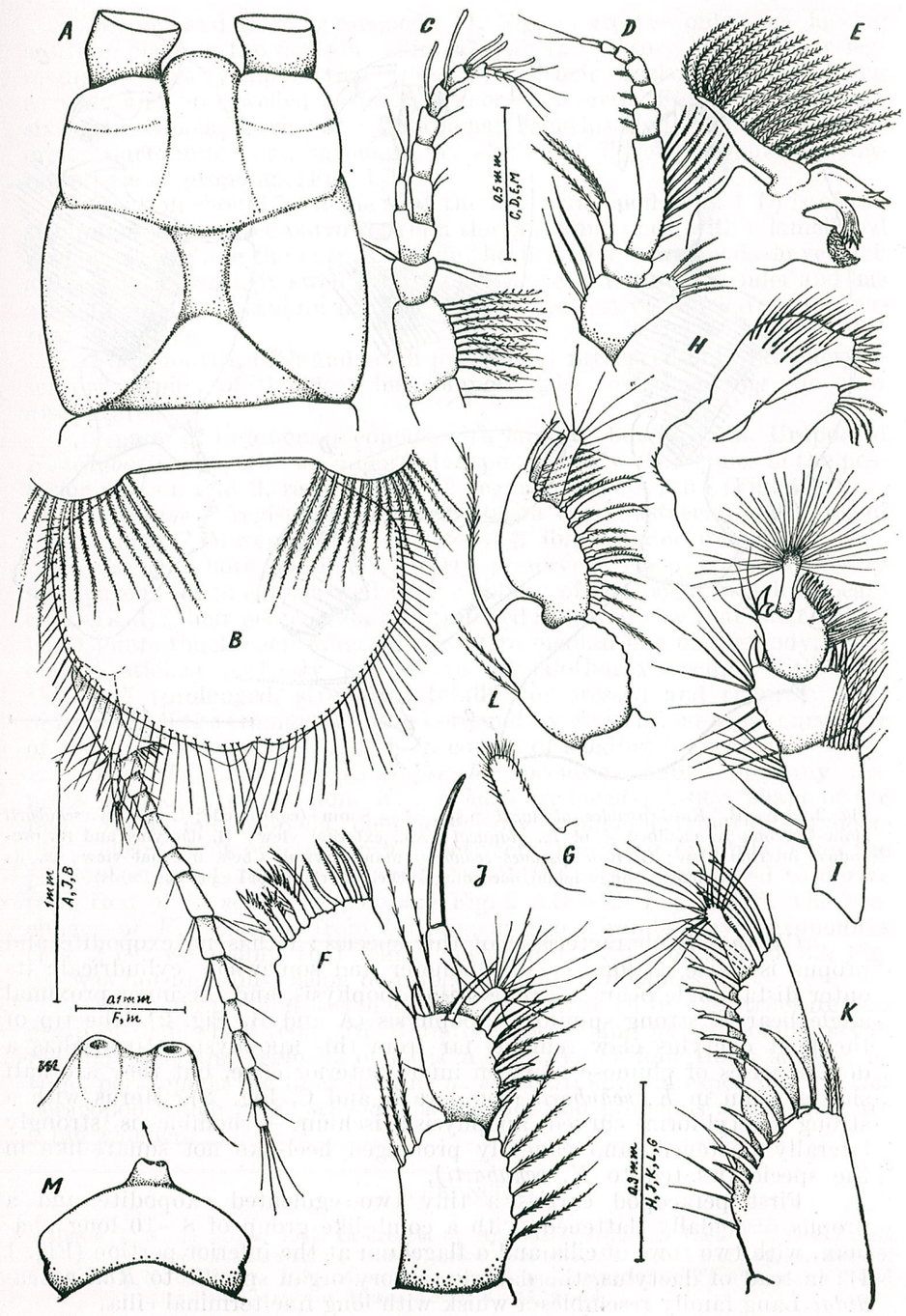

Fig. 1. — *Kalliapseudes gianucai* s.sp., ♂ = 8 mm. A, Carapace seen from above ; B, telson and uropod ; C, antenna I ; D, antenna II ; E, mandible ; G, one of the labial palps ; H, maxilla, F, its external endite ; I—L, peraeopod I, II, IV and VI ; M, sexual tubercle ; N, its two openings, magnified.

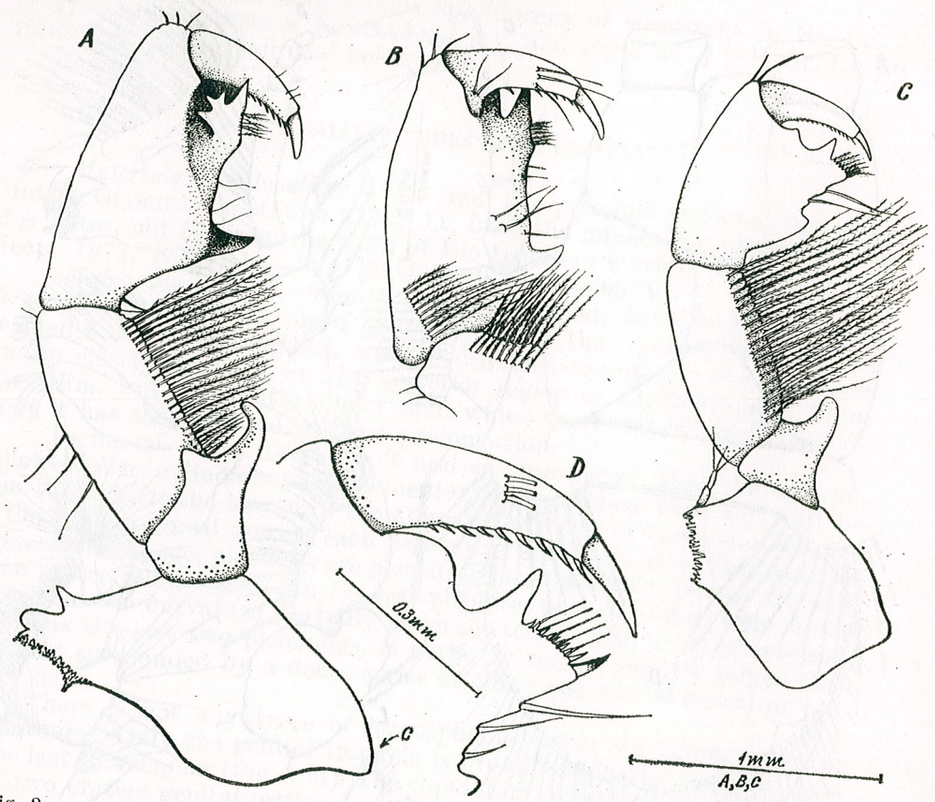

Fig. 2. — A—B, *Kalliapseudes gianucai* n.sp., ♂ = 8 mm (continued); C—D, *K. schubarti* Mané-Garzon; A, cheliped ♂ of *K. gianucai* n.sp., external view; B, dactylus and its propodus, internal view; C, *Kalliapseudes schubarti* Mané-Garzon, Chela internal view; D, its prehensile end (original, according to the material from Lagoa dos Patos).

Cheliped is characteristic of this species: it has no exopodite and propus is twice as long as its diameter and somewhat cylindrical; its outer distal angle bears a strong trifid apophysis, and the inner proximal angle bears a strong spiniform apophysis (A and B, Fig. 2). The tip of the bent dactylus claw remains far from this apophysis. Carpus has a double series of plumose hairs on infero-anterior edge, but they are half shorter than in *K. schubarti* (compare A and C, Fig. 2). Merus with a strong dactyliform curved apophysis; ischium ± rhombic is strongly laterally flattened (and caudally prolonged heel-like not square-like in the species related to *K. schubarti).*

First peraeopod carries a tiny two-segmented exopodite and a propus discoidally flattened, with a comb-like group of 8—10 long phanera, with two rows of cilia and a flagellum at the inferior portion (Fig. 1 I); instead of dactylus, the dactylo-sensory organ specific to *Kalliapseudidae* Lang family resembles a whisk with long fine terminal cilia.

Second and third peraeopoda (J, Fig. 1) are the only ones having a strong claw on the dactylus twinned with the sensory organ. Their segments are clearly longer than wide; even their basis is twice as long as wide and not swelled as in *K. schubarti*. Fourth (Fig. 1 K), fifth and sixth peraeopoda have not a functional dactylus, but only the sensory organ, surrounded and surpassed by sickle-like flagellated phanera characteristic of propodus (Fig. 1 L).

Mention should be made that the last peraeopod (Fig. 1 L) is of the swimming type, twice narrower than the preceding ones, with a lamellated propodus oval like the carpus. While the first five peraeopoda have each a big ischium strongly swelled to the basis, the sixth one is slender and has a long cylindrical ischium nearly as big as the rest of lamellated transparent segments.

First, fourth, fifth and sixth peraeopods preserved only the dactylo-sensory papilla of the dactylus complex; the rest, including the claw disappeared.

5 pairs of biramous pleopods with largely obovate rami. Uropod of *K. schubarti* type, with an inner distal spur armed with 4 spines in the posterior portion and 2, respectively 12 segments at its rami (Fig. 1 B).

Holotype ♂ registered under nº 501 in the Crustacean collection of "Gr. Antipa" Museum, 3 paratypes ♂♂, ibid., under nº 502.

Ecology: both specimens clearly preserve a clean, soft and nearly transparent tegument, as well as the rolling of the body (which appears cylindrical); their peraeopoda are stretched in such a way that their ischio-basal joints touch each other on the infero-median line of the body, while chelae, sticked perfectly parallel to cephalothorax occupy with their "elbows" (prolonged, strongly laterally compressed and rhombic parts of ischios) all the triangular space bordered by mero-ischio-basal curvature of the first peraeopod. One ♂ is in course of sheding its coat.

Remarks: The new *Kalliapseudes* species resembles in many respects its biotop companion, *K. schubarti* (reduced pilosity, shape of the body, telson and uropoda, dactylo-sensory papillae distribution), indicating a clear phyllogenetic kinship between them. For example, even the particular morphology of cheliped ♂ could be readily supposed to derive from that of *K. schubarti* (compare Fig. 2 AB with Fig. 2 CD). The specimens of *K. schubarti* from the lagoon Rio Grande have exopodites (Fig. 2 C); we assume that Lang's specimen (*l.c.*, p. 250 and Pl. 33, Fig. 28) was not an adult, being described without exopodites.

The main peculiarities of *K. gianucai* are given by the structure and grasp of male chela (compare B and C, Fig. 2) and by lack of palpus in maxilla; the first segment of basis A_1 is three or four times as long as wide, peraeopoda are finer than in *K. schubarti* species, especially the last peraeopod which in *K. schubarti* is rougher and propodus and carpus are a bit widened.

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MOTASIONA — UN NOUVEAU GENRE DE *PAPILIONINI (LEPIDOPTERA. PAPILIONIDAE)*

PAR

E. V. NICULESCU

The author describes a new genus of the Papilioni tribe (Lepidoptera-Papilionidae): Motasiona, which is obviously different from the Papilio genus because it has another structural plane especially clear-cut in the short and bifid superuncus and the nontiered uncus appearing as a long dorsal-convexity plate. Species *zagreus* Doubl. and *bachus* Felder belong to this genus.

Dans son travail sur les Papilionidae [1] publié en 1961, E. Munroe divise le genre *Papilio* en cinq sections. La dernière section comprend trois groupes d'espèces (18 espèces) : 1. *zagreus (zagreus* Doubleday, *neyi* Niepelt, *ascolius* Felder, *bachus* Felder); 2. *scamander (hellanichus* Hewitson, *scamander* Boisduval, *birchalli* Hewitson); 3. *homerus (victorinus* Doubleday, *cephalus* Godman et Salvin, *cleotas* Gray, *aristeus* Cramer, *judicael* Oberthür, *garamas* Hübner, *homerus* Fabricius, *warscewiczi* Hopffer, *cacicus* Lucas, *euterpinus* Godman et Salvin).

En examinant les genitalia chez les espèces *zagreus*, *bachus*, *scamander*, *cleotas* et *aristeus* nous avons constaté que ces espèces ne sont pas congénériques, qu'elles ne sont pas, toutes, équivalents morphologiquement et taxonomiquement. Ainsi les espèces *zagreus* et *bachus* ont un plan de structure [2] qui diffère profondément de celui des autres espèces appartenant aux groupes *scamander* et *homerus*.

Les espèces *scamander*, *cleotas* et *aristeus* ont le plan de structure caractéristique au genre *Papilio* c'est-à-dire un superuncus [1] long, recourbé ventralement et l'uncus double bilobé (uncus en « étage ») ayant un étage supérieur et un étage inférieur.

Le plan de structure des espèces *zagreus*, et *bachus* est tout autre : le superuncus est court, massif et profondément bifide, l'uncus est non étagé, formé d'une plaque large, convexe sur sa partie dorsale, un peu bifide à l'extrémité distale.

Ce sont les motifs qui nous ont déterminé de séparer les espèces *zagreus* et *bachus* dans un nouveau genre que nous nommons

Genus *Motasiona nov.*

Typus generis : *Papilio zagreus* Doubleday

Les caractères de l'armure génitale de ce genre sont les suivants (fig. 1—6) :

Superuncus court et bifide.

Uncus large, convexe-concave, non étagé.

[1] Le superuncus est nommé par Munroe [1] pseudouncus ; c'est évidemment une erreur.

Valva profondément concave.
Harpe longue, terminée en pointe.
Sacculus présent.
Pseudosacculus horizontal.

Pour mettre mieux en évidence ces caractères et justifier ainsi la création d'un nouveau genre, nous présentons, ci-joint, un tableau synoptique en comparant le genre *Motasiona* avec le genre *Papilio* (les espèces *scamander*, *cleotas* et *aristeus*).

Tableau synoptique montrant les différences dans l'armure génitale entre les genres Motasiona nov. et Papilio

Caractères génériques	*Motasiona*	*Papilio*
1. Superuncus	Court, large, massif, bifide à l'extrémité	Long, étroit, aplati dorso-ventralement et recourbé en bas
2. Uncus	Large, convexe-concave, non étagé, un peu bifide	Double bilobé, en « étage » (étage supérieur et étage inférieur)
3. Valva	Profondément concave	Moins concave
4. Harpe	Terminée par une seule pointe	Terminée par deux ou plusieurs pointes
5. Sacculus	Présent	Absent
6. Pseudosacculus	Horizontal	Très oblique et large

Dans ce tableau nous avons inclus seulement les sclérites qui présentent des caractères génériques, communs à toutes les espèces du genre. C'est pourquoi le pénis, la fultura inférieure et le saccus ne sont pas mentionnés dans le tableau puisqu'ils ne fournissent pas de bons caractères génériques.

L'habitus n'a pas été non plus mentionné, car il est déficient à niveau générique. Dans les genres *Parides*, *Eurytides* et *Papilio*, l'habitus ne peut pas être utilisé pour caractériser le genre puisqu'il est extrêmement varié, ces genres ayant un grand nombre de types du dessin. Nous avons nommé ce phénomène *divergence intragénérique* [3]. Même dans l'intérieur d'une section il y a une grande divergence du graphisme. Les espèces de la V^e^ section, à savoir, *zagreus* Doubleday, *hellanichus* Hewitson, *scamander* Boisduval, *birchalli* Hewitson, *cleotas* Gray, *homerus* Fabricius, *warscewiczi* Hopffer, *cacicus* Lucas, *euterpinus* Godman et Salvin ont les types du dessin profondément différents l'un de l'autre. Dans ces conditions il est impossible de caractériser le genre par le graphisme ainsi comme c'est le cas pour d'autres genres (*Colias*, *Erebia*, *Polygonia*, *Melitaea*, *Argynnis*, *Zygaena*, etc.). Le graphisme chez les Papilionini ne nous dit rien à niveau générique. D'autre part, il y a aussi une grande convergence entre les espèces (convergence interspécifique), les genres (convergence intergénérique) et même les familles (convergence interfamiliale). Ainsi dans le groupe

zagreus nous constatons une intéressante convergence interfamiliale, les espèces de ce groupe ressemblant aux Heliconiides.

Le nouveau genre a été nommé *Motasiona* en l'honneur du prof. C. Motaș, le doyen d'âge des zoologistes roumains.

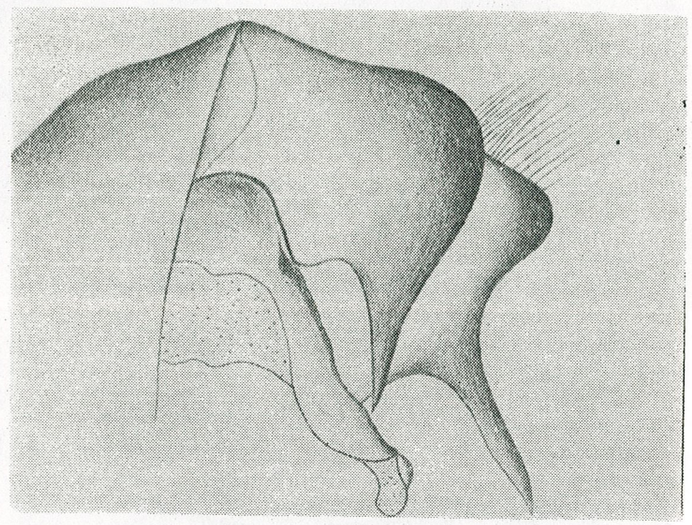

Fig. 1. — *Motasiona zagreus* Doubleday. Pars dorsalis en vue latérale.

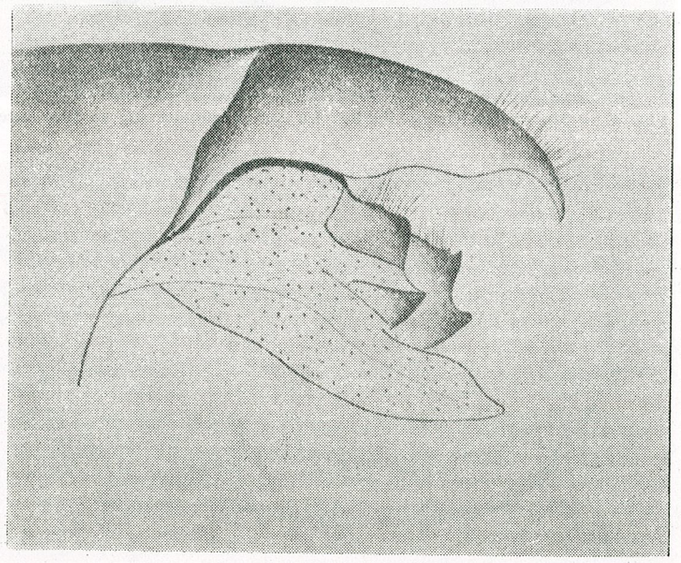

Fig. 2. — *Papilio scamander* Bsdv. Pars dorsalis en vue latérale.

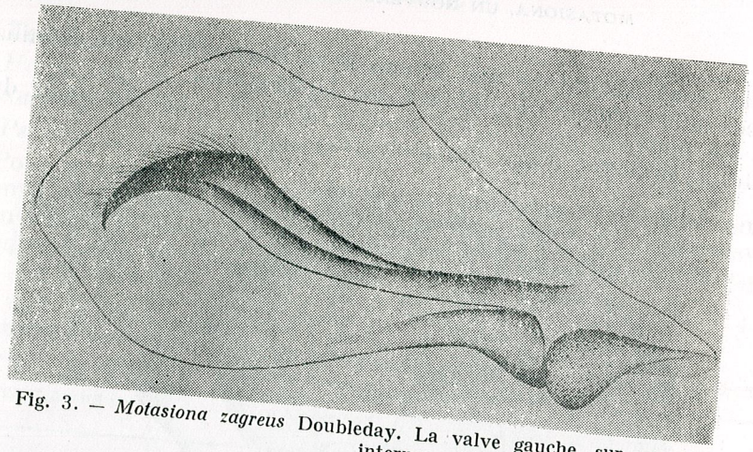

Fig. 3. — *Motasiona zagreus* Doubleday. La valve gauche sur sa face interne.

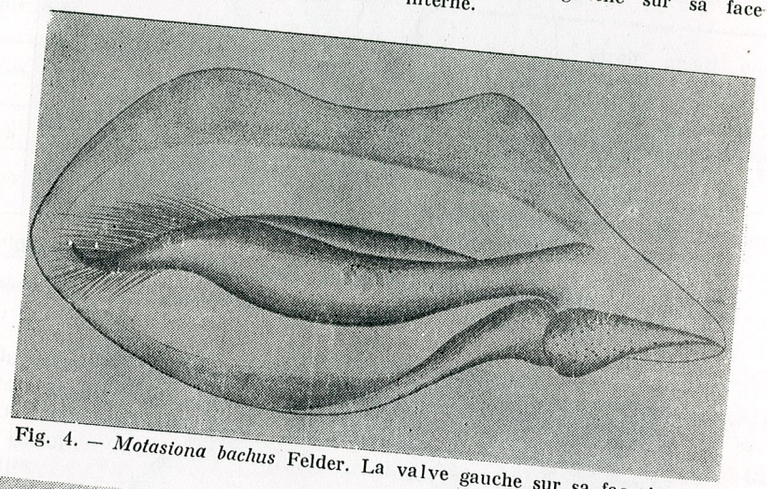

Fig. 4. — *Motasiona bachus* Felder. La valve gauche sur sa face interne.

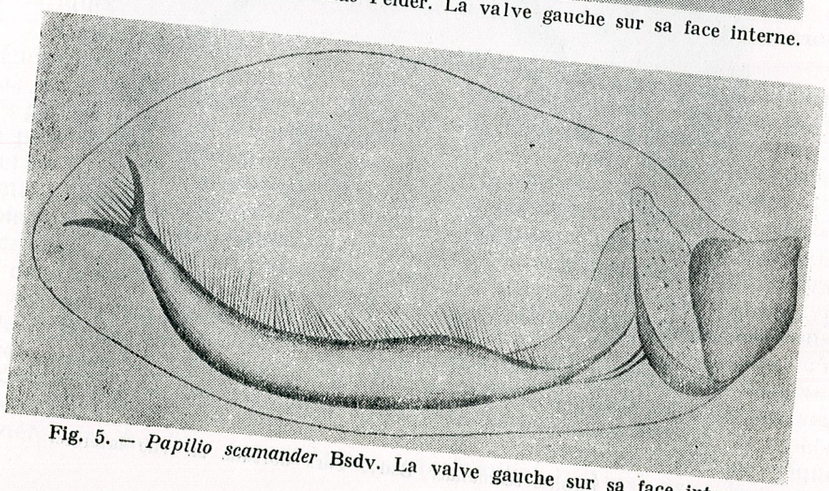

Fig. 5. — *Papilio scamander* Bsdv. La valve gauche sur sa face interne.

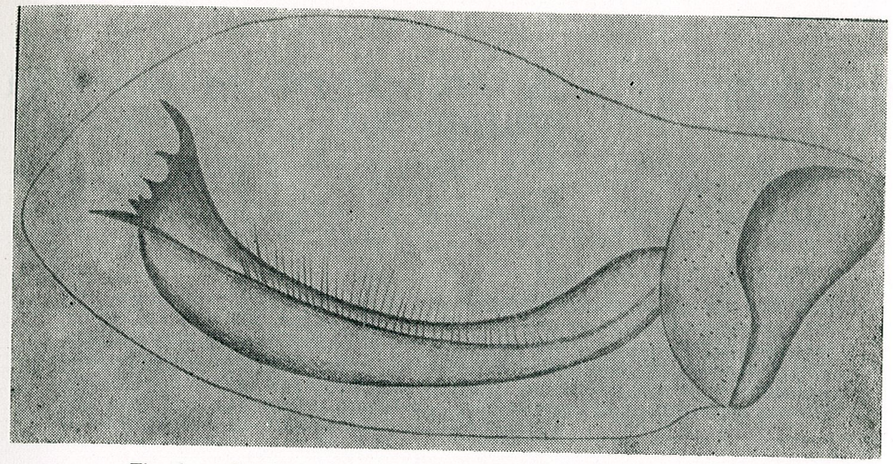

Fig. 6. — *Papilio aristeus* Cr. La valve gauche sur sa face interne.

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Reçu le 20 juillet 1978

LA NOTION DE « POPULATION » ET LA SYSTÉMATIQUE ZOOLOGIQUE

PAR

C. ALMAÇA

La « population » représente un niveau d'organisation de la vie qui peut être perspectivé sous des angles divers. Ceci fait que les auteurs visualisent les « populations » d'accord avec leurs intérêts. Mais, dans la plupart des espèces, les « populations » constituent des communautés génétiques et, de ce fait, elles présentent des caractères biologiques généraux, écologiques, ou biométriques qui leur sont propres. Dans ces conditions, l'analyse biométrique permettra souvent la localisation et la délimitation des « populations ».

Dans un article récent, Botosaneanu [5] a critiqué l'usage peu précis qu'on fait souvent du terme « population » en Zoologie. Je crois très justes quelques remarques de Botosaneanu et je voudrais, dans cette note, contribuer dans la mesure du possible à préciser davantage ce très important problème. J'aborderai, premièrement, la définition de « population » et, ensuite, la délimitation des « populations ».

Botosaneanu [5] a cité plusieurs travaux où la définition de « population » est vague ou ambiguë. Je ne reviendrais pas sur ces travaux ; je crois plus utile, sans la prétention d'être exhaustif, de mentionner d'autres où cette définition me semble plus précise et pourra donc placer plus loin le début de la discussion.

Beaucoup d'entités biologiques semblent offrir des réalités différentes selon les optiques à travers lesquelles ces entités sont visualisées. Ceci est particulièrement valable quand on considère les « populations ». La terminologie suggérée par Gilmour & Gregor [7] en est un exemple. Le *deme* ou « population locale » est « un ensemble d'individus ayant des rapports étroits », mais, selon l'importance qu'on attache à ses caractères génétiques, géographiques ou écologiques, on aura une même entité biologique nommée de *gamodeme*, *topodeme* ou *ecodeme*. Heslop-Harrison [8] a développé la terminologie du *deme*, suggérant d'autres caractères différentiels qui permettraient la diversification des désignations. Le même auteur [8], présente une définition de «population» : « . . . an aggregate of individuals considered together because of their common habitation of a given area at a given time ». Mayr [9] a très clairement démontré que le *deme* est la « population locale », réalité biologique du plus haut intérêt aux points de vue taxinomique et évolutif. Allee *et al.* [1], utilisant des critères sociologiques, biologiques et biométriques, présentent plusieurs définitions formelles de « population ». Dobzhansky [6], se basant sur les caractères génétiques, considère la « population mendélienne » — communauté reproductive d'individus partageant le même *pool* génétique.

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Almaça [2], [3] cherche à montrer que les définitions formelles de Allee *et al.* [1] correspondent à une même et seule réalité, laquelle ne diffère point de la « population » envisagée sous la perspective génétique. En effet, n'importe quelle caractéristique qu'un auteur ou un autre mettent en évidence est conséquence du fait qu'une population à reproduction sexuée est un ensemble d'individus habitant une aire donnée pendant une période donnée, c'est-à-dire une communauté génétique. Toute communauté génétique présente, puisque les individus la composant partagent du même *pool* de gènes, des caractères biologiques généraux, écologiques ou biométriques déterminés. Il est donc justifiable que les auteurs envisagent la « population » sous la perspective leur convenant le mieux.

Tout ceci semble très simple et clairement compréhensible. Le vrai problème est de type pratique. Comment délimiter, dans la Nature, les « populations » d'une espèce? C'est encore le critère génétique qui, premièrement, pourra nous guider dans ce domaine. À vrai dire, quand on essaie la délimitation des « populations » d'une espèce on devait, plus précisément, se référer aux « populations locales », car l'isolement reproductif étant le seul critère biologique valable pour les espèces à reproduction sexuée, chaque espèce constitue une seule « population mendélienne », la plus grande possible [6]. Mais, la Nature offre une très grande diversité de situations dont la continuité rend arbitraire le découpage. En termes génétiques cette gradation peut être décrite par l'expression de Wright [10] relative à la pression d'immigration :

$$\Delta q = -m\,(q - q_i)$$

où, q est la fréquence d'un gène donné dans une « population locale » q_i la fréquence du même gène parmi les immigrants et m le coefficient d'immigration (mesure de la quantité de remplacement de la population par les immigrants par génération). m est fonction du nombre d'individus de la « population locale » et du nombre d'immigrants. Si $m = 0$, la « population locale » forme un isolat [4]. Les valeurs absolues croissantes de m dénoncent l'existence d'un flux génétique croissant entre la « population locale » et les « populations immigrantes ». Par exemple : $m = 0{,}5$ signifie que, en une seule génération, la fréquence d'un gène deviendra la somme des fréquences du même gène dans la « population locale » et parmi les immigrants.

Au point de vue pratique qu'est-ce que cela signifie? Si l'on possède des marqueurs génétiques l'étude des fréquences des gènes les contrôlant peut nous renseigner sur la délimitation des « populations locales » et l'importance du flux génétique entre ces populations. Mais, ce n'est pas usuellement le cas. Il nous reste donc d'uutiliser des critères biométriques, qui, n'étant pas aussi précis, sont bien plus pratiques et peuvent, le croisje, résoudre la plupart des problèmes concernant la délimitation des « populations ». En fait, les caractères biométriques présentent, tels que les autres, une composante génétique les déterminant avec plus ou moins de plasticité [2], [3]. Si l'on utilise plusieurs caractères biométriques — surtout ceux qui, se basant sur les caractéristiques les plus constantes de l'espèce en question, nous assurent d'une réalisation phénotypique moins influencée par l'environnement — on pourra toujours essayer la délimi-

tation des « populations locales ». Ceci n'est évidemment, pas vrai que si l'échantillonnage peut être réalisé de façon valable pour l'application des tests statistiques.

Je crois donc que la recherche biométrique, bien que fastidieuse et dépendante de l'échantillonnage qui, dans la pratique, n'est pas toujours si facile qu'on peut le songer, permettra de résoudre beaucoup de problèmes concernés dans les exemples cités par Botosaneanu [5]. Malgré les difficultés d'un travail de ce type, la recherche en Systématique zoologique ne peut pas s'en passer. En effet, comment envisager les problèmes si importants de la Systématique actuelle, tels que la structure des espèces, la spéciation, la nature de la variation, etc., sans un travail préalable de délimitation des « populations locales »? En dépit de la justesse des commentaires de Botosaneanu [5] sur l'emploi peu précis du mot « population », je ne partage pas de son pessimisme en ce qui concerne l'éventuelle impossibilité de reconnaître et délimiter les « populations » de quelques espèces. Ce qui me semble possible c'est que quelques espèces ne comprennent qu'une seule « population ». Ceci dépend de plusieurs facteurs, parmi lesquels ceux qui déterminent la vitesse et les directions du flux génétique, la formation de barrières spatiales, l'étendue de l'aire géographique, l'organisation territoriale et d'autres.

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HISTOLOGICAL STRUCTURE OF THE INTERRENAL TISSUE IN *PHALACROCORAX CARBO* L.

BY

ANCA PETRESCU-RAIANU

The localization and external morphology of adrenal gland, as well as the interrenal cords arrangement and structure in *Phalacrocorax carbo* were studied. They were similar to those encountered in other birds, but the cell arrangement in cords was more regular. Besides interrenal and chromaffin cells, some small, undifferentiated ones with strong basophilic nuclei were found. They were different from the lymphoid elements sometimes infiltrated among the interrenal cords. In peripheral zone of the gland there were some very large, intense Feulgen-positive, probably polyploid, nuclei. The lipofuscins were frequently encountered, but their amount and localization were variable from one individual to another.

In spite of the considerable variability in anatomical localization and external morphology of adrenal constitutive tissues in different vertebrates, the physiology and secretory products are characterized by a remarkable similitude.

Adrenal glands in *Phalacrocorax carbo* are an excellent material to study the avian interrenal and chromaffin tissues, because the arrangement of interrenal cells in cords is very regular and the staining properties are characteristic enough. Therefore, it is easy to differentiate the two constitutive tissues, even using routine histological methods.

MATERIAL AND METHODS

The birds, *Phalacrocorax carbo*, were captured in autumn by hunting in order to avoid stress situation. Adrenal glands from 40 individuals (males and females) were immediately removed and immersed in routine histological fixatives (Bouin-Hollande, Carnoy, Zenker, Helly). After the paraffin embedding, the sections were stained with hemalum and eosin, Heidenhain's azan stain, and iron hematoxylin. In order to prove the pigment nature, the periodic acid-Schiff (PAS) before and after salivary amylase and Schmorl's reactions were performed. The DNA was demonstrated using Feulgen reaction.

RESULTS

The adrenal glands in *Phalacrocorax carbo* are situated in visceral cavity, on both sides of abdominal aorta and inferior vena cava, at the anterior poles of the kidneys. During the sexual rest season, in males, the testes partially cover the ventral surface of the adrenals. In females, the left gland is entirely covered by the ovary.

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Connective tissue thin layers separate the adrenals from gonads or kidneys. The gland has a pyramidal shape and the colour is variable depending on individual, from light yellow to orange or brown.

The adrenal gland is surrounded by a connective tissue capsule which has an inner, proper part, made up of 2—6 cell layers and a peri-capsular one composed of a loose connective tissue. In the latter, there are arterioles, venules, numerous large vegetative ganglia and bundles of nerve fibres.

The interrenal cells are radially arranged in cord-like groups. The cords are sinuous, irregularly disposed, branched out and anastomosed. They form a network in whose spaces there are capillaries and groups of chromaffin cells. Immediately beneath the capsule, the cords are disposed in the form of arches but a little deeper they run in various directions and anastomose (Fig. 1).

The interrenal cells in *Phalacrocorax carbo* are prismatic in shape, the basal pole being directed towards the capillary that borders the cord. Most of the cells have spherical nuclei. The nuclei are situated in the apical third of the cells, never near the basement membrane (Fig. 2). In the sub-capsular cords there are numerous larger nuclei, with diameters between 8 and 11 μ.

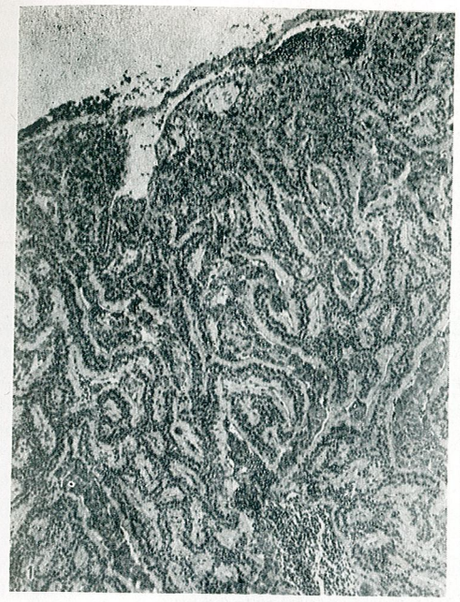
In sections stained with routine histological methods significant amounts of yellow-brown pigments may be observed. When the adequate histochemical reactions were performed, it was proved that these pigments are lipofuscins (Fig. 3). They are not an obligatory constituent of the interrenal cells in *Phalacrocorax carbo*. When present, they showed morphological variability from one individual to another. They may appear either as isolated granules or as clusters lying in the middle third of the cell, between the nucleus and basement membrane (Fig. 3). The lipofuscins were more frequently encountered in the peripheral zone; however in many individuals they were also found in the deeper part of the gland.

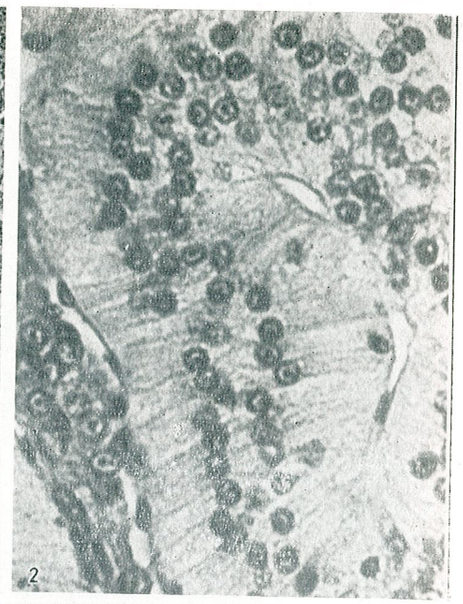
Within adrenal glands in *Phalacrocorax carbo*, groups of small cells with strong basophilic nuclei and little cytoplasm were found. In fact, it is possible to ascertain that some of them are frequently located beneath the capsule and are constituted of young, undifferentiated elements; they probably become mature as the functional cells degenerate (Fig. 4). The others are constituted of lymphoid elements, infiltrated between glandular parenchymal cells (Fig. 5).

In some individuals we saw numerous blood red cells that penetrated into interrenal cords. In these places the basement membrane of the cord appears to be interrupted. Interrenal cells all around the red cells clusters are flat and seem to separate the clusters from the remainder interrenal tissue (Figs 6, 7). This process might be the result of a stress stimulus.

DISCUSSION

Comparing the information stored up to the present date about adrenal structure and physiology in mammals, on the one hand, and interrenal tissue in remainder vertebrates, on the other hand, the considerable





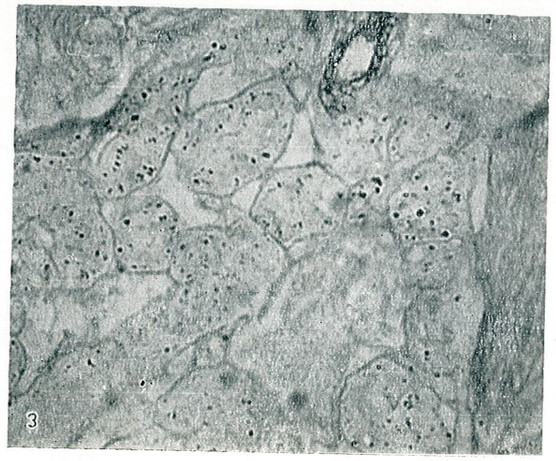


Fig. 1. — Section showing sinuous, irregularly disposed and anastomosed interrenal cords. They form a network in the spaces of which there are capillaries and groups of chromaffin cells.

Fig. 2. — Prismatic interrenal cells radially arranged in cord. The nuclei are situated in the apical third of the cells.

Fig. 3. — The PAS-positive reaction after salivary amylase demonstrating lipofuscins.

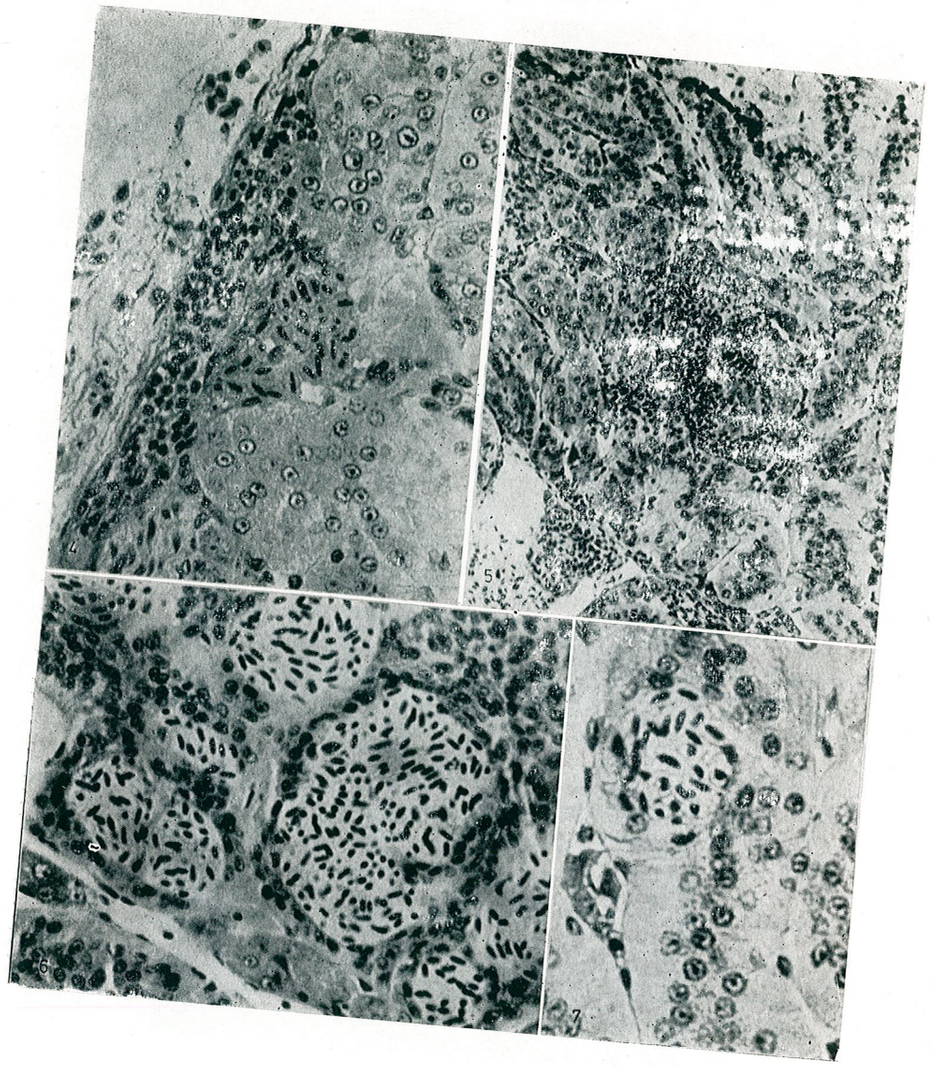


Fig. 4. — Group constituted of small, incompletely differentiated cells disposed immediately beneath the capsule.
Fig. 5. — Lymphoid elements infiltrated between glandular parenchymal cells.
Figs 6 and 7. — Blood red cells penetrated into interrenal cords. Note flat interrenal cells all around the red cells clusters.

variability of structure and the uniformity of synthesis and secretion processes are both evident. On this basis, we consider that our results about adrenal gland in *Phalacrocorax carbo* can be generalized to a certain extent for most birds.

Though some species show fused adrenals, most of them display two anatomical independent glands [9]. In this respect *Phalacrocorax carbo* is placed in the last group.

The interrenal cords in *Phalacrocorax carbo* display a structural resemblance with those described in *Pelecanus occidentalis* [9], [11], [21]. They are characterized by a radial arrangement of the interrenal cells which are very elongated and slightly narrower towards the central part of the cord. In the other species of birds the interrenal cells are less high, showing a cubical *(Passer domesticus)* [2], or polyhedral cellular outline *(Zonotrichia leucophrys gambelii)* [15]. In pigeon, the interrenal cells are never as high as in *Phalacrocorax carbo* or *Pelecanus occidentalis* [1]. In this respect the interrenal cells appearance in the last two species is more similar to that encountered in *Crocodilus niloticus* [5], than the characteristic one for Passeriformes. One may assume that the interrenal cell shape has changed along the evolution, from a very elongated form to a cubical or polyhedral one, specific to Passeriformes and similar to the characteristic one for Mammalia.

Taking into account the occurrence of large nuclei in peripheral zone (with diameters between 8 and 11 μ), displaying a more intense positive Feulgen reaction, discernible even without cytophotometric measurement, the hypothesis that they may be polyploid does not appear impossible. Moreover, polyploid nuclei in adrenal gland of *Peromyscus leucopus* were reported [4]. Also, giant adrenocortical cells with voluminous nuclei were described in rat embryo and newborns [12], [13]. Certainly, the occurrence of polyploid nuclei in *Phalacrocorax carbo* interrenal tissue can be confirmed only by cytophotometric study. Significant changes of Feulgen reaction intensity in adrenal cortex were reported, under normal [3], [6], [7], and experimental conditions, as: exposure to cold [17], ACTH administration [16], dehydration [17]. These variations of Feulgen reaction intensity are probably connected with certain modifications of chromatin molecules under special functional conditions, being possible to take them into account, in appreciating the cellular activity.

There are only few studies dealing with interrenal cells regeneration birds. Sivaram considers that the replacement of depreciated interrenal cells is achieved by cellular proliferation in the adrenal peripheral zone and migration of the new cells towards deeper zone [22]. Cellular proliferation in mammalian adrenal gland was studied to a larger extent. According to Planel *et al.* the new cortical cells originate in incomplete differentiated cell groups, located in the connective capsule of the gland and forming regenerative blastema [18], [19]. After unilateral adrenalectomy, Grzybek noted dedifferentiated cells with a clear proliferative capacity in the capsule of the remainder gland [8]. Studies carried out with thymidine-^{3}H indicated glomerular and outer fascicular zones as sites where proliferative activity reaches its peak [10], [14], [20], [23], [24]. The groups of small, undifferentiated cells, with preponderant localization in

peripheral zone might represent a reserve which gradually substitutes the degenerating cells. At first sight they can be easily confused with lymphoid cell groups found among interrenal cords too.

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NUCLEAR ULTRASTRUCTURAL MODIFICATIONS OF THE ADENOVIRUS 3-INFECTED CELLS OF THE H. Ep. 2 LINE

BY

GR. MIHĂESCU, D. MIŞCALENCU and M.D. IONESCU

In the adenovirús 3-infected cells of the H.Ep. 2 line deposits of materials of paracrystalline structure occur. These materials do not participate in the viral morphogenesis process and represent viral inclusions. The nucleolar material segregates in voluminous, granular and dense peripheral fibrillar zones. The nucleolus disintegrates gradually. In the advanced stage of infection, crystalloid formations appear in the nucleus of infected cells; these are formed of parallel fibres of uniform thickness. The role and origin of crystalloid and paracrystalline inclusions have not been precisely established.

The adenoviruses are assembled in the nucleus of the host cell but the specific viral proteins are synthesized in the cytoplasm and then they migrate to the nucleus.

The infection with adenoviruses alters significantly the activity of some cytoplasmic and nuclear enzymes [1]. From the metabolic point of view, the infected cells are characterized by a high level anaerobic glycolysis, resulting organic acids that determine the rapid acidification of the culture medium.

The replication of cellular DNA is inhibited, some hours later after infection [2], and the synthesis of the RNAr decreases up to 20% [8].

The study of the nuclear morphological modifications in the infected cells provides information regarding the dynamics of the viral components accumulation, as well as the degree of their using in the process of viral assembly.

MATERIAL AND METHODS

The cells of the H.Ep. 2 line, from the collection of the Laboratory of Cell Cultures within the "I. Cantacuzino" Institute, were grown on the Eagle nutritive medium with an addition of 10% calf serum.

The cells were infected with adenovirus 3 and after 24h the double fixation with glutaraldehyde 2% and osmium tetraoxide 1% was performed, then doubly stained with uranyl acetate and lead citrate.

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RESULTS

The cells infected with adenovirus 3 exhibit obvious ultrastructural nuclear modifications. A peripheral nuclear disposition of the chromatin and its electronoptic increase density take place. Large areas of the nucleoplasma become clear or are occupied by a finely granulated low density substance, that represents the viral proteins. The virions seem to be isolated or associated in groups, rarely giving crystalline accumulations (Fig. 3). The accumulations of the constituents induced by the virus are divided into two groups.

A first group is represented by the granular accumulations with a lax arrangement without a precise delimitation from the rest of the nuclear material. These accumulations are the active centres in which the assembly of virions takes place and represent the source of precursor proteins, available for the production of progen virus. The virions are present only in the accumulations with lax structure and these are the real structural proteins.

The second group of components is represented by accumulations probably of protein origin, with a compact paracrystalline structure (Figs. 1, 2) inside which one can never see the presence of viral particles.

Sometimes the paracrystalline components are quantitatively preponderant, but the absence of viral particles in their mass denotes the impossibility of utilization of such components in the process of viral assembly.

The two usual components, the fibrillar and granular ones, of the hypertrophied nucleolonema, segregate into a voluminous granular mass and a fibrillar electrondense peripheral mass (Figs. 1, 2). Then, the nucleolus exhibits a disintegration process. The fibrillar component decreases quantitatively and separates from the granular one, which becomes compact (Fig. 4). The fibrillar component completely disappears (Fig. 5) and the granular component breaks up step by step and condensates, still remaining like some dense inclusions, diseminated in the whole mass of the nucleus (Fig. 6).

In some cases in the nucleus there appear crystalloid formations, composed of parallel fibrilles with a uniform thickness (Fig. 7). These formations have a close spatial relation with the viral particles (Fig. 8).

DISCUSSIONS

Besides viral particles, sometimes with crystalline arrangement, the accumulation of large quantities of materials, representing viral precursors, takes place in the nucleus of infected cells.

The accumulations of lax granular structure represent proteins participating in the process of viral assembly because the virions are associated only with this category of components, that represent the real viral structural proteins.

The large accumulations with paracrystalline structure do not represent precursors of viral material because we have not found virions in their mass. These materials, probably of protein origin, could be syn-

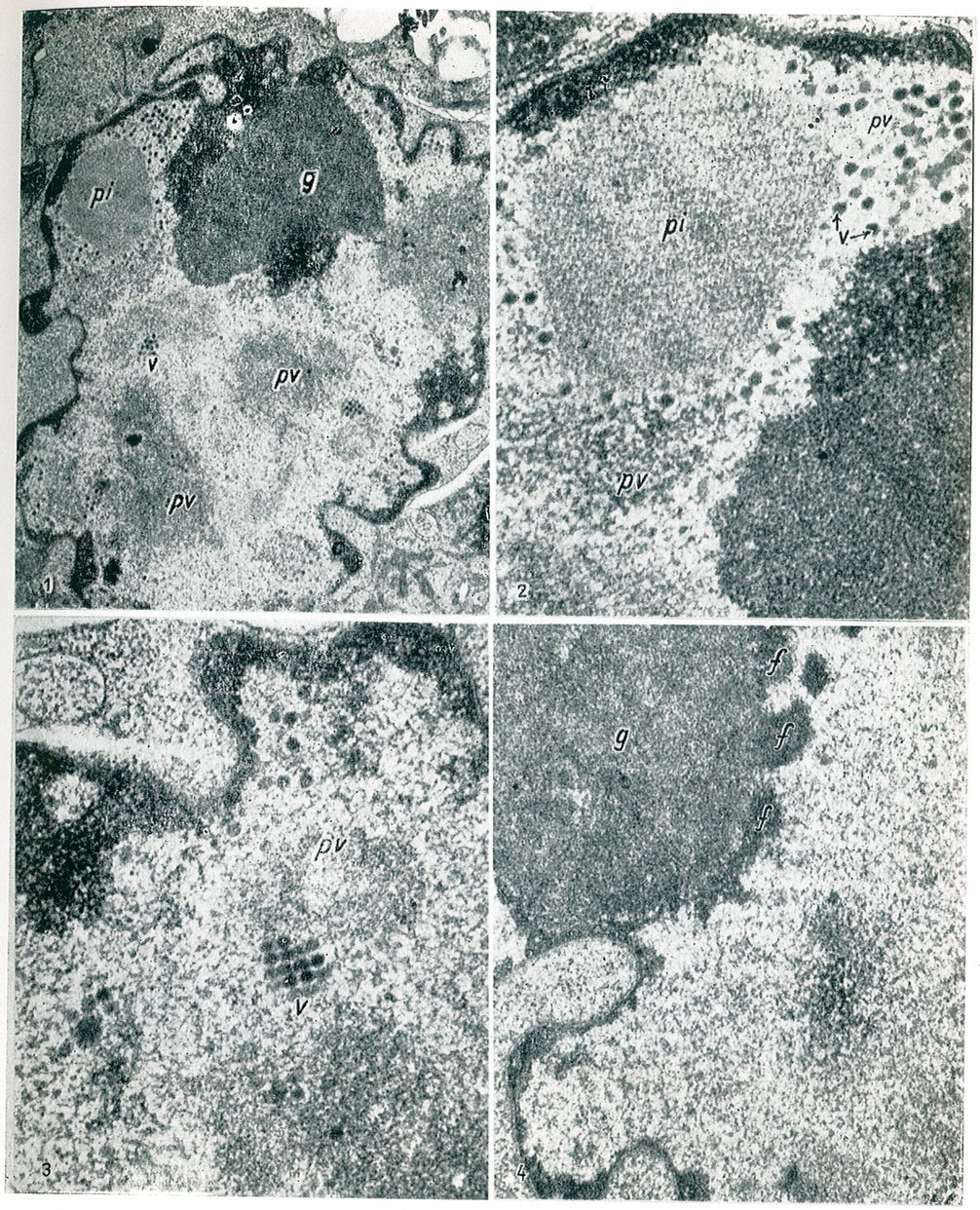

Fig. 1. — Chromatin(c) is dense and disposed at the periphery of the cell. The viral proteins(pv) occupy large areas. The virions (v) with disordered arrangement surround a paracrystalline inclusion (p.i). The nucleolonema segregates into a voluminous, dense, granular mass(g) and a fibrous(f) mass × 14 500.

Fig. 2. — The virions are assembled in the lax viral protein accumulations but they are not present in the mass of the paracrystalline inclusion × 45 000.

Fig. 3. — The virions with crystalline arrangement into an area occupied by viral proteins × 45 000.

Fig. 4. — The fibrillar component of the nucleolus disintegrates and migrates in the nucleoplasm. The granular component is compact × 30 000.

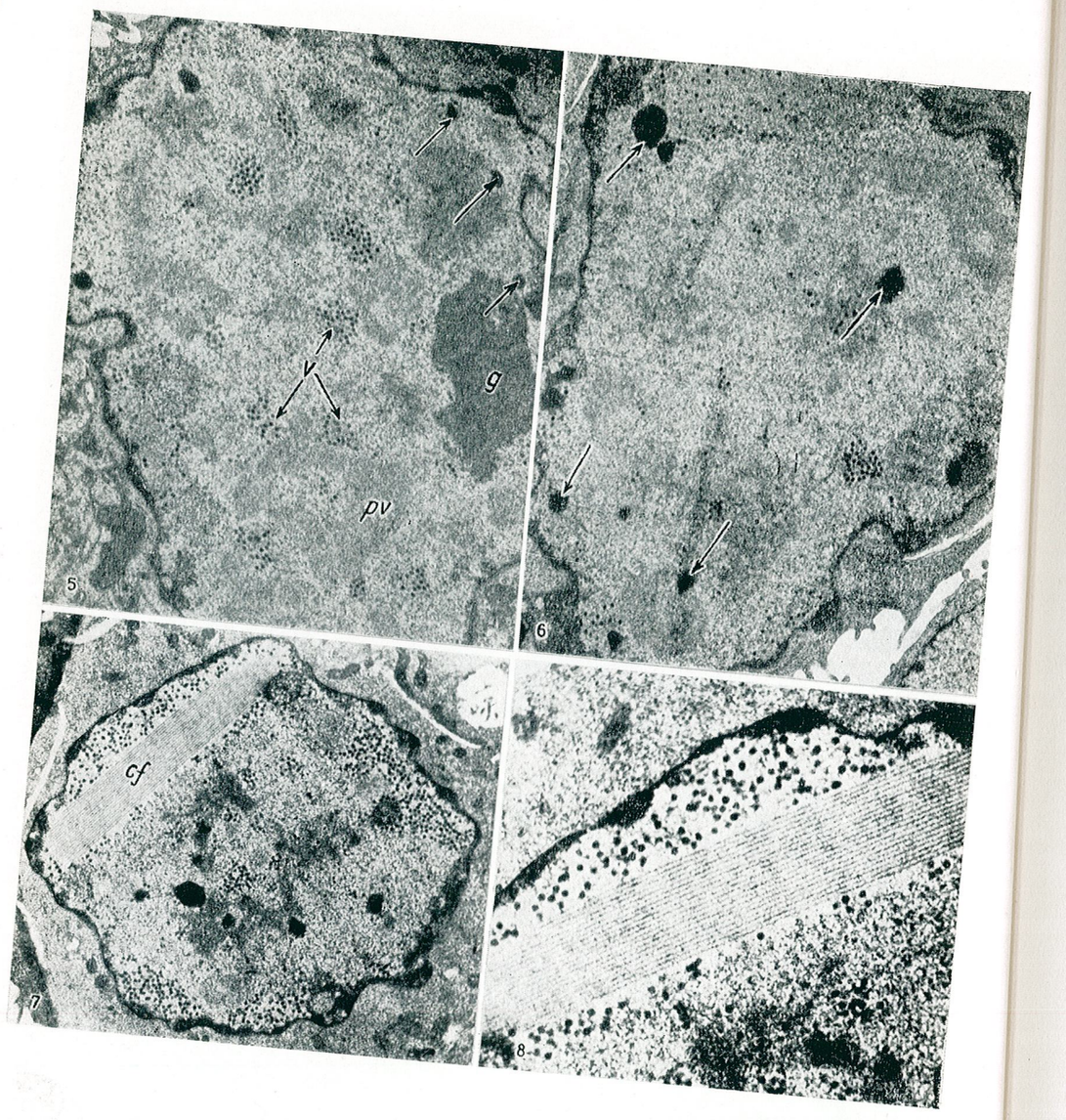

Fig. 5. — The granular component of the nucleolus begins to disintegrate (arrows). The fibrillar component is absent. Viral proteins and virions are all over the nucleus ×14 500.
Fig. 6. — Fragments of the disintegrated nucleolus (arrows) × 14 500.
Fig. 7. — Fibrillar crystalloid formations (c.f.) and nucleolar disintegration × 12 600.
Fig. 8. — Detail of Fig. 7 × 30 000.

thesized by the viral genetic information and then they would have the same biochemical nature with the structural proteins. The fact that they are not used in the viral morphogenesis could be due to an accumulation above the limit concentration, that would cause the condensation and their transformation into an inert state, unavailable for the viral assembly.

On the other hand, the paracrystalline inclusions could be proteins of cytoplasmic origin transferred to the nucleus.

The process of disintegration of the nucleolus is not absolutely coordinated with the accumulation of a given quantity of virions. The segregation of the components and then their disintegration up to the complete loss of the structure unit of the nucleolus could be interpreted in terms the of RNAr synthesis blockage [6].

The nucleolar disintegration appears after the inhibition of the DNA synthesis [2], but such nucleolar modifications are specific both to the viral infection, as well as to some chemical agents that inhibit the DNA-dependent RNA synthesis [7], [9].

The crystalloid formations encountered only in the nuclei of the infected cells have always occurred in the advanced stage of disintegration of the nuclear material. They are proteins and it is considered that their synthesis could be codified by viral genetic information, or could be a result of the depression of some cell genes, as a result of viral infection, and which would induce the reorganization of the preexisting nuclear structures [4].

Other types of intranuclear inclusions [3], [5] with a variable architecture have also been described; they appear after the infection with adenoviruses, that reflects the complexity of the virus — host cell relationship. Their variability is dependent both on the nature of the infecting virus and also on other internal conditions of the cell, yet unknown.

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SCANNING ELECTRON MICROSCOPY (SEM) IN THE STUDY OF SOME LEUKEMIC CELLS IN DOMESTIC MAMMALS AND FOWL*

BY

N. MANOLESCU, V. CIOCNITU, N. AVRAM and C. DIMITRIU

The paper describes the leukemic cells from the peripheral blood of cattle, sheep, horses and fowl studied by the scanning electron microscopy technique (SEM) on a number of 205 cases.
The data yielded were corroborated by findings on blood smears under the optic microscope. In this way, the form, size and surface structure particularities of the cell could be defined and the following leukemia cellular forms distinguished: "B" lymphocytal, "T" lymphocytal, monocytal and histiomonocytal.

Diagnosing the presence of leukemic cells in the peripheral blood of animals or in the haematopoietic central organs (which defines leukosis) made the object of numerous communications at home and abroad. A wide range of methods have been employed, each with its advantages and disadvantages.

In 1973 Polliak [5] published his first work on the scanning electron microscopy technique in the study of leukemic cells in humans.

No SEM investigations have been reported so far in the study of normal or leukemic cells in animals. The aim of the present paper is to make known the results of such an investigation [1]—[4], [6]—[11].

MATERIAL AND METHOD

Samples from the peripheral blood and the haematogenous marrow were collected on K_2EDTA.

The plasma was centrifuged for 10 min at 1000 r.p.m. The sediment obtained — leukocytary concentrate — was embedded first in 1% glutaraldehyde and next in 1% osmium tetraoxide (for 30 min each).

Dehydration was performed progressively in ethyl alcohol baths over an 18 hrs interval. Metallization was achieved in a "Vacuum Evaporator" with the help of gold.

The samples were studied in the JEM-JEOL 100 C electron microscope at a tension of 20 kW; double views of the same cells were taken by the "normal contrast" and "high contrast" techniques in order to have the elements of external morphostructure markedly improved.

Leukosis cases were diagnosed by means of haematological and cytomorphological procedures corroborated by clinical observations.

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* Paper presented at the Vth Symposium of Electron Microscopy.

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RESULTS AND DISCUSSION

Scanning electron microscopy provides precise information on the size, form and architecture of the cells otherwise undetectable under the optic microscope.

In case of bovine or avian leukemia, AIE and malignant lymphopathies in swine, the following cellular forms were studied and diagnosed :

— chronic lymphoid leukemia with type "T" cells ;
— chronic lymphoid leukemia with type "B" cells ;
— acute lymphoid leukemia with type "B" cells ;
— cytemic lymphosarcoma ;
— monocytal and histiomonocytary leukemia.

If in the cases of lymphoid leukemia with type "T" cells, the aspect of the cells is normal, showing frequent surface thickenings and having an incidence of 80—95 per cent, in chronic lymphoid leukemia with type "B" cells this kind of cells occur in proportion of 85—90 per cent and show also surface structural modifications ; gradually, the microvilli grow thicker anastomizing in the form of muffs or shields of variable thicknesses (Figs. 1, 2, 3).

In cases of acute lymphoid leukemia, a pure lymphoblastic culture develops.

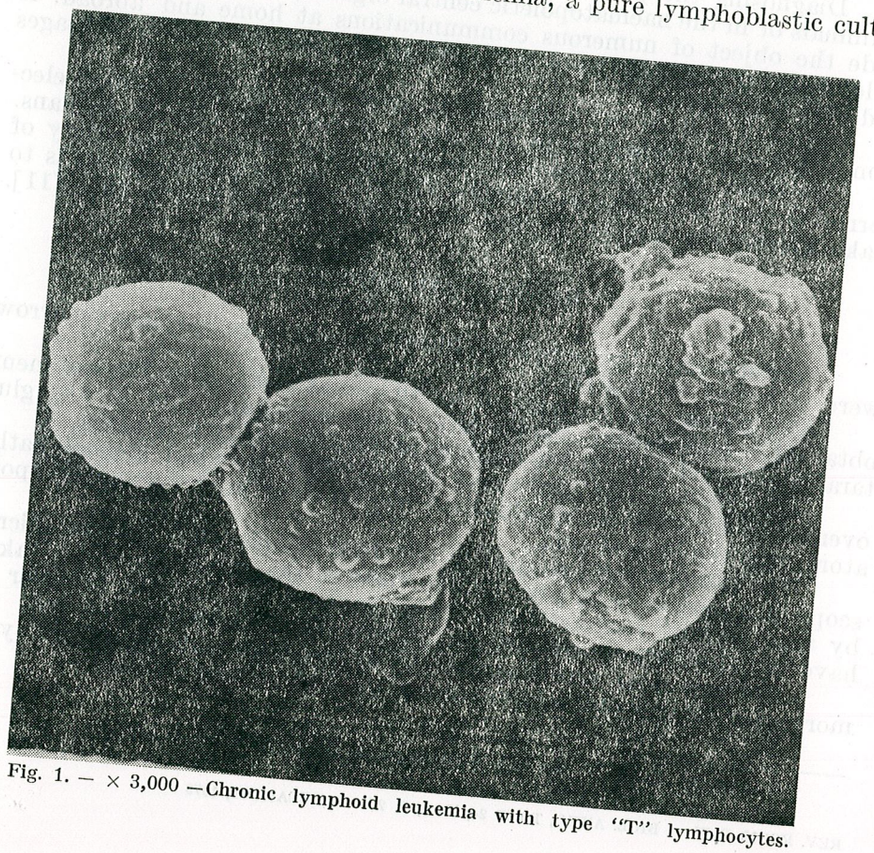


Fig. 1. — × 3,000 — Chronic lymphoid leukemia with type "T" lymphocytes.

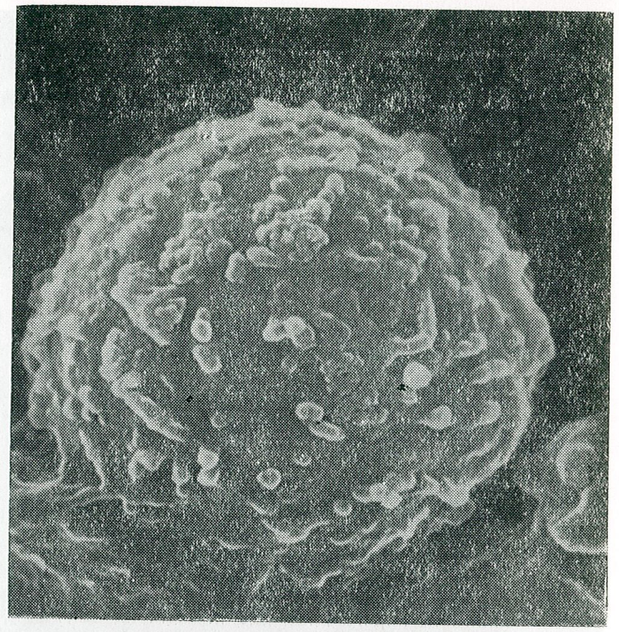


Fig. 2. — × 10,500 — Chronic lymphoid leukemia with type "B" lymphocytes.

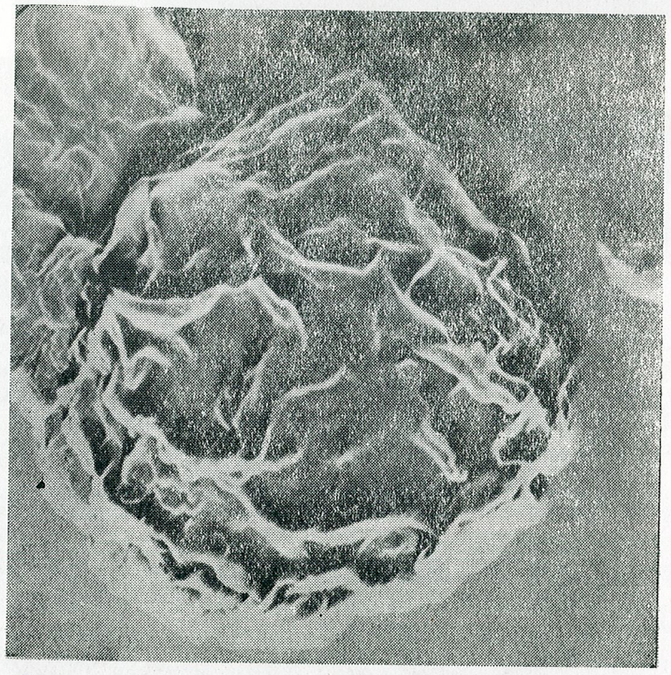


Fig. 3. — × 12,000 — Acute lymphoid leukemia with type "B" lymphoblasts (High contrast).

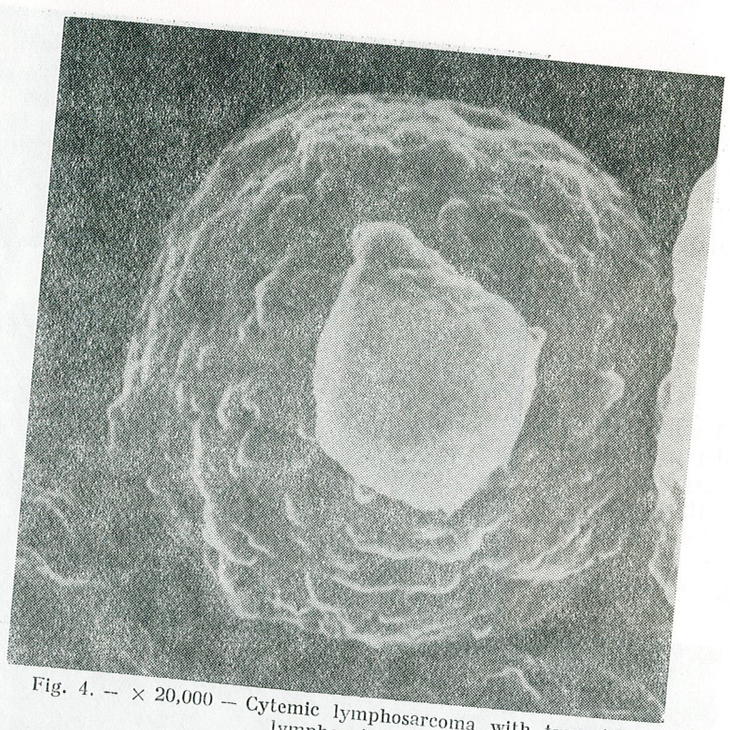


Fig. 4. — × 20,000 — Cytemic lymphosarcoma with type "T" lymphocytes.

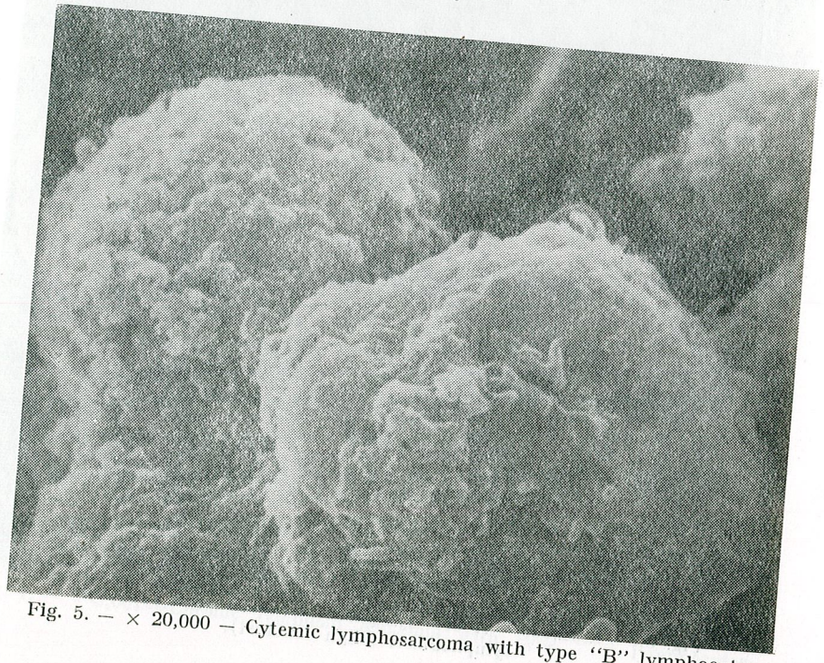
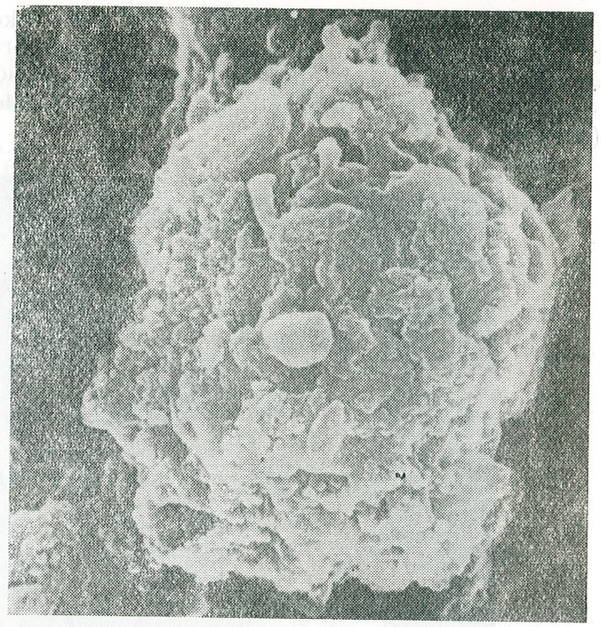


Fig. 5. — × 20,000 — Cytemic lymphosarcoma with type "B" lymphocytes.

Fig. 6. — × 8,500 — Cytemic lymphosarcoma with type "B" lymphocytes. A monstruous, cauli flower-like cell.



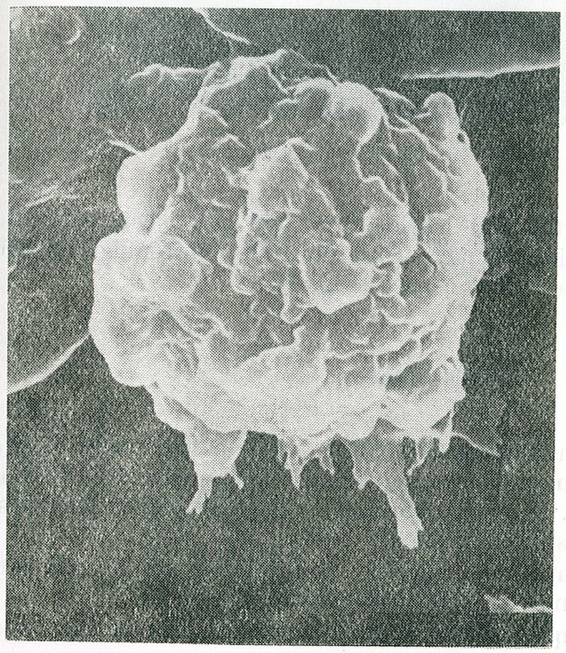


Fig. 7. — × 13,000 — Cytemic histiosarcoma.

In cases of lymphosarcoma, the cells take queer, variously-sized anaplastic forms (the monomorphism of the chronic lymphoid leukemia disappearing). The cellular elements show various surface thickenings, which largely deform the cells, as well as frequent cytoplasmic elongations (Figs. 4, 5, 6, 7).

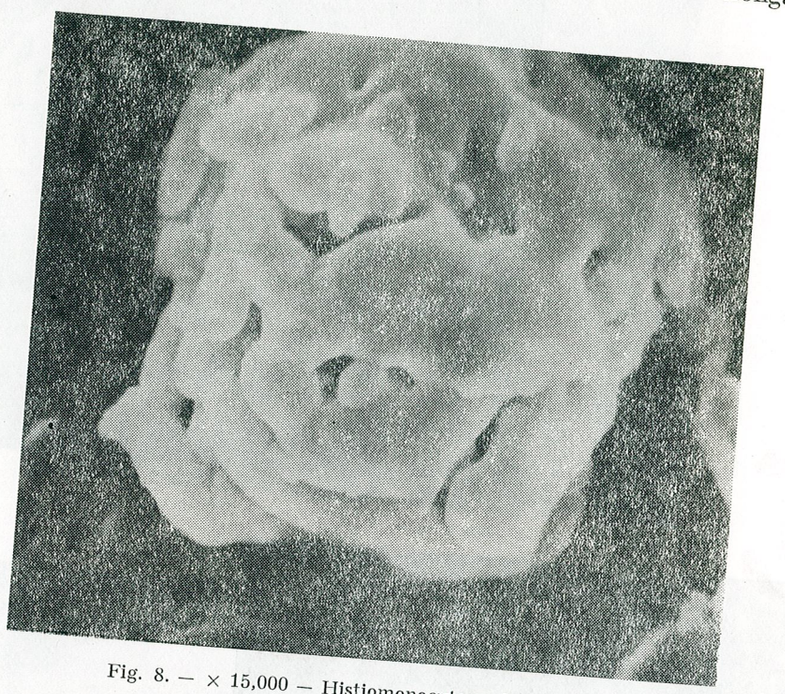


Fig. 8. — × 15,000 — Histiomonocytary leukemia.

In cases of monocytal and histiocytary leukemia such elements prevail, some of them being atypical with multiple thickenings on the surface; they acquire an aberrant aspect (Fig. 8).

CONCLUSIONS

1. Scanning electron microscopy (SEM) used in the study of the blood cells is a practical method recommendable in the diagnosis of leukoses in domestic animals. It is corroborated by haematological methods, supplementing and diversifying them.

2. In malignant lymphomas, specific modifications of the cell surface occur as against normal aspects, irrespective of species.

3. The SEM technique enables a good differentiation of the cytologic forms of mononuclear leukosis.

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ON THE MECHANISM OF MAINTENANCE OF NORMAL MEMBRANE EXCITABILITY

BY

ȘTEFAN AGRIGOROAEI, GABRIELA AGRIGOROAEI and FLORIN PATAPIE-RAICU

Considering that extracellular Na ions stabilize the phospholipid micellar structures of the external layer of the membrane, thus maintaining its normal excitability, the action of Na^+ substitutes : TMFA, TEA and TPA on sartorius fiber resting potential and depolarization by high external K^+ is best explained as a result of a superstabilization of cationic phospholipidic micella from this layer of the membrane.

In this superstabilization, which means a reduction in the normal excitability by increased resistance to "disturbing" factors, the hydrophobic properties of these organic cations are involved.

The involvement of extracellular Na^+ in maintenance of normal excitability of the nervous and muscular fibers was reported long time ago, but no explanation for the mechanism by which the ions exert their role was given.

Recent studies [4], [18] have shown that Na^+ behaves differently on the external and internal layer of the membrane.

On the other hand, Lorente de Nó [12] and subsequently many other investigators [6], [11], [13] pointed out that some quaternary ammonium ions (such as tetraethylammonium — TEA) and their hydroxylated derivatives can substitute the external Na^+ in certain concentration limits without sensibly affecting the membrane resting potential. However, in very high concentrations, these compounds block the impulse propagation along the fibers.

Some of these agents have also a blocking effect on the impulse transmission through the ganglionic synapses achieved by a mechanism of "stabilization" of postsynaptic membrane, that is raising its excitation threshold [14], [15].

However, the Na^+ substitutes have not been classified among the true stabilizers of the membrane as the local anesthetics and some other factors have [5], [7], [17]. These are defined as agents unable to modify the membrane resting potential and at the same time are opposing the factors which have the tendency to modify it.

A new point of view on the membrane stabilization and stabilizers has been advanced lately, considering the stabilizers as factors which normally ensure the unity of membrane structure [8]. On this line, however, only some general remarks have been made.

Taking into account that some resemblance existed between the Na^+ action, Na^+ substitutes and the true stabilizers in connection with membrane excitability, this common basis was investigated.

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In this paper, the effects of some quaternary ammonium ions (trimethylphenylammonium — TMFA; tetraethylammonium — TEA and tetrapropylammonium — TPA) on the resting potential of striated muscular fiber membrane as well as its modification by high external K^+ are presented.

The interpretation of the results was based on the "2—M.S.I." concept on membrane organization and function [2].

MATERIAL AND METHOD

The experiments were performed on frog sartorius fiber membrane using glass intracellular microelectrodes, at room temperature, a normal external pH (7.4) and a constant external Cl^- concentration.

The action of quaternary ammonium ions (TMFA, TEA, TPA) on RP was studied at two concentrations, 25 mM/l and 50mM/l, by equimolar substitutions of Na^+ in Ringer.

The membrane depolarization by high external K^+ was achieved with 50 mM K^+/l in Ringer solution, also by equimolar Na^+ substitution.

The effects of quaternary ammonium ions on the membrane depolarization by high external K^+ were followed in Ringer solutions with 50 mM K^+/l and 25 mM/l or 50 mM/l organic ions, by equimolar substitution of Na^+. The time course of membrane depolarization was registered by measurements at 5—10 minutes intervals for one hour.

RESULTS

The values of resting potential registered in our experiments were in the range of 88.50 mV and 90.50 mV.

Experiments, where 25 or 50 mM Na^+/l in Ringer were equimolarly substituted by TMFA, TEA or TPA, showed that in these conditions the quaternary ammonium ions had no evident effect on the membrane resting potential (Table 1).

During the membrane depolarization with high external K^+ (50 mM/l Ringer), the membrane potential decreased during one hour from 89.58 mV to 27.00 mV (S.E. = ±0.32 mV) (Figs. 1 and 2: the broken line).

In the presence of quaternary ammonium ions, the depolarization with K^+ was affected in respect to both its velocity of installation and amplitude. In experiments where the organic ion concentration in the depolarizing solution was 25 mM/l Ringer, the final resting potential value was 31.00 mV (S.E. = ±0.38 mV) for TMFA (Fig. 1, curve *1*), 32.00 mV (S.E. = ±0.22 mV) for TEA (Fig. 1, curve *2*) and 34.00 mV (S.E. = ± 0.32 mV) for TPA (Fig. 1, curve *3*) compared with 27.00 mV in the control experiment.

In the other series of experiments (50 mM/l quaternary ammonium ion in high K^+ Ringer) the final value of the membrane potential was 35.00 mV (S.E. = ±0.22 mV) for TMFA (Fig. 2, curve *1*), 36.50 mV(S.E. = ±0.29 mV) for TEA (Fig. 2, curve *2*) and 40.00 mV (S.E. = ±0.21 mV) for TPA (Fig. 2, curve *3*), compared with 27.00 mV in the control experiment.

Therefore, at the concentrations used in this study, all the organic ions behaved as good Na^+ substitutes, not affecting the value of the membrane potential, but opposing to some extent to its variation imposed by the increase of external K^+ concentration. This effect is dependent also upon the agent concentration and the hydrophobic properties of its chemical structure.

Table 1

The resting potential of frog sartorius fiber membrane in normal external medium and in medium with quaternary ammonium ions

External medium	Number of animals	Number of determinations	Membrane potential (mV)	S.E. (+ mV)
Normal Ringer	6	50	89.17	0.20
Ringer 25 mM TMFA	6	96	89.15	0.17
Ringer 50 mM TMFA	6	96	89.17	0.19
Normal Ringer	6	60	88.50	0.20
Ringer 25 mM TEA	6	102	88.42	0.19
Ringer 50 mM TEA	6	98	88.43	0.21
Normal Ringer	5	49	89.58	0.14
Ringer 25 mM TPA	5	91	89.60	0.16
Ringer 50 mM TPA	5	88	89.62	0.17

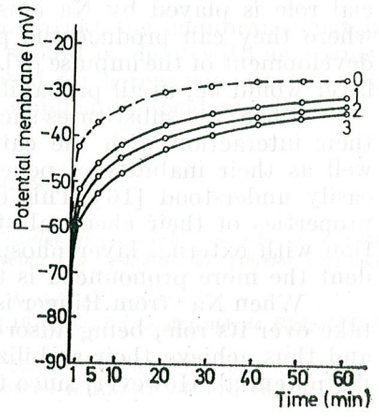

Fig. 1. — Membrane depolarization in: (K—50 mM) Ringer — curve 0; (K—50 mM, TMFA—25 mM) Ringer — — curve *1*; (K—50 mM, TEA — 25 mM) Ringer — — curve *2*; (K—50 mM, TPA — 25 mM) Ringer — — curve *3*;

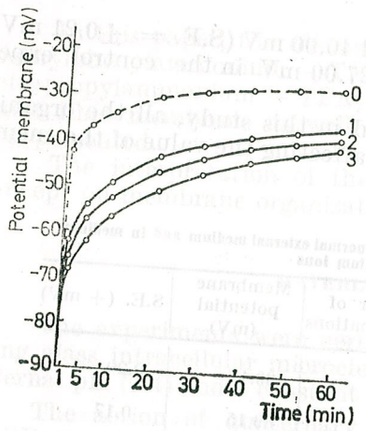

Fig. 2. — Membrane depolarization in: (K—50 mM) Ringer — curve 0; (K—50 mM, TMFA — 50 mM) Ringer — curve *1*; (K—50 mM, TEA — 50 mM) Ringer — curve *2*; (K—50 mM, TPA — 50 mM) Ringer — curve *3*.

DISCUSSION

A previous paper [1] presented our point of view regarding the essential role played by membrane phospholipids in the bioelectric and ionic permeability phenomena, which have well defined ion exchange properties [9].

The micellar structure of phospholipids organized as liophobic sols requires for its very existence ensuring of the essential condition for physicochemical stability. Ion adsorption from the aqueous substrate on the micelle surface, resulting in a protective electrical sphere, represents the basic way providing their stability.

Since the phospholipids in the external layer of the membrane are predominantly cationic, most of them will be stabilized by cations from the extracellular medium. According to adsorption selectivity and ion exchange laws, the only cation in the external medium which can ensure their stability in normal resting conditions is sodium.

Thus, it is admitted that Na carries out its role in maintaining the normal excitability of the membrane just by this stabilizing effect. A special role is played by Na ions at the level of membrane internal layer where they can produce the phase transition typical for the automatic development of the impulse [3]. The stabilization of the membrane internal layer would be, in all probability, due to a small intracellular anion.

Since Na^+ substitutes such as TMFA, TEA, TPA are organic cations, their interaction with the cationic external layer of the membrane, as well as their inability of penetrating into the intracellular medium are easily understood [16]. This difficulty is enhanced by the hydrophobic properties of their chemical structure which also explains their interaction with external layer phospholipids. This interaction is the more evident the more pronounced is the molecule hydrophobia [10].

When Na^+ from Ringer is substituted for such organic cations, these take over its role, being adsorbed on the cationic phospholipidic micellae and thus achieve their stabilization without a marked effect on the resting potential. However, since these organic ions have also lipophilic properties, their interaction with phospholipidic micella is stronger compared to that of Na^+. In this way, the stabilization of the phospholipidic micella is greater than that supported by an equimolar amount of Na ions, that is they are superstabilized. In this way, the phospholipidic micella become more resistant (decreased excitability) to factors determining their phase transition with all the consequences on the electrical charge and selective permeability to ions.

It is evident from our experiments that the quaternary ammonium ions studied by us reduce the transitory effect of high external K^+, the more so as their hydrophobia is increasing. This effect is stronger with the increase in ion concentration, when a higher density of adsorbed particles on the surface of each micella is ensured.

On the basis of the mechanism proposed by us, the increase in the excitation threshold of the membrane treated with TEA [11], even when its concentration did not result in blocking the impulse, could be explained. A stronger excitant is needed to ensure the penetration of an amount of Na, toward the internal layer of the membrane, able to start a phase transition at this level [3].

We explain the complete blockage of the impulse, when the organic ion had substituted Na^+ from Ringer [11] almost completely (over 89%) by the decrease of external concentration below the critical value required to start the excitation. The quaternary ammonium ions being unable to be transported to the internal layer cannot substitute Na in order to achieve its secondary role.

It has also been proved that a feature of the quaternary ammonium ion blocking action at the level of ganglionic synapses based on a stabilization mechanism [15] is well justified.

The conclusion can be drawn that such an ion is a more suitable substitute for Na^+ as its lipophilia is more reduced. In this way it produces a stabilization of the membrane more similar to that produced by Na^+ itself, reducing only slightly its excitability. In general, it can be stated that the quaternary ammonium ions superstabilize the external layer of the membrane (reducing its excitability), also decreasing Na^+ efficiency in the internal layer during stimulation by decreasing its concentration in the external medium.

Evidently, our point of view requires that the membrane "stabilization" phenomenon by various agents should be dealt with as a superstabilization phenomenon and also the need for a reconsideration of the classic concept on stabilizers (generally applied to local anesthetics).

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IN VITRO EFFECT OF INSULIN ON THE GLUCOSE UPTAKE BY ISOLATED MANTLE PIECES OF *MYTILUS GALLOPROVINCIALIS* (L.)

BY

IOSIF MADAR, NINA ŞILDAN, EUGEN A. PORA and LUCIANO HERNANDEZ

Isolated mantle pieces from various age-groups of the sea mussels were incubated for 2 hours in glucose containing saline solution in the presence or absence of glucagon-free ox-insulin (10^{-1} U.I./ml). It has been established that insulin enhances significantly the glucose uptake by the mantle tissues. The possible role of insulin in the carbohydrate metabolism in this species is discussed.

The physiological role of insulin and the presence of insulin-like substances and insulin-like producing cells in some bivalve mollusc species have convincingly been demonstrated only in the last years [1], [2], [3], [7], [8], [9]. In spite of these facts there is no evidence referring to the insulin-responsiveness of isolated tissues in molluscs.

Our recent data have demonstrated that the hypoglycemic effect of recrystallized insulin in sea mussels is age-related [7].

Starting from the above considerations the *in vitro* effect of insulin upon the glucose uptake by the isolated mantle pieces of *Mytilus galloprovincialis* of various ages has been studied in the present work.

MATERIALS AND METHODS

For experiments performed in August 1977, sea mussels of various sizes corresponding to different age-groups (3.67 cm mean length $\simeq$ 1.5-year-old; 5.19 cm, $\simeq$ 2.5-year old; and 7.05 cm, $\simeq$ 4.0-year old) were used. The mussels were collected from the low water of the Romanian sea-shore of the Black Sea, and kept for 24 hours, prior to the experiments in sand-filtered water, aerated with compressed air, at 22°C in the laboratory (IRCM Constanţa-Agigea).

Two equal pieces (each weighing approximately 100—120 mg) from the marginal part of the mantle (without the germinal part) were quickly excised, blotted with filter paper and thereafter incubated simultaneously in a thermostated shaking device [5], in a final volume of 1 ml filtered and sterilized natural sea-water, containing 1 mg glucose ("UCB", Belgium), 2 mg calf-skin gelatine ("Merck") in the absence or presence of 10^{-1} units insulin/ml. For this purpose recrystallized glucagon-free ox-insulin ("Calbiochem", San Diego, Calif., Lot 201248, grade B, potency 26.1 I.U./mg) was used.

The incubation was carried out for 2 hours at 22°C with a shaking velocity of 90 oscillations per minute and with an amplitude of 5 cm, the gaseous phase in the incubation flasks being a mixture of 95% O_2 + 5% CO_2.

The initial and final concentrations of glucose in the medium were determined from 100 microliters solution, according to the glucoseoxidase-peroxidase method of H.A. Krebs *et al.* [4]. The samples were measured at 545 nm, using a VSU-2G spectrophotometer (Carl Zeiss, Jena).

The results are expressed as μmole glucose uptake by 1g wet tissue/2 hours, and the insulin-stimulated net glucose consumption was evaluated by the pair method, i.e. by subtracting the basal glucose uptake of tissue pieces, incubated without insulin, from that of their corresponding pair incubated in the presence of hormone.

The data are compared according to the Student *t* test, the differences between the mean values being considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSIONS

Elsewhere we pointed out [6] that the circulating glucose level in the haemolymph of sea mussels is age-related. On this basis we suggested that in the metabolic and hormonal regulation of glucose production and

Table 1

***In vitro* glucose uptake by the mantle pieces of *Mytilus galloprovincialis* of various ages. BAS = basal glucose uptake; INS = glucose uptake in the presence of insulin (10−1 U.I./ml); Δ INS − BAS = insulin stimulated net glucose uptake**

Age of animals	μmole glucose uptake by 1g wet weight tissue/2 hours		
	BAS	INS	Δ INS-BAS
≃ 1.5-year-old	5.03 ± 0.41 (8)	7.19 ± 0.50 (8)	2.16 ± 0.33 (8)
	$P < 0.01$		
≃ 2.5-year-old	6.03 ± 0.55 (8)	7.97 ± 0.57 (8)	1.94 ± 0.24 (8)
	$P < 0.05$		$P > 0.50$ *
≃ 4.0-year-old	5.11 ± 0.39 (8)	7.18 ± 0.62 (8)	2.07 ± 0.58 (8)
	$P < 0.02$		$P > 0.50$ *

Results are expressed as means ± S.E. Number of experiments is given in parentheses.
* Compared with the values obtained in 1.5-year-old age-group.

in the *in vivo* glucose consumption by the tissues the age of animals plays a conditioning role.

The data summarized in table 1 indicate that the isolated mantle pieces of *Mytilus galloprovincialis* are able to metabolize glucose, from

glucose containing saline solution, at a rate of 5.03 — 6.03 μ moles glucose/g wet tissue per 2 hours, and that this phenomenon at a relatively high glucose concentration (1 mg/ml) does not depend on the age of individuals.

When recrystallized glucagon-free ox-insulin is added to the incubation medium in a concentration of 10^{-1} units per ml, the rate of glucose penetration from the solution into the tissue pieces is significantly enhanced as compared to the basal values, the hormone stimulated net glucose uptake varying between 1.94 and 2.16 μ moles per g fresh tissue/2 hours. These data suggest that the tissues of the marginal part of the mantle are "insulin-responsive", and that insulin is involved in the glucose metabolism. From this point of view the above data agree with our findings concerning the hypoglycemic effect of insulin on *Mytilus galloprovincialis* [7]. On the other hand, the present data underline the possible physiological role of the insulin-producing cells in carbohydrate metabolism in this species [8] as well as in other bivalve molluscs in which the presence of insulin-like or insulin-producing cells has been recently demonstrated [2], [3], [9].

CONCLUSIONS

1. The mantle pieces of *Mytilus galloprovincialis* are able to metabolize glucose under *in vitro* conditions, regardless of the age of animals.
2. The *in vitro* stimulatory effect of insulin upon the rate of glucose penetration from the incubation medium into the mantle pieces of sea mussels indicates that the marginal part of the mantle is insulin-responsive.

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A NEW MODALITY OF CORRECTING OXYGEN CONSUMPTION IN FISHES *

BY

AL. G. MARINESCU and E. A. PORA

A new modality of correcting oxygen consumption values in fishes is suggested. On the basis of the general metabolism/body weight relation ($Y = a \cdot X^b$), the authors used two variants of modifying the registered data by avoiding the influence of body size. The new "expected" values are closer to a real dependence of metabolism on temperature and nutritional state (variant I) and on season (variant II), respectively.

The metabolism of poikilotherms is dependent on numerous endo- and especially exogenous factors [14], [13], [11], [6], [12], [10], etc. of which individual body size (weight, length, surface) (Rubner, 1902, [5], etc.) is the most important one. Taking oxygen consumption as metabolic indicator, we tried to find a means for eliminating the influence of size variation in batches where inviduals showed distinct body weight values.

MATERIAL AND METHOD

The general relation of metabolic dependence on body weight takes the form : $M = kW^n$ (allometric growth, Huxley 1932, cited after [6]), or $Q = aW^k$ (3) or $Y = a\,X^b$[17] where M(Q or Y) represents the so-called "metabolic magnitude" (oxygen consumption /24 hrs/ individual), or as designated more recently, oxygen consumption/h ; $W(X)$ is the body weight ; $k(a)$ a constant of this ratio ; $n(k$ or $b)$ the regression coefficient between metabolism and body weight, called also weight exponent. This indicator (known mostly as $b)$ was considered by various authors to be an individual index with specific, evolutive significance [18], [15], [5], [4], [6] and having a variability characteristic under various physiological states or function of different environmental factors [17], [13], [7], [8], [9], [10].

Hemmingsen [5] found the value 0.75 for the weight exponent (b) presumably common to all animal species, while Vinberg [14] noted for several fish groups a relatively constant value of 0.81.

In the metabolism body weight dependence relation, weight stands for the variable, while coefficient b and index a are the known values for

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all the data recorded in a given batch, and are characteristic of them in the respective conditions.

If we assume that the weight exponent may show a certain degree of variability with one and the same species in relation to temperature, season, state of nutrition, etc., it becomes quite obvious that index a is the "specific" constant of the oxygen consumption values.

With the help of the metabolism body weight relations, we devised a modality of correcting oxygen consumption values by eliminating the influence of the individual growth factor. In this way, we could evidence more accurately the metabolic index variation in respect to the given factors (season and temperature).

Furthermore, two variants are given as to the use of oxygen consumption correction.

RESULTS AND DISCUSSION

1) OXYGEN CONSUMPTION VARIATION IN TERMS OF TEMPERATURE AND STATE OF NUTRITION

Since the mean values recorded at the three acclimation temperatures ($\Delta T = ET : 25°$, 15° and 5°C) in both control ("fed") and starved batches (Table 1) correspond to different average body weight values

Table 1

Correction of oxygen consumption in *Idus idus* L.

Batch		Average body weight	Average oxygen consumption (registered) (ml O_2/kg/h)	Constant a (calculated)	Body weight exponent b	"Corrected" oxygen consumption (ml O_2/kg/h)
25°C	Fed	6.80	305.76	0.4934	0.75	324.07
	Starved	4.80	85.66	0.1267	0.75	83.14
15°C	Fed	7.04	75.68	0.1233	0.75	80.91
	Starved	4.34	47.08	0.0639	0.75	42.3
5°C	Fed	5.56	20.62	0.0318	0.75	20.83
	Starved	4.40	23.95	0.0330	0.75	21.59

(Fig. 1), we could not make a comparison between oxygen consumption levels in the investigated species (*Idus idus* L.), under our experimental conditions.

In order to correct these values we first calculated index a value for each batch, which in controls, for example, (25°C) was found to be of 0.4934, and next, we determined the average body weight common in all the batches studied ($G = 5.40$). Taking the value of 0.75 as body weight index (b), assumed by the majority of authors as most frequently

recorded in the metabolism / body weight ratio, we *re-calculated the oxygen consumption for a body-weight of 5.40 g as well as index (a) value, initially determined.* In the above example (control batch at 25°C) the corrected value of oxygen consumption is 324.07 ml O_2/kg/h as against the registered value of 305.76.

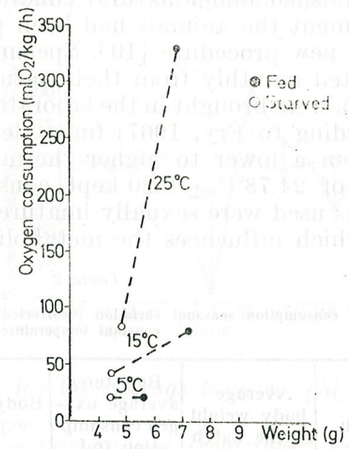

Fig. 1. — Influence of the body-weight factor on oxygen consumption variation in terms of acclimation temperature and inanition.

In this way, no changes were made in the oxygen consumption /body weight dependence relation (maintaining value a as registered and a non-modified index b) and we could obtain the "expected" oxygen consumption value for a given body weight. The two oxygen consumption values (registered and corrected) are listed in Fig. 2.

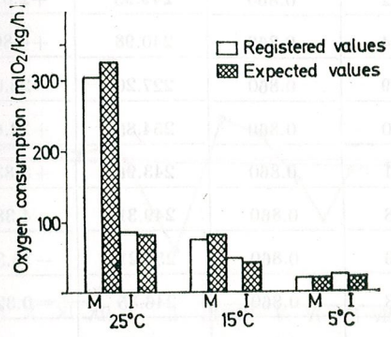

Fig. 2. — Correction of oxygen consumption in *Idus idus* L.

By this procedure, having eliminated the influence of the body weight factor, we have found the "real" dependence ratio of the metabolism on thermal and starvation factors.

2) SEASONAL VARIATION OF OXYGEN CONSUMPTION

Determination of the seasonal variation of the energy metabolism in poikilothermic animals, in general, and in fishes, in particular, is a very delicate problem because the seasonal variation of the environment is

very much influenced. Since oxygen consumption variation was strongly affected by seasonal temperature changes, some assayings were performed under conditions of constant temperature being maintained the year round ([16], Knauthe, 1899, cited after [1], [2]).

In order to avoid the development of a new rhythm, given that the one established under natural conditions was diminishing with time from the moment the animals had been placed at constant temperature, we devised a new procedure [10]. Specimens of *Carassius auratus gibelio* Bloch, collected monthly from their natural setting (under normal conditions of life), were brought in the laboratory and left to accomodate (acclimation according to Fry, 1967) for at least four days (enough time for transition from a lower to higher thermal level) to the experimental temperature of 24.78°C ± 0.20 kept constant the year round. All the test specimens used were sexually imature so as to eliminate the reproduction factor which influences the metabolism heavily [2], [6], [17].

Table 2

Real oxygen consumption seasonal variation ("corrected") in *Carassius auratus gibelio* Bloch at constant temperature

Month	Average body weight of the batch (g)	Registered average oxygen consumption (ml O_2/kg/h)	Body weight exponent *b*	Expected oxygen consumption (ml O_2/kg/h)	Per cent difference (%)
Sept./66	23.52	251.53	0.860	244.93	+2.69
Oct.	24.12	254.36	0.860	244.01	+4.25
Nov.	20.31	255.12	0.860	249.95	+2.07
Dec.	24.40	252.54	0.860	240.98	+4.80
Feb./67	40.14	170.39	0.860	227.20	−25.01
March	17.73	287.10	0.860	254.85	+12.65
April	24.14	265.51	0.860	243.96	+8.83
May	20.68	238.38	0.860	249.31	−4.38
June	18.49	214.56	0.860	253.21	−15.30
July	22.54	245.58	0.860	246.35	−0.32
Aug.	17.69	257.45	0.860	249.93	+1.03

The results scored under these conditions corresponded to some different monthly body weight averages in these batches, a fact that prevents the exact appraisal of the direct influence of seasonal factors, besides changes due to the body weight variation (Table 2).

These data are used in correcting the registered values with a view to eliminating the influence of body weight variations between the different test fish-batches used.

All the experimental data are processed statistically and the regression line coefficient is established (weight exponent) : $b = 0.860$. Index a values have also been assessed monthly for each batch as previously reported (*Idus idus* L.).

Similarly, using the value of 0.860 for exponent b we *calculated*, by means of values a computed according to the registered data, the "*expected*" *oxygen consumption values for the average body weight of the respective batch.*

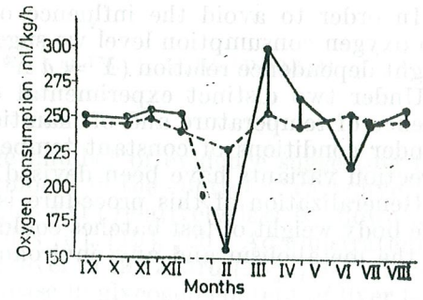

Fig. 3. — Seasonal oxygen consumption in *Carassius auratus gibelio* Bloch at constant temperature (registered values).

A comparison between the two values (registered and corrected) (Fig. 3) yields the per cent difference between the experimentally determined oxygen consumption and the expected level for a given body weight. This per cent difference (calculated monthly) *represents the real seasonal variation of the oxygen consumption obtained by correcting the influence of the body weight factor* (Fig. 4).

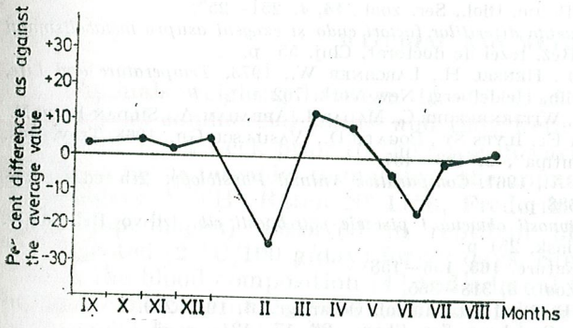

Fig. 4. — Seasonal variation of oxygen consumption in Carassius at constant temperature (corrected values).

It is easily observable that in this latter example of respiratory metabolism level correction, we proceeded differently from the former instance, maintaining, this time, the average body weight of the respective batch. In this way, the column of "expected" values shows a constant level which should be registered with one and the same temperature (body weight values remaining constant) were it not for a seasonal metabolic rhythmicity independent of the direct action of the thermal factor. Thus, the differences between the two thermal values evidence just the interfer-

ence of seasonal variation when eliminating first the influence of environmental temperature and next, by correction, the impact of individual body weight.

CONCLUSIONS

1) In order to avoid the influence of the individual body weight factor on oxygen consumption level we suggest the following metabolism/body weight dependence relation ($Y = a\,X^b$).

2) Under two distinct experimental conditions (on the one hand, the influence of temperature and of inanition and, on the other hand, of season under conditions of constant temperature) two oxygen consumption correction variants have been devised.

3) Generalization of this procedure is recommended in all cases in which the body weight of test batches could change (or hide) the relations between the metabolism and any environmental factor.

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EFFECT OF ACTH ON CARBOHYDRATE METABOLISM IN THE INDIAN GARDEN LIZARD, *CALOTES VERSICOLOR* (DAUDIN)

BY

D. M. DUBEWAR * and S. A. SURYAWANSHI

Although there is a large number of evidence suggesting the involvement of adrenocorticoid hormones in the control of carbohydrate metabolism in poikilothermic animals [11] relatively less is known about the role of these hormones in reptiles [6], [12], [20]. Administration of cortisol increased the blood glucose level in the lizard [13], [20], snake [7] and alligator [5]. Moreover, an increase in glycogen content of liver is reported in the snake, *Diadophis punctatus* [10].

The existence of a pituitary-adrenal axis has been well established in reptiles [1], [2]. Hypophysectomy produced a decline in blood glucose level in the snake, *Natrix piscator* [16] and the lizard, *Varanus monitor* [15] whereas Suryawanshi and Rangnekar [21] failed to observe any change in blood sugar level in hypophysectomized lizard, *Uromastix hardwickii*. Therefore, this study was carried out in order to examine the effects of ACTH on carbohydrate metabolism in the lizard *Calotes versicolor*.

MATERIAL AND METHOD

Animals weighing between 10—25 g of either sex were obtained from a local commercial source and they were acclimatized to laboratory conditions for a week prior to their use in various experiments; care was taken, as far as possible, to maintain the animals in natural environment. *Mammalian* ACTH (Batch N° 1156, Frederiksberg Chemical Laboratory Ltd., Copenhagen) dissolved in reptilian physiological saline (0.8%) was injected (2 IU/100 g/day) for 20 days. Since there is seasonal variation in the blood composition [4] and adrenal histology [9] of this lizard, parallel control animals were used and injected with equivalent amount of hormone free carrier. Two sets of experiments were conducted in the month of October, 1972 (Temp. 20—30°C) and May, 1972 (Temp. 27—41°C). Day length at the start of experiment in May was 12.30L : 11. 70D and in October 11.45L : 12.55 D.

Animals from both control and experimental groups were slain after 10 and 20 days, respectively. Blood samples were obtained in hepa-

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rinized centrifuge tubes and blood glucose [18] and tissue gly
were measured by employing standard methods. The excised adrenal
gland was weighed and fixed in Bouin's fluid for histological pre
Cell and nuclear diameters of cortical tissue were measured by u
meter (PZO, Poland). Dunnett's procedure [19] was used for t
mination of level of significance.

OBSERVATIONS

The adrenal weight and histology did not show any significant change during winter. Although liver glycogen increased significantly after administration of ACTH, blood glucose remained unaffected (Table 1).

In contrast to this, weight of adrenal, histology and physiological parameters showed conspicuous changes in summer. Though the body weight was not affected significantly, adrenal glands showed an increase in the weight. Both cell and nuclear diameters of cortical tissue were increased significantly, however, chromaffin tissue remained unchanged. Physiological parameters, liver glycogen and blood glucose levels, were elevated significantly after 10 and 20 days, while muscle glycogen did not show any change (Table 1).

DISCUSSION

In the present study mammalian ACTH (2 IU/100 g/day) has no effect on body, weight and adrenal weight in the winter season, whereas the same dose of ACTH resulted in an increase in the adrenal weight in summer. These findings are similar to earlier observations made in lizards [22] and snakes[17]. In contrast to the ability of ACTH to increase hepatic glycogen in winter, no changes were observed in the blood glucose level in the experiment conducted during winter season, while the same dose of ACTH in summer showed a significant increase in hepatic glycogen and blood sugar level. It appears that the action of ACTH requires a higher environmental temperature.

Glycogenic action of ACTH upon the liver glycogen accompanied with increased activity of the adrenal in this lizard is similar to mammals. In mammals, ACTH elevates the circulating blood glucose level *via* glucocorticoid [8] and increases the hepatic glycogen concentration by gluconeogenesis [14].

The results of the present investigation suggest the existence of pituitary-adrenal axis in *Calotes* and it appears that ACTH acts through the adrenocorticosteroids, since the administration of ACTH produces hyperactivity of the adrenal gland. These results are in good accord with the earlier observations made in the reptilian species in which adrenocorticosteroids increased the blood glucose [5],[7],[13] and led to glycogen deposition [10]. Gist [6] also observed an increase in hepatic glycogen after ACTH treatment along with an increase in adrenal weight during summer season, but he did not measure the blood glucose level and hence, it is difficult to know whether ACTH elevated liver glycogen concentration

Table 1

Effect of ACTH on body and adrenal weight, liver and muscle glycogen, blood glucose and adrenal histology of the garden lizard, *Calotes versicolor*

Duration		Winter		Summer	
		Control	Experimental	Control	Experimental
10 days	Initial body wt (g)	18.7 ± 2.6	20.0 ± 2.0 *	13.4 ± 0.8	16.00 ± 2.0 *
	% change in body wt	−2.0 ± 0.36	+1.0 ± 0.2 *	+3.0 ± 1.8	+2.0 ± 1.40
	Adrenal wt (% body wt)	0.021 ± 0.002	0.023 ± 0.001 *	0.018 ± 0.0017	0.022 ± 0.001 *
	Liver glycogen (mg/100 mg)	2.20 ± 0.80	2.10 ± 0.60 *	2.23 ± 0.78	3.28 ± 1.00 *
	Muscle glycogen (mg/100 mg)	0.78 ± 0.06	0.80 ± 0.04 *	1.1 ± 0.10	1.0 ± 0.09 *
	Blood glucose (mg/100 ml)	88.0 ± 6.5	85.0 ± 5.6 *	80.0 ± 4.5	100.0 ± 11.5 *
	Cortical cell diameter (μm)	8.2 ± 1.1	8.5 ± 0.9 *	8.6 ± 0.23	8.2 ± 0.46 *
	Cortical nuclear diameter (μm)	3.4 ± 0.24	3.8 ± 0.40 *	3.6 ± 0.5	4.2 ± 0.23 *
20 days	Initial body wt (g)	22.0 ± 2.4	16.60 ± 1.8 *	24.0 ± 6.0	23.0 ± 8.4 *
	% change in body wt	+4.0 ± 2.1	+2.20 ± 0.8 *	−2.4 ± 0.6	4.0 ± 2.1
	Adrenal wt (% body wt)	0.016 ± 0.007	0.019 ± 0.005 *	0.017 ± 0.002	0.031 ± 0.001 **
	Liver glycogen (mg/100 mg)	2.60 ± 0.16	5.20 ± 1.30 **	2.40 ± 0.60	5.60 ± 0.20 **
	Muscle glycogen (mg/100 mg)	1.20 ± 0.20	0.92 ± 0.32 *	0.86 ± 0.12	0.92 ± 0.10 *
	Blood glucose (mg/100 ml)	88.0 ± 4.5	100.0 ± 8.4 *	82.0 ± 6.2	119.0 ± 5.6 **
	Cortical cell diameter (μm)	7.60 ± 0.87	7.20 ± 0.90 *	6.70 ± 0.20	8.78 ± 0.14 **
	Cortical nuclear diameter (μm)	3.20 ± 0.27	3.90 ± 0.50 *	3.36 ± 0.40	5.60 ± 0.22 **

Five animals were used in each experiment.
* NS
** $P = 0.05$.

with a concomitant increase in blood glucose level in *Caiman sclerops*. In contrast to these results Coulson and Hernandez [5] did not observe any measurable hyperglycemia after ACTH treatment in alligator. These differences may be due to the species specificity or existence of a very weak pituitary adrenal axis in the alligator.

CONCLUSIONS

The effects of the administration of mammalian ACTH for 20 days on carbohydrate metabolism in the garden lizard *Calotes versicolor* are reported. Mammalian ACTH (2 IU/100 g/day) failed to produce any changes in physiological parameters and adrenal histology during winter but the same dose significantly increased the blood glucose and hepatic glycogen levels and also altered adrenal histology during summer. It seems that the mammalian ACTH manifests its action in reptiles at higher temperature. Similarly the present investigation gives further support to the existence of the pituitary adrenal axis in reptiles.

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BIOCHEMICAL CHANGES IN THE THYMUS AND THE SPLEEN OF FEMALE AND NEWBORN RATS FOLLOWING ADMINISTRATION OF AN ANABOLIC STEROID TO MOTHER DURING PREGNANCY

BY

N. BUCUR and A. D. ABRAHAM

An acute or chronic treatment with methyl-androstenediol (Madiol) was applied to both intact adult female and pregnant rats. The thymus and spleen reactivities were studied by the determination of free cholesterol, ascorbate and glycogen content and of the uptake of ^{14}C-acetate by lipids and proteins. Madiol has thymolytic activity in the case of adult female rats. Pseudohypertrophy of the thymus was observed with young animals born of treated mothers. The spleen of treated animals reacts in a different way to the administration of Madiol.

There are only few data in the literature about the effect of anabolic steroids upon lymphatic system and lymphoid organs of adult female animals and upon embryogenesis. Some experiments were made concerning the action of these steroids for elucidating the mechanisms by which androgen steroids and related substances influence the metabolism of proteins, lipids and glucides, particularly in the case of male animals [1], [5], [6], [11].

In this paper we conducted experiments to study the action of 17α-methyl-androstenediol (Madiol) upon the thymus and the spleen metabolism of adult female rats and 21-day-old rats born of mothers treated during pregnancy.

MATERIAL AND METHODS

3–4 months old female Wistar rats kept under standard conditions were used 30 animals were injected i.m. with 5 mg Madiol per day (total dose : 15 mg) (U.M.B. Bucharest). Other 30 animals were treated per os with a total dose of 200 mg Madiol for 28 days. 20 pregnant females were treated with the same dose after separation from males (on the 5th day). Newborn rats were killed at the 21th day after birth. The thymus and the spleen were removed quickly and weighed. The concentration of ascorbate was determined using Klimov's method [4] and the glycogen content with Montgomery's technique [10]. The concentration of free cholesterol and the rate of biosynthesis were measured by the radiochromatographic method using 2–^{14}C-acetate as precursor injected i.p. 1 hour before killing. The isolation of free cholesterol was made on Silicagel F_{254} plates (0.2 mm thickness) purchased from E. Merck (Darmstadt). The spe-

cific radioactivity of lipids and proteins was determined with the aid of a "Betaszint" Liquid Scintillation Spectrometer BF-5003 as described earlier [3].

RESULTS

The acute treatment (3 days) with Madiol determined a significant decrease of the thymus weight (-20.66% ; $p < 0.05$) of adult female rats. Concomitantly the concentration of ascorbate and glycogen decreased from 101.2 ± 3.7 to 88.0 ± 6.2 µg/100 mg wet tissue (13.05%, $p < 0.05$), respectively from 158.4 ± 6.3 to 123.0 ± 8.8 µg/100 mg wet tissue (-18.99% ; $p < 0.01$). At the same time a significant decrease of the glycogen content in the spleen of adult female rats from 199.3 ± 20.65 to 78.9 ± 9.6 µg (-60.43% ; $p < 0.001$) was observed.

After a prolonged per os treatment with Madiol, no significant changes of absolute or relative weight of the thymus or the spleen of adult female rats were observed. Madiol did not influence ascorbate or glycogen content of the thymus and the spleen (Tables 1, 2), but significant modifi-

Table 1

Concentration of ascorbate in the thymus of adult female rats and of young rats born of Madiol treated mothers (µg per 100 mg wet tissue)

	ADULT		YOUNG	
	Control	Treated	Control	Treated
$\overline{X}$	92.41	83.82	79.02	75.62
S.E.	±4.00	±2.01	±2.87	±4.66
n	8	6	8	7
$D\%$	—	−9.24	—	−4.30
P	—	> 0.25	—	> 0.5

$\overline{X}$ = arithmetical media; S.E. = standard error; n = number of experiments;
$D\%$ = per cent difference between control and treated groups;
P = Student P.

Table 2

Concentration of glycogen in the thymus and the spleen of adult female rats and young rats born of Madiol treated mothers (µg per 100 mg wet tissue)

ADULT FEMALES				
	THYMUS		SPLEEN	
	Control	Treated	Control	Treated
$\overline{X}$	136.1	114.8	211.9	226.8
S.E.	±15.3	± 6.8	±10.8	± 9.9
n	11	11	9	9
$D\%$	—	−15.67	—	+7.03
P	—	>0.1	—	>0.25
YOUNG RATS				
$\overline{X}$	191.5	188.3	217.0	177.6
S.E.	±10.9	±13.8	±10.2	±16.1
n	10	10	11	12
$D\%$	—	−1.69	—	−18.13
P	—	> 0.5	—	$0.1 > P > 0.05$

cations were observed concerning the concentration of free cholesterol and its biosynthesis in the thymus of adult or young female rats (Figs. 1 and 2). The incorporation of labelled acetate into lipids and proteins is inhibited by the administration of Madiol in the thymus of adult female rats (Fig. 3) and acetate uptake by lipids of the thymus of young rats born of treated mothers. It is important to note that the thymus weight

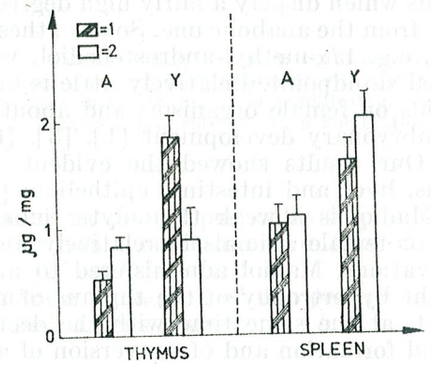

Fig. 1. — Action of prolonged administration of Madiol on the concentration of free cholesterol in the thymus and the spleen of adult female rats (A) and young rats (Y). (1 — control; 2 — after Madiol administration; µg/mg = = µg cholesterol per mg wet tissue).

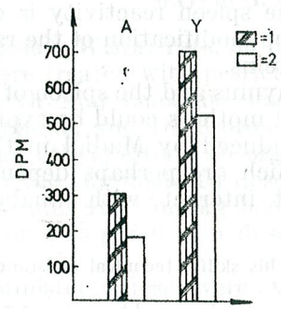

Fig. 2. — Specific radioactivity of free cholesterol isolated from the thymus of adult female (A) and young rats (Y). (1 — control; 2 — after Madiol administration).

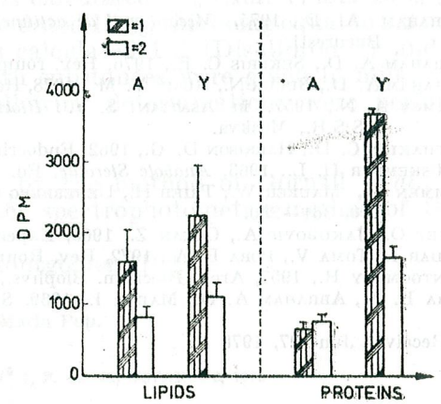

Fig. 3. — Specific radioactivity of lipids and proteins isolated from the thymus of adult female (A) and young rats (Y). (1 — control; 2 — after Madiol administration).

of new-born rats has increased with 17.72% ($p < 0.02$), which indicates a state of pseudohypertrophy, the biosynthesis of proteins and lipids being under inhibition.

DISCUSSION

Chemical modifications of androgen hormones have led to some compounds which display a fairly high degree of dissociation of the androgenic effect from the anabolic one. Some of these hormones can be orally administered, e.g. 17α-methyl-androstenediol, which is a great advantage from clinical standpoint. Relatively little is known about the effect of anabolic steroids on female organisms and about the action of hormonal steroids on embryonary development [1], [3], [6], [7].

Our results showed the evident reaction of adrenals, lymphoid organs, liver and intestinal epithelium [1], [3], which differ function of sex. Madiol is a weak thymolytic substance [1] when administered to male or female animals in relatively high doses. In contrast with these observations, Madiol administered to mothers during pregnancy caused a slight hypertrophy of the thymus of newborn rats, i.e. the increase in weight, at the same time with the decrease of cholesterol biosynthesis, of lipid formation and of conversion of acetate into proteins.

The spleen of adult female rats reacts moderately to the acute or chronic administration of Madiol. The spleen reactivity is characterized by degradation of glycogen without the modification of the rate of uptake of labelled acetate into lipids or proteins.

The different reactivity of the thymus and the spleen of adult female rats and young rats born from treated mothers could be explained taking into account the different changes induced by Madiol in the endocrine system function of these animals which are perhaps dependent on the presence of hormonal receptors that interact with anabolic steroids [1], [6].

Acknowledgements. We thank I. Ilyés for his skilful technical assistance.

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REACTION OF THE THYMUS OF WISTAR RATS TREATED WITH ALAR AND DISULFOTON

BY

RODICA GIURGEA *

Female Wistar rats aged 30 days were treated during 3 respectively 6 months with Alar or Disulfoton, each in two doses. After a 3 months treatment with Alar in the small dose there appears a stress state in the thymus. The modifications in thymus after a 6 months treatment are reduced, probably due to a habituation phenomenon.

The application of an increased number of pesticides to plants and animals has raised in the past few years numerous problems of their action on the mammalian organism [2], [3], [4].

This determined us to investigate the action of two pesticides, Alar and Disulfoton, insufficiently studied in this respect.

MATERIAL AND METHODS

Adult female Wistar rats, 30 days old, maintained under standard conditions, were treated with pesticides for 3 and 6 months respectively, as follows: with Alar (succinic acid 2,2 dimethyl hydrazide) a growth inhibitor for plants, and Disulfoton (phosphorodithionic acid 0,0 diethyl 5 [2-(ethylthio) ethyl] ester), an organophosphorous pesticide.

For Alar the administered dose was 200 mg/kg body weight/day for the small dose and 1000 mg/kg body weight/day for the high dose.

Disulfoton was given in a dose of 22 respectively 110 μg/kg body weight/day.

The administered doses were experimented after a preliminary determination of LD_0 for Alar, which was equivalent to 1000 mg/kg body weight. From this quantity it was calculated 2% (Alar I) and 10% (Alar II). For Disulfoton it has been calculated LD_{50}, this being equal to 2.2 mg/kg body weight, from which it was calculated 1% (Disulfoton I) and 5% (Disulfoton II), respectively. Both substances were given in milk.

After decapitation, the following determinations were performed on the thymus:

— Fresh organ weight (TW);
— Total proteins content (TP), by the method of Gornall *et al.*, [5];
— RNA and DNA content, by the spectrophotometric method of Spirin [10];
— Glycogen content (G), after Montgomery [6].

* With the technical assistance of Maria Pop.

Table 1

Organ weight (TW), and concentration of RNA, DNA, total proteins (TP) and glycogen content (G), in the thymus of control, Alar- as well as Disulfoton-treated rats

Parameters		3 MONTHS TREATMENT					6 MONTHS TREATMENT				
		Control	Alar I	Alar II	Disulfoton I	Disulfoton II	Control	Alar I	Alar II	Disulfoton I	Disulfoton II
TW (mg)	x	352.50	265.75	314.14	382.29	396.81	253.37	273.93	237.57	245.81	289.75
	SE±	25.58	23.24	41.44	21.00	34.22	28.68	43.07	44.27	29.67	107.16
	±%	–	−25	−11	+8	+12	–	+8	−7	−3	+14
	p	–	< 0.02	–	–	–	–	–	–	–	–
RNA (mg/g)	x	27.11	12.76	14.65	15.58	17.68	6.20	8.14	6.76	6.34	9.92
	SE±	4.27	0.87	0.93	0.94	0.89	0.56	0.56	0.57	0.20	1.17
	±%	–	−53	−46	−43	−35	–	+31	+9	+2	+60
	p	–	< 0.01	< 0.02	–	< 0.05	–	< 0.01	–	–	< 0.01
DNA (mg/g)	x	6.24	8.17	6.47	6.84	11.89	9.54	10.38	11.80	10.87	11.53
	SE±	1.19	0.63	0.87	0.34	2.38	0.67	0.98	0.40	0.85	0.70
	±%	–	+30	+3	+9	+90	–	+8	+17	+13	+20
	p	–	–	–	–	< 0.05	–	–	< 0.02	–	< 0.02
TP (mg/g)	x	187.88	183.20	150.30	188.67	159.02	174.56	172.57	171.66	173.92	173.41
	SE±	7.97	6.12	9.97	11.84	6.97	17.68	13.86	5.21	10.94	9.04
	±%	–	−3	−21	0	−16	–	−2	−2	−1	−1
	p	–	–	< 0.01	–	< 0.02	–	–	–	–	–
G (mg/ 100γ)	x	53.00	65.00	59.00	45.00	44.00	67.00	55.00	68.00	75.00	84.00
	SE±	1.51	4.54	4.79	2.48	1.63	4.28	2.83	3.90	3.90	8.92
	±%	–	+22	+11	−16	−17	–	−18	+1	+11	+25
	p	–	< 0.01	–	< 0.02	< 0.01	–	< 0.01	–	–	$0.05 < p < 0.01$

Alar I = 200 mg/kg body weight/day; Alar II = 1000 mg/kg body weight/day; Disulfoton I = 22 μg/kg body weight/day; Disulfoton II = =110μg/kg body weight/day. x = mean values; SE ± = standard errors; p = statistic significance; ± % = percentage differences against the control values, For each group of 8 animals,

RESULTS AND DISCUSSIONS

The results were expressed as percentages against the mean values of the control. Student's "t" test was used to estimate the statistical significance of differences (Table 1).

The first observation refers to the toxicity of the two pesticides; Alar has a very low toxicity, while Disulfoton has a high one [11].

Nevertheless the small dose of Alar given daily for 3 months induces involutive phenomena in the thymus. So the thymus weight decreases with 25% ($p < 0.01$) and the glycogen content increases with 22% ($p < 0.01$) in comparison with the control group.

The increase of the glycogen content in a lymph organ which involutes under the action of different stress factors is a known phenomenon [8], [9].

The increase of the glycogen content in a lymph organ which involutes under the action of different stress factors is a known phenomenon [8], [9].

However, in the high dose group the modifications are in the same sense but substantially milder.

For Disulfoton in both doses the glycogen content in thymus is significantly decreased.

In what concerns the variation of the thymic proteins after a 3 months treatment there is a decrease only after high doses of both substances.

A common phenomenon in all groups is a significant RNA decrease that may be due to an activity inhibition of the RNA-polymerase in thymus [1]. The increase of the DNA content after a 3 months treatment with Disulfoton is difficult to interprete.

Another observation can be made: After a 6 months treatment, the thymus presents very reduced modifications and these are generally of inverse sense than after 3 months. This may be due to an accomodation process, but the intervention of the age of animals is also very likely. Popov [7] in a recent paper has reported about habituation phenomena after treatment with some pesticides.

The way of action of these substances is difficult to establish. It is however very presumably that they act directly on the thymus and not *via* the adrenals, as the ascorbic acid content of the latter has been found to be increased in all the treated groups [11].

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CHANGES OF NUCLEIC ACID AND PROTEIN METABOLISM OF THE THYMUS AND THE LIVER BY X-IRRADIATED A2G MICE AFTER TREATMENT WITH MADIOL

BY

ŞTEFANIA MANCIULEA, MARIA BORŞA and A. D. ABRAHAM

Male A2G mice were irradiated with 200 R or pretreated with Madiol and irradiated. After 1 or 14 days the animals were killed, and DNA, RNA and protein concentration of the thymus and the liver were measured. Concomitantly the rate of incorporation of ^{14}C into proteins from 2-^{14}C-acetate was determined. The results obtained showed a radioprotector effect of Madiol especially on the thymus level, where the stimulation of recovery systems was observed.

Recent findings have shown that ionizing radiations cause irreversible changes in the animal organism. Radioprotectors are known as chemical substances which have the capacity to prevent or decrease the effect of radiation energy. A great number of radioprotectors (about 15 000) have been discovered and studied, but most of them exerted toxic effects in the doses administered [6] [7].

In this paper, we have studied the action of a nontoxic drug, Madiol, which is an anabolizing steroid [4], [10]. This steroid exerts a proteins anabolic effect and concomitantly lymphotropic actions on the level of the thymus and small intestine [5].

It is worth mentioning that the thymus and the liver must be regarded as "target-organs" for steroid hormones, containing specific receptors for androgen steroids [2], [4], [12].

MATERIAL AND METHODS

Male A2G mice weighing 30—40 g were kept under standard conditions and with food and water *ad libitum*. Madiol (17-β-methyl-and-rosta-5-ene 3-α,17α-diol) (Biofarm, Bucharest) was administered per os through 30 days in a total dose of 30 mg (per 100 g b.w.). Animals were irradiated with 200 R (with the aid of a radiotherapeutical apparatus type TUR, 180 kV, 10 mA, 1 mm Cu-filter, focus-skin distance of 40 cm).

The groups of animals were: control group (N), Madiol-treated group (M), irradiated group killed 1 day after exposure (IR-1d) and after 14 days (IR-14d). Other groups were pretreated with Madiol and irradiated and killed after 1 day (M-IR-1d) and 14 days (M-IR-14d) of exposure. It was determined the concentration of DNA and RNA in the thymus [1]

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and in the liver [14], and the concentration of proteins [11]. The rate of incorporation of radiocarbon from 2-^{14}C-acetate into proteins (injected 1 hr before killing) was measured with the aid of a Liquid Scintillation Spectrometer type Betaszint BF-5003.

RESULTS AND DISCUSSION

Treatment with Madiol determined a significant decrease of DNA and RNA concentration in the thymus (Fig. 1). RNA/DNA ratio has increased suggesting a diminution of DNA concentration, which is probably in

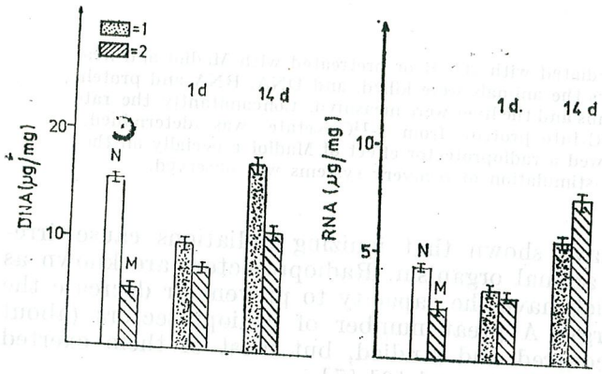

Fig. 1. — Changes of the concentration of DNA and RNA in the thymus of Madiol-treated mice (M), irradiated mice (1) and Madiol-pretreated and irradiated mice (2) as against the control (N).

relation with the release or destruction of small lymphocytes in the thymic cortex. Irradiation has caused same effects on the thymus level (Fig. 1) demonstrated by other authors too [8], [9], [16].

Administration of Madiol to irradiated mice after 1 day of exposure (M-IR-1d) caused no significant changes in comparison with the IR-1d group, but the concentration of nucleic acids was appropriated to normal levels by the IR-14d and M-IR-14d groups.

Our results concerning the rate of incorporation of radiocarbon from 2-^{14}C-acetate into thymic proteins confirmed that regeneration processes take place after administration of Madiol. The specific radioactivity of proteins increased by the IR-1d group (1247 $\pm$ 101 DPM per 100 mg proteins) and the M-IR-1d group (1002 $\pm$ 93 DPM) as against the control group (497 $\pm$ 36 DPM); a decrease of specific radioactivity by the IR-14d group (624 $\pm$ 125 DPM) and a significant increase by the M-IR-14d group (1646 $\pm$ 146 DPM; $p < 0.001$) were observed.

In the liver, which is considered an organ relatively radioresistant, no significant changes were observed after irradiation or after treatment with Madiol and irradiation (Fig. 2). The results concerning DNA, RNA and protein concentration showed no modifications after exposure to 200 R, or after treatment with Madiol. In contrast with these results a significant increase of the rate of conversion of acetate into proteins was observed in the case of IR-1d and M-IR-1d groups (+52% respectively 70.74%), as against the control (N). By the groups IR-14-d and

M-IR-14d significant decreases, of 50.5% ($p < 0.001$) and respectively 57% ($p < 0.001$) of the rate of conversion were observed, which suggest significant changes in the activity of liver enzymes after this period of time.

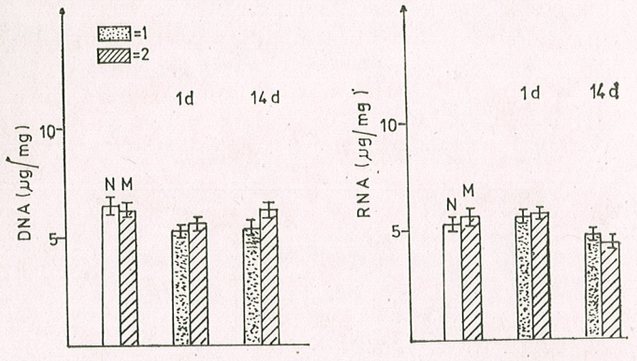

Fig. 2. — Changes of the concentration of DNA and RNA in the liver of Madiol-treated mice (M), irradiated mice (1) and Madiol-pretreated and irradiated mice (2) as against the control (N).

Our results showed that administration of Madiol to irradiated animals could prevent some biochemical alterations induced by radiation energy at the level of the thymus and the liver, by its stimulatory effects exerted on the cellular recovery systems [3], [13], [15].

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L'EFFET DE LA LEUCOTROPHINE SUR LE REPEUPLEMENT DE LA RATE AVEC DES CELLULES FONCTIONNELLES CHEZ LES SOURIS IRRADIÉES

PAR

MARIA SENA CRIVII, Z. URAY, MONICA CRIȘAN et ROZALIA AJTAI

Using the migration inhibition test *in vitro* in irradiated mice we have shown the decrease of migration capacity of cells in the splenic explant and the lack of migration inhibition factor (MIF).
The animals treated with leucotrophine 6 days after irradiation exhibit the repopulation of the spleen with functional cells, as shown by the increase of migration capacity and the presence of the migration inhibition factor.
The behaviour of irradiated and leucotrophine treated animals was similar with that of control nonirradiated and treated animals.

Le test de l'inhibition de la migration des leucocytes est souvent employé pour la détection de l'immunité cellulaire chez l'homme et chez les animaux. Ainsi, il est appliqué dans l'étude de l'hypersensibilité retardée [3], [6], l'autoimmunité [1], l'immunité antitumorale [9], l'immunité de transplant.

Certains auteurs ont trouvé que les différents extraits du thymus ont un effet stimulateur sur la lympho-leucoérythropoïèse et sur l'immunité [7], [13]. Des études récentes viennent de montrer que la leucotrophine, extrait protéique du thymus, a un effet radioprotecteur et stimulateur sur la leucoérythropoïèse [2], [11]. Sur la base de ces données, nous avons utilisé le test de l'inhibition directe de la migration des cellules spléniques pour obtenir des indications cytophysiologiques sur les éléments cellulaires impliqués dans ce procès, à la suite d'un traitement des souris irradiées avec de la leucotrophine.

MATÉRIEL ET MÉTHODE

Nous avons effectué les expériences sur des souris DBA, femelles, d'un poids de 20 ± 1 g, soumises à un régime standard.
Les animaux ont été partagés en 4 lots :

(a) témoins non traités
(b) témoins traités
(c) irradiés non traités
(d) irradiés traités.

L'irradiation a été effectuée en boîtes spéciales de plastique sous un appareil de radiothérapie TUR-I (180 kv, 10 mA, filtre 1 Cu, DEP =

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= 40 cm, débit 23 R/min), la dose totale étant de 400 radiations gamma. Après l'irradiation les animaux ont été injectés i.p. avec 0,2 ml leucotrophine (coresp. à 50 mg de glande fraîche), pendant 6 jours. Trois jours après, les animaux ont été sacrifiés.

Le test de l'inhibition directe de la migration a été effectué d'après la méthode de Sandru G. [12]. On a employé le milieu de culture IC-65, enrichi en sérum homologue inactivé à 56°C, 30 minutes, des antibiotiques et de la leucotrophine en concentration de 250 μg/ml.

Après la projection des surfaces de migration sur papier Wattman 1, avec un microscope MC-1, les résultats exprimés en mg ont été calculés pour leur signification statistique par le test «t» de Student.

L'index de la migration a été calculé d'après la formule suivante :

$$IM = 100 \times \frac{\text{surface de migration en présence de la leucotrophine}}{\text{surface de migration du témoin}}$$

La positivité du MIF commence pour IM $< 80\%$.

On a introduit également le coefficient de corrélation qui peut nous montrer si les deux phénomènes observés (la migration et l'inhibition de la migration) sont interdépendants.

RÉSULTATS ET DISCUSSIONS

Les résultats obtenus sont présentés dans le tableau 1 et les figures 1 et 2.

Le tableau 1 montre que les témoins non traités ont une moyenne des surfaces de migration de $\bar{x} = 159{,}69$ mg et l'index de migration IM = 103,34 % n'indique aucune influence significative ($p > 0{,}05$) si la leucotrophine est ajoutée au milieu de culture.

Les témoins traités pendant six jours ont une moyenne de 160,99 mg et IM = 68,9 %, ce qui peut indiquer que le facteur de l'inhibition de la migration (MIF) a été sécrété, c'est-à-dire qu'il s'agit d'une réponse lymphocytaire splénique ($p < 0{,}02$).

Les souris irradiées et non traitées présentaient des surfaces de migration diminuées, $\bar{x} = 53{,}99$ mg, ce qui montre une déplétion en cellules migratoires et/ou le baissement de la capacité physique de migration. De plus, chez ces animaux l'inhibition de la migration n'est pas présente ($\bar{x} = 56{,}83$, IM = 107,49 %, $p > 0{,}5$).

Les cellules spléniques des animaux irradiés et traités à la leucotrophine présentent une capacité de migration augmentée $\bar{x} = 119{,}72$, ainsi que la sécrétion de la lymphokine respective (IM = 60,78 %, $p < 0{,}01$), qui est mise en évidence par la présence de l'inhibition.

Ce phénomène peut être mieux étudié si on calcule les coefficients de corrélation entre les surfaces de migration en absence et en présence de la L, ainsi que l'aspect de la pente de régression linéaire. De cette manière nous avons obtenu des coefficients de corrélation très près de 1 (0,91—0,99), ce qui dénote l'interdépendance entre les deux phénomènes.

Tableau 1

La moyenne de la migration, l'index de migration (IM), le coefficient de corrélation et la pente de regression

GROUPE	La moyenne de la migration *		L'index de migration (IM)	Le coefficient de corrélation «r» et la pente de régression
	Sans L	Avec L		
1. Témoins non traités (n = 5)	215,83 * 215,00 86,66 102,33 178,66	225,50 192,00 102,66 108,25 176,33	104,48 89,30 118,46 105,78 98,69	r = 0,975 ($p < 0{,}01$) y = 0,847 x + 25,80
	$\bar{X}$ = 159,69 ES = ±27,63	$\bar{X}$ = 160,94 ES = ±24,08	$\bar{X}$ = 103,34 ES = ±4,77	
	t = 0,034	p > 0,5		
2. Témoins traités à la leucotrophine (n = 5)	197,33 188,00 145,33 128,66 145,66	140,00 131,66 97,00 95,33 91,33	70,94 70,03 66,74 74,09 62,70	r = 0,964 ($p < 0{,}01$) y = 0,738 x − 7,77
	$\bar{X}$ = 160,99 ES = ±13,40	$\bar{X}$ = 111,06 ES = ±10,26	$\bar{X}$ = 68,90 ES = ±1,94	
	t = 2,96	p < 0,02		
3. Irradiés non traités (n = 6)	48,33 42,66 38,33 39,00 80,33 75,33	50,66 45,00 47,00 44,66 77,66 76,00	104,82 105,48 122,61 114,51 96,67 100,88	r = 0,99 p < 0,001 y = 0,822 x + 12,436
	$\bar{X}$ = 53,99 ES = ±8,41	$\bar{X}$ = 56,83 ES = ±6,98	$\bar{X}$ = 107,49 ES = ±3,88	
	t = 0,28	p > 0,5		
4. Irradiés traités à la leucotrophine (n = 6)	83,33 85,00 138,00 147,00 131,66 133,33	48,33 53,33 91,33 75,33 84,33 83,33	57,99 62,74 66,18 51,24 64,05 62,49	r = 0,907 ($p < 0{,}01$) y = 0,574 x + 4,065
	$\bar{X}$ = 119,72 ES = ±11,49	$\bar{X}$ = 60,78 ES = ±7,26	$\bar{X}$ = 60,78 ES = ±2,20	
	t = 3,94	p < 0,01		

* — chaque valeur représente la moyenne pour 3 surfaces de migration ; $\bar{X}$ — la moyenne ; ES — l'erreur standard ;

La figure 2 nous montre que chez les animaux non traités à la leucotrophine les pentes de régression sont parallèles et disposées sous un angle de $\cong 40°$.

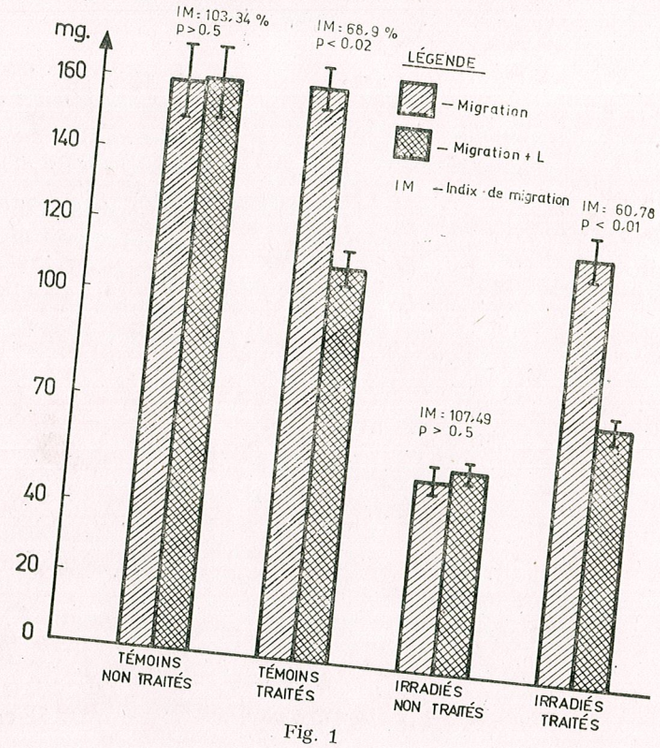

Fig. 1

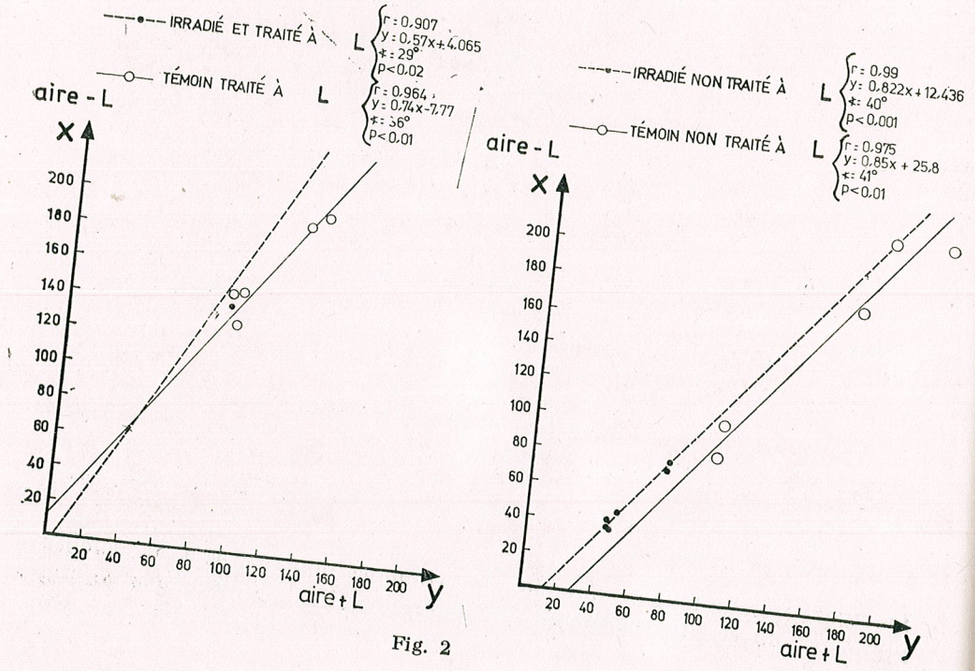

Fig. 2

Au cas des animaux traités le coefficient de corrélation est statistiquement significatif, mais d'après la pente de régression on peut observer que les animaux traités répondent plus fortement au traitement ($\measuredangle = 29°$, $p < 0{,}01$) que les animaux non irradiés ($\measuredangle = 36°$, $p < 0{,}02$).

Basés sur les données de la littérature nous pouvons supposer que l'augmentation de la surface de migration peut être provoquée par l'augmentation du nombre de cellules migratoires d'une part et par l'augmentation de leur capacité physique de migration d'autre part.

Nous pouvons corréler nos données avec celles de Rădulescu et collab. [11] et Uray [14], qui ont constaté dans la rate des souris irradiées une déplétion lymphocytaire accentuée, qui a comme suite la diminution et même la disparition des follicules lymphoïdes et l'estompation de la structure caractéristique de l'organe. Ces animaux présentent une petite capacité de migration (33,80% de la valeur du témoin). Le traitement à L apporte la restauration partielle du follicule lymphoïde et dans la poulpe rouge on voit de nouveau les lymphocytes; en même temps on observe la présence de centres de myélopoïèse et érythropoïèse. Kiricuta et collab. [2] et Coucourde [4] ont montré que la L a une action antileucopénique chez les malades.

La présence de l'inhibition de la migration est une preuve que le MIF a été élaboré par les lymphocytes venus pour le repeuplement de la rate des animaux irradiés. Ainsi, sous l'aspect de l'immunité cellulaire le test de l'inhibition directe de la migration peut indiquer la fonctionnalité des cellules impliquées, c'est-à-dire les granulocytes et les macrophages comme des éléments migratoires, d'une part, et les lymphocytes comme des éléments qui produisent le MIF, d'autre part.

On connaît également que la leucotrophine, tout comme la thymosine, [10] [7] stimule chez les cancéreux et chez les adultes normaux les lymphocytes T, et que la thymostérine [5] stimule les lymphocytes B chez les souris thymectomisées à la naissance.

CONCLUSIONS

Par le test de l'inhibition directe de la migration des cellules spléniques nous avons constaté :

— l'augmentation des surfaces de migration chez les souris irradiées et traitées à la leucotrophine ;

— la présence de l'inhibition de la migration chez les souris irradiées et traitées ;

— ces données peuvent être interprétées commes des épreuves en ce qui concerne l'effet de la leucotrophine sur le repeuplement de la rate des souris irradiées avec des cellules fonctionnelles.

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MODIFICATIONS MÉTABOLIQUES DU CERVEAU DES RATS BLANCS APRÈS L'ÉTABLISSEMENT D'UN COMPORTEMENT D'ÉVITEMENT

PAR

M. POP et A. D. ABRAHAM

Metabolic modifications in the brain of white rats, after stabilization of an avoidance behaviour. Some metabolic values were determined in the brain of white rats after 10-days of systematic training on an avoidance conditioned reflex. Conditioned rats showed, versus controls, an increased amount of labeled acetate incorporated in proteins, fats and acid soluble substances of the supernatant fluid of the brain tissue. A correlation was put into evidence between the acetate incorporation in brain proteins and the rate of stabilization of reflexes. An analogous correlation with the incorporation into brains lipids was uncertain.

Les activations du système nerveux central entraînent des modifications plastiques et métaboliques des neurones et des synapses [3] [4] [5] [9] [15]. Ces modifications sont particulièrement importantes en relation avec l'apprentissage et la mémoire. On cite souvent les implications de la synthèse de RNA et des protéines cérébrales dans les mécanismes de la mémoire [2] [7] [8] [10] [11] [12] [14] [16] [17]. D'autres modifications d'un caractère plus général sont également signalées en rapport avec l'apprentissage des animaux [13] [16] [17] [18] [23].

Les recherches de Hydén et Lange (1972), de Kazakhashvili (1974), Shumskaya et Korochkin (1975) et de Segal et Mats (1978) [14] [20] [21] ont établi que les activations métaboliques des structures cérébrales hippocampiques sont plus nettes dans la première étape de l'apprentissage des rats. Vraisemblablement, le nombre plus grand d'erreurs en sont la cause [14]. De toute façon, les modifications électriques de l'hippocampe pendant le conditionnement des chats marquent cette étape [1] [7].

Le but de nos expériences était d'établir certaines modifications métaboliques du cerveau des rats blancs après l'établissement complet (automatisation des réponses) d'un comportement d'évitement et de saisir les éventuelles corrélations avec les performances individuelles des rats.

MATÉRIEL ET MÉTHODES

Nos investigations ont porté sur 12 rats blancs ♂ de 200—220 g. Six rats ont été entraînés systématiquement dans une cage de réflexes conditionnés pendant 10 jours. Le stimulus conditionnel (SC) était une sonnette dont l'action précédait de 5 sec le courant électrique envoyé

au plancher de la cage. L'intervalle entre les tests était de 60 sec et la durée totale d'une séance expérimentale d'une heure.

Après 7 séances expérimentales (1 h), le comportement d'évitement est stabilisé. Des entraînements sans renforcements ont été poursuivis jusqu'au 10^{e} jour en comptant dès le 1er jour de nos expériences. Les rats ont été sacrifiés par décapitation et les cerveaux (sans cervelet et lobes olfactifs) ont été prélevés pour les analyses biochimiques.

La technique consiste à comparer la radioactivité spécifique de l'acétate ^{14}C dans les protéines, lipides et substances acidosolubles du surnageant liquide du tissu cérébral après injection intrapéritonéale de l'acétate ^{14}C, une heure avant la sacrification des animaux. On a évalué la radioactivité spécifique des substances isolées par la technique de la scintillation liquide *(Betaszint, BF — 5003)*. Les polypeptides libres ont été déterminés par la méthode de Gornall et collab. [6].

RÉSULTATS ET DISCUSSIONS

Il résulte d'une première analyse que le temps de stabilisation des réflexes conditionnés est très différent chez les divers rats. Comme critère de classification nous avons choisi le nombre total des séances nécessaires pour aboutir à la stabilisation des réponses (leur automatisation) multipliés par le nombre des réponses positives de chaque individu.

Les analyses biochimiques montrent une élévation du taux de l'incorporation de l'acétate radioactif dans les protéines, lipides et substances acidosolubles du surnageant liquide du tissu cérébral par rapport aux témoins. La concentration des polypeptides libres est aussi augmentée (tableau 1).

Tableau 1

Modifications biochimiques dans les cerveaux des rats conditionnés, par rapport aux témoins

	Radioactivité du surnageant DPM/lg tissu	Concentration des polypeptides libres. micro g/lg tissu	Radioactivité spécifique des protéines. DPM/100 mg	Radioactivité spécifique des lipides. DPM/100 mg
TÉMOINS	$\overline{X} = 391{,}6$ $\pm$ 15,7	$\overline{X} = 2\,365$ $\pm$ 52	$\overline{X} = 292{,}1$ $\pm$ 39,3	$\overline{X} = 707{,}6$ $\pm$ 78,7
CONDITIONNÉS	$\overline{X} = 543{,}7$ $\pm$ 39,2 $\%$ + 38,84 $P < 0{,}001$	$\overline{X} = 2\,480$ $\pm$ 121 $\%$ + 4,86 $P > 0{,}1$	$\overline{X} = 498{,}0$ $\pm$ 67,0 $\%$ + 70,49 $P < 0{,}02$	$\overline{X} = 757{,}2$ $\pm$ 58,6 $\%$ 7,01 $P > 0{,}1$

Les résultats tendent à montrer l'existence d'une corrélation négative significative ($r = 0{,}79$; $p = 0{,}05$) entre les variations des performances individuelles et le degré de l'incorporation de l'acétate dans les protéines cérébrales des rats conditionnés. L'existence d'une corrélation positive suggère également l'incorporation de l'acétate dans les lipides, mais elle n'est pas statistiquement significative pour le nombre d'individus étudiés ($r = 0{,}56$; $p > 0{,}05$).

Toute une série d'expériences montre que la synthèse de RNA et des protéines dans l'hippocampe des rats conditionnés est liée surtout à la première phase de l'élaboration d'un conditionnement ; les premiers 6—7 jours [14] [20], ou bien, même la première séance d'un conditionnement alimentaire [21]. Certains auteurs n'ont pas remarqué de modifications significatives du taux de l'acétylcholinestérase et butyrylcholinestérase dans le cerveau des rats conditionnés, même après 10 jours d'entraînement [13].

Nos résultats mettent en évidence certaines modifications métaboliques dans les cerveaux des rats après 10 jours d'entraînement en un comportement d'évitement (3—4 jours après stabilisation complète des réflexes). les modifications montrent des variations individuelles dont certaines sont en corrélation avec la vitesse de l'acquisition des réponses automatisées stables (sans aucun renforcement).

L'évidence de la corrélation négative entre les performances des divers rats (vitesse d'acquisition des réponses conditionnées stables) et l'incorporation de l'acétate dans les protéines du cerveau, constatée dans nos expériences tient selon toute vraisemblance à l'intensification de la synthèse des protéines pendant la phase d'élaboration des réflexes conditionnés dont la durée est différente d'un individu à l'autre. Plus

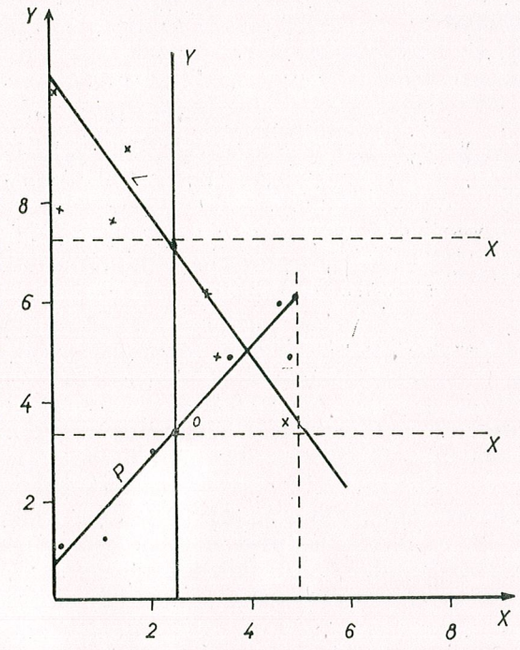

Fig. 1. — Corrélation des performances comportementales des rats blancs avec l'incorporation de l'acétate ^{14}C dans les protéines (P) et lipides (L) du cerveau. X = les performances en ordre décroissant ; Y = l'incorporation de l'acétate radioactif dans les protéines et les lipides cérébrales (courbe de régression).

la phase de stabilisation des réflexes est brève, plus l'incorporation de l'acétate dans les protéines cérébrales, enregistrée au bout de nos expériences (10 jours), est relativement faible (fig. 1).

Nos expériences ne permettent toutefois pas de conclure si les autres modifications enregistrées ont la même signification.

En résumé, l'automatisation du comportement d'évitement des rats blancs est accompagnée par certaines modifications biochimiques du cerveau. Une corrélation négative entre l'incorporation de l'acétate radioactif dans les protéines cérébrales et le degré des performances individuelles a été enregistrée. Cette corrélation semble être due à la durée différente de la phase d'élaboration des réflexes conditionnés des divers rats en rapport avec le nombre total des séances d'entraînement (10 jours).

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THE CYTOGENETIC EFFECTS OF ETHIDIUM BROMIDE (EB) *IN VITRO* AND *IN VIVO*

BY

LILIANA GEORGIAN and ZOLTAN LENGHEL

The *in vitro* and *in vivo* treatments with ethidium bromide (EB) were performed on the peripheral human blood cultures and respectively on the bone marrow cells and Guerin T_8 ascite cells of Wistar rats.
Corresponding to the drug administration during the last 2—6 hr before cell harvesting the chromosome crossbanding has been observed both in the *in vitro* and in the *in vivo* treatments. The possible significance of these observations is discussed.

INTRODUCTION

Since the direct action of ethidium bromide (EB) on the nucleic acids has been described [1],[8], the possible genetic effect of this drug has become of great interest. A clear chromosome crossbanding has been observed in the mitotic chromosomes of the *in vitro* treated human and mammalian cells [2],[4], and also in early meiotic prophase of the *in vivo* treated Chinese hamster males [9]. Moreover, the continuous induction of chromatid lesions was described in the *in vitro* treatments, even though the drug has been removed from the medium [5]. Also, the presence of EB significantly enhanced the mutagenic activity of methylmethanosulphonate in barley [6].

MATERIAL AND METHODS

The *in vitro* treatments with EB, 2 ; 5 ; 10 and 20 μg/ml were applied to human lymphocyte cultures. The drug was added for 24 ; 6 ; 4 ; and 2 hr before fixation in the 72 hr cultures. Colchicine treatment (5 μg/ml) was followed by the hypotony with a KCl 0.75 p.c. solution in distilled water for 12—14 minutes. Three successive fixations were performed in a mixture of methanol-acetic acid, 3/1, for 30 minutes at 4°C. The flame dried slides were coloured with Giemsa.

The *in vivo* treatments with BE, 10 μg/g.b.w., and respectively 20 μg/g.o.w., intraperitoneally administered were performed on the Wistar rats bearing T_8 Guerin ascite tumour 2,4 and 6 hrs before fixation. The chromosomes preparations from the bone marrow cells and from the ascite cells have been obtained.

Colchicine treatment (5 μg/g.b.w.) for 90 minutes was followed by hypotony with KCl 0.75% in distilled water, 35 minutes for the bone

marrow cells and with distilled water 45 minutes for the ascite cells. The fixations, slide obtaining and coloration were the same as for the blood cultures cells. As in the *in vivo* experiments, in bone marrow cells and in the ascitic cells, in the *in vitro* experiments chromosome observations were also made in treatments of 2,4 and 6 hr before fixations without colchicine, and also with colchicine.

RESULTS

In the *in vitro* experiments, the tested doses were 2.5 ; 5 ; 10 ; 12.5 μg BE/ml culture medium. These doses applied to 20 human blood cultures, 24 hrs before fixation did not induce any chromosome aberrations comparatively to the 5 control cultures. The dose of 10 μg BE/ml applied for 2 ; 4 ; and 6 hrs before fixation in 15 blood cultures induced a clearly drawing band-like sequence on the chromosomes with uncoiled, stretched appearance of chromatid when colchicine was also supplied. The same doses given at the same laps of time, without colchicine treatment induced the arrest of the cell cycle at the prophase.

The *in vivo* treatments on Wistar rats were performed with doses of 10 and 20 μg/g.b.w., intraperitoneally applied to 20 normal rats and 20 rats bearing T_8 Guerin ascite for 2 ; 4 ; and 6 hrs before fixation. Similar experiments have been made with and without colchicine treatment. As in the *in vitro* experiments, in the bone marrow cells and in the ascite cells stretched, decondensed chromosomes with the like-band sequence of morphological differences along the chromatid have been observed. In the experiments performed without colchicine treatment the cell cycle inhibition at prophase was also observed.

DISCUSSIONS

Due to its interpolation on the DNA molecules and also to its inhibitory action on the DNA and RNA synthesis, ethidium bromide (EB) could be able to affect the chromosomes by two ways : (a) by attaching itself to DNA molecule, it could structurally disturb the normal relationship within the chromosomal nucleo-protein complexes, and (b) by inhibiting the DNA dependent RNA synthesis, EB could prevent also the synthesis of certain chromosome proteins, necessary for chromosome preparation for mitosis. These two possible mechanisms were supposed as a result of consecutive cytogenetic observations after the *in vitro* administration of EB and other drugs, related by their action, on the mammalian and human chromosomes [2]; [4].

Previous experiments on the plant *Scilla sibirica* [3] and on the cultured Chinese hamster cells and human cells [4] had revealed the appearance of R bands on the chromosomes consecutively to the EB treatments. In our experiments the stretched appearance of metaphase chromosomes might be due to the maintenance of a coiling degree corresponding to the prophase, after the cell spindle breakage. Possibly this effect is not the same on the whole length of chromosomes, thus inducing the

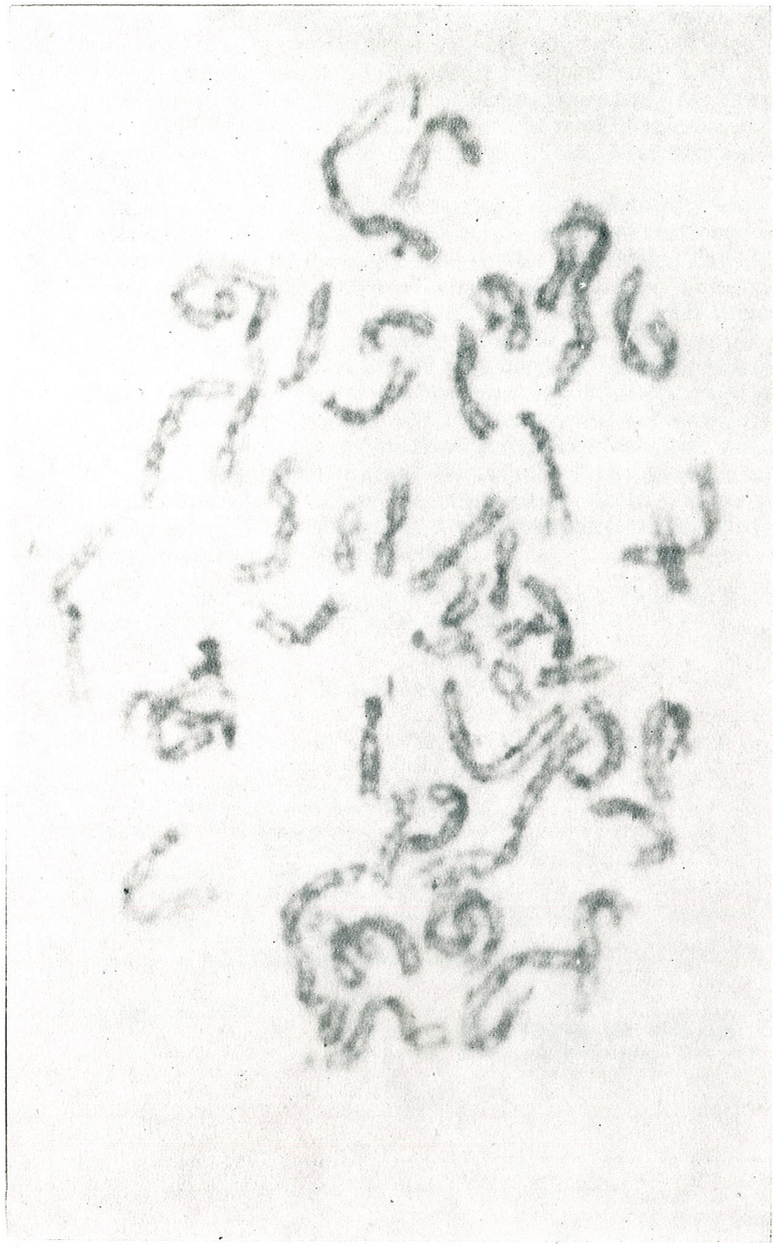

Fig. 1. — Metaphase from the ascite Guerin T_8 *in vivo* treated with ethidium bromide (EB); the band-like differentiations along the chromatids are evident.

band-like differentiations along the chromosomes. The *in vitro* BUdR induced crossbanding, considered by Zakharov [11] to be the effect of the differential action of the drug on the interphase DNA, could present possible similarities with our own observations. The cell cycle arrest in prophase observed by us as well in the *in vitro* and in the *in vivo* experiments without colchicine could be the result of interfering with the synthesis of certain chromosomal protein constituents, necessary for the normal onset of mitosis [4]. Thus, the prophase arrest results from a defective chromosome condensation induced by the physical interpolation of BE in DNA molecules [7].

In our *in vivo* and *in vitro* experiments, the crossbanding was observed only in the 6; 4 and 2 hr treatments before cells harvesting, i.e. when the drug has been added in the G_2 phase of the cell cycle. The fact that the BE induced crossbanding was observed *in vitro* and *in vivo* in animals from different species [3]; [4]; [5] in mitotic [4]; [5] and meiotic [9] chromosomes in normal and neoplastic cells (in our own experiments) provides an incontestable evidence for the general character of the phenomenon. The observation that the *in vitro* BE addition continuously induced chromatid lesions even though the drug was removed from the medium [5] permits the supposition that the BE interaction with chromosomal DNA also affects the normal behaviour of the genetic material. All these observations lead us to the supposition of the same possible correlations between the crossbanding, the physiology of the chromatine and chromosome damaging susceptibility.

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SEASONAL VARIATIONS IN THE FEEDING ACTIVITY OF THE MILLIPEDE *IULUS SCANDINAVIUS* (LATZEL) IN NATURAL HABITAT *

BY

A. M. KHEIRALLAH

The faecal pellets produced by a population of *Iulus scandinavius* from natural habitat showed that this millipede is most active in litter breakdown during autumn. Feeding activity decreased in the other seasons but was more pronounced in winter than in spring and summer. This variation in feeding activity is associated with temperature and precipitation of the Woodland.

1. INTRODUCTION

Variation in the activities of millipedes in the field has been studied by Barlow [2], [3]. According to Barlow the physical factors of the environment are more influential ones, however, other factors such as hibernation and reproduction periodicity are probably primarily responsible for the incidence of activity variation. Banerjee [1] noted an association of locomotor activity with the breeding cycle in the millipedes he studied.

As far as I am aware no work has been performed on seasonal activity in feeding behaviour of millipedes in the field; all the previous studies were made in the laboratory. The purpose of the present paper is to examine the seasonal variations in the feeding activity of a population of *Iulus scandinavius* in the field.

METHODS

Various stadia of *Iulus scandinavius* were collected by hand from the grounds of mature sycamore-ash Woodland, Cheshire once each month during 1964 and part of 1965, as near to the middle of the month as possible and two hours before sunset. Preliminary observations had shown that the bulk of faecal pellets were produced during this twelve-hour period. Each animal caught was immediately placed in a separate tube lined with tap-water agar and closed with a cork, and in the laboratory they were placed at a constant room temperature of 16°C and left to empty the gut contents.

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* The research was carried out within the Department of Zoology, University of Manchester, England

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Discrimination of the various stadia has been done according to Blower and Gabbutt [5]. All the stadia of *Iulus scandinavius* are not present at all times of the year [6]. In order to study the seasonal changes in feeding activity all stadia were included which could be found in most of the months of the year. Accordingly, adult male, eleventh stadium female, ninth stadium (males and females) and eighth stadium (males and females) were taken.

RESULTS

Table 1 shows the monthly variation in the number of faecal pellets produced by the various stadia. The largest number was produced in September, October and November. Fig. 1 indicates the fluctuations

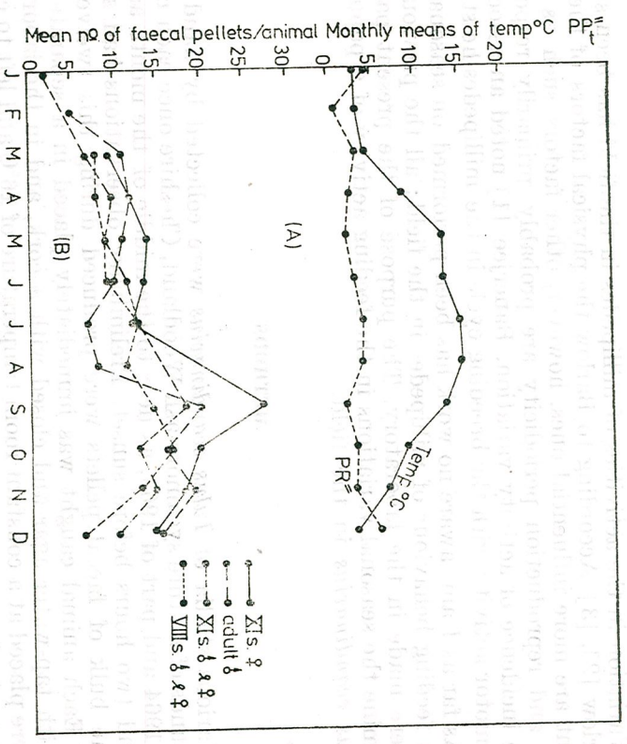

Fig. 1. — (A) Monthly mean air temperature and precipitation of the Woodland. (B) Monthly variation in the mean number of faecal pellets produced by the various stadia of *Iulus scandinavius*.

in the feeding activity of the various stadia as well as temperature and precipitation means throughout the year. Feeding activity increased during the autumn and fell off gradually on both sides of this optimum but was more pronounced in winter than in spring and summer.

Table 1

Mean number of faecal pellets produced by the various stadia of the millipede *Iulus scandinavius* (Latzel) during different months of the year. Monthly mean air temperature and total rainfall of the Woodland were also included

Month and year	Sex and Stadium					
	Adult male	XI (♀)	IX (♀♀ & ♂♂)	VIII (♀♀ & ♂♂)		
	Mean nº (±S.E) of faecal pellets per animal	Mean nº (±S.E) of faecal pellets per animal	Mean nº (±S.E.) of faecal pellets per animal	Mean nº (±S.E.) of faecal pellets per animal	Mean monthly air temperature (°C)	Total rainfall (inches)
March, 1964	11.1 ± 2.2(10)	9.5 ± 2.4(7)	7.2 ± 0.9(12)	8.0 ± 1.2(17)	4.3	3.54
April, 1964	12.0 ± 0.7(21)	12.1 ± 1.5(13)	9.7 ± 0.5(22)	8.1 ± 0.6(22)	8.8	2.65
May, 1964	10.8 ± 1.2(11)	13.9 ± 1.6(10)	8.9 ± 0.6(29)	9.4 ± 0.7(27)	13.5	2.49
June, 1964	9.9 ± 1.5(14)	13.5 ± 1.4(8)	11.4 ± 2.1(9)	9.2 ± 2.7(5)	13.5	3.31
July, 1964	6.8 ± 3.2(4)	11.8 ± 3.0(4)	12.5 ± 2.2(4)	13.0 ± 4.6(3)	15.5	4.26
Aug., 1964	8.0 ± 2.2(3)	/	/	11.4 ± 1.1(15)	15.3	4.28
Sept., 1964	19.8 ± 0.9(4)	27.5 ± 3.5(2)	18.5 ± 0.5(3)	14.6 ± 2.2(9)	13.8	2.26
Oct., 1964	15.8 ± 1.4(7)	19.7 ± 1.1(21)	12.4 ± 1.1(9)	16.5 ± 5.4(2)	9.1	3.37
Nov., 1964	18.7 ± 1.6(9)	18.2 ± 1.4(4)	14.5 ± 1.0(14)	12.1 ± 1.5(8)	7.0	3.00
Dec., 1964	14.9 ± 1.3(11)	14.5 ± 1.0(5)	10.0 ± 1.1(10)	6.0 (1)	3.3	5.93
Jan., 1965	—	—	2.0 (I)	—	3.4	4.80
Feb., 1965	5.0 (1)	—	—	—	3.5	1.10

The figures in brackets represent number of animals collected.
Symbol — indicates that no animals were found in the litter due to overwintering.
Symbol / indicates that no animals of the given stadium were present during this time due to moulting into the next stadium or death of the animal.

DISCUSSION

Barlow [2] found two peaks of locomotor activity during the year for the millipede *Iulus scandinavius*, one in the spring and one in the autumn. These periods of locomotor activity may have resulted from a change in the climatic factors or from a change in the physiological state of the animal associated with the assumption of reproductive conditions. The feeding activity may be related to these two peaks of locomotor activity. Nevertheless, the results given in table 1 would indicate that the sexed young and the adults exhibit only one peak of feeding during the autumn months.

If the low feeding of the adults during spring is related to the mating behaviour, one would get a peak of feeding in the sexed young.

Temperature may be one of the factors that has an effect on the feeding activity. This is reasonable if one considers the nature of changes in temperature and precipitation during the year (Fig. 1). Seasonal changes in temperature consist of long and steady trends, rising in spring to a maximum in summer and then falling to a minimum in winter. The low feeding of the various stadia during winter months is perhaps related to the temperature of the Woodland floor. In laboratory experiments conducted by the author [7] on the effect of temperature on the rate of uptake of food, feeding ceased at 3.5°C. The mean air temperature of the Woodland from December, 1964 to February, 1965 was 3.4°C (Table 1). In fact the number of the various stadia collected during winter months was very small to give reliable figures on the feeding activity but from the evidence presented above temperature affected feeding during winter months. The animals migrate into the soil to avoid the relatively cooler surroundings of the litter layer. This is because the humus and soil layers maintain higher temperatures than that of the litter [4]. Precipitation shows also trends (Fig. 1), tending to be highest during summer months and this is possibly one of the factors which affected feeding in summer. It would appear from table 1 that temperature during the autumn is higher than during the spring (average during autumn months (September, October and November) is 9.96°C and during spring months (March, April and May) is 8.9°C. Although precipitation is almost the same during spring and autumn (average 2.89 inches in the spring months and 2.8 inches in the autumn months), fluctuations in temperature are more radical.

Apart from the climatic factors which affected feeding, it should be mentioned that variation in the age of leaf litter available to the animal in the field could also affect feeding. *I. Scandinavius* exhibit a preference for leaves of different ages [7]. However, if the preference of the animal to leaves is taken into consideration, it will be noticed that this is insignificant during November and December. But more faecal pellets were produced in November than in December and this variation must be due to a difference in temperature. It appears that for the initiation of a peak of feeding in a population of *Iulus scandinavius* in natural habitat a higher temperature and moderate precipitation are necessary.

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A SEX ATTRACTANT FOR *AUTOGRAPHA (PLUSIA) GAMMA* L. MOTH

BY

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In previous trials, made in apple orchards, the lures containing *cis*-7-dodecenyl-acetate, added for enhancing the specificity of synthetic *trans*-8-*trans*-10-dodecadien-1-ol (the sex-pheromone of *Laspeyresia pomonella* L.), attracted adults of the noctuid moth *Autographa (Plusia) gamma* L. Two experiments, made in the 1978 year by field screening, in an apple orchard and in a red clover field, demonstrated that *cis*-7-dodecenyl-acetate, and not *trans*-8-*trans*-10-dodecadien-1-ol, is the attractant for *A. gamma* L. moth. That this attraction is a sex-attraction was demonstrated by the fact that all the caught *A. gamma* L. moths, sexed by genitalia, were males.

The *Autographa (Plusia) gamma* L. moth is an important polyphagous pest, damaging clover, cabbage, lettuce, sugar beet, bean, potato, and even cereals and young fruit trees in the nurseries. Its larvae gnaw the leaves of plants, generally at the edges of the limb or perforate it, but in the course of hard attacks they devour all the limb, except the nervures.

In Romania the species is bivoltine, its first flight period being from May to the end of June, and the second from the middle of August to the middle of September. The most damaging is the feeding activity of the larvae of the first generation, in July [1], [6]. The pest is presently kept under control by sprays with contact insecticides in the second half of June and the first half of July, but the short residual activity of these compounds (necessary because much of the damaged plants are vegetables which are consumed fresh) makes necessary the effective timing of the sprays. This can be effected by the flight survey of the moth by sex-attractant traps.

MATERIALS AND METHODS

Previous trials, made in apple orchards, showed that ATRAPOM I (the first Romanian synthetic sex-pheromone for codling moth, *Laspeyresia pomonella* L.), containing *trans-8-trans*-10-dodecadien-1-ol, attracted also adults of *Hedya nubiferana* Haw. moth (Fig. 1), while the Pherocon™ CM caps, produced by Zoëcon Corp. (USA), attracted only the adults of *L. pomonella* L. moth (Fig. 2).

Consequently, it was tried to enhance the attraction specificity of ATRAPOM I by adding to the lures other substances, possible inhibitors of the *Hedya nubiferana* Haw. attraction. Among these substances

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cis-7-dodecenyl-acetate (*cis*-7-DDA) was tested, which demonstrated in Switzerland inhibitory effects to the attraction of *Hedya nubiferana* Haw. by *trans*-8-*trans*-10-dodecadien-1-ol [2]. Surprisingly, in the late summer of the 1977 year we observed that in the traps baited with this mixture were caught many adults of *Autographa gamma* L. moth (Fig. 3).

Since in the literature [3], [4], [5], [7], the *cis*-7-DDA is indicated as sex-pheromone or sex attractant for two tortricid, one gelechiid, one pyralid, and nine noctuid moths from the subfamily of *Plussiinae: Trichoplusia ni* Hübner, *Anagrapha falcifera* Kirby, *Chrysaspidia contexta* Grote, *Plusia aereoides* Grote, *Pseudoplusia includens* Walker, *Rachiplusia ou* Guénée, *Autographa biloba* Stephens, *Autographa californica* Speyer, and *Autographa ampla* Walker, we supposed that *cis*-7-DDA may be a sex attractant also for *Autographa gamma* L. (belonging to the same subfamily).

Subsequently, in the 1978 year, for catching *A. gamma* L. moths two field screening experiments were made, one in a sprayed apple orchard, using IAC-1M sticky traps, and one in a sprayed red clover field, using IAC-4 sticky traps. The traps were baited with lures containing *trans*-8-*trans*-10-dodecadien-1-ol, *cis*-7-dodecenyl-acetate, mixtures between these two substances, and other two dodecenyl-acetates: the *cis*-8-DDA and the *cis*-9-DDA. For assuring equal catch conditions to all the traps, they were daily permuted circularly. The attractancy of the lures was estimated by the total number of moths caught in single traps.

RESULTS AND DISCUSSIONS

Table 1, containing the principal results of the first experiment, indicates that the *A. gamma* L. moths were caught only by the trape baited with *cis*-7-DDA, alone or in mixture with *trans*-8-*trans*-10-dodecadien-1-ol (see also Figs. 4 and 5). The lures containing only *trans*-8-*trans*-10-dodecadien-1-ol attracted only *Laspeyresia pomonella* L. and no *A. gamma* L. moths, and those containing mixtures of *trans*-8-*trans*-10-dodecadien-1-ol with different amounts of *cis*-7-DDA attracted both *L. pomonella* L. and *A. gamma* L. moths (Fig. 5). At the same time, the lures containing *cis*-8-DDA and *cis*-9-DDA attracted no adults of either two species.

The results of the second experiment, contained in Table 2, confirmed the results of the first, and showed that the optimum dose for catching *A. gamma* L. moths is 1 mg of *cis*-7-DDA per lure.

The two tables show that the lures containing only *cis*-7-DDA were more attractant for *A. gamma* L. moths than those containing mixtures with *trans*-8-*trans*-10-dodecadien-1-ol. Consequently, the attractant for *A. gamma* L. is undoubtedly the *cis*-7-dodecenyl-acetate, and not the *trans*-8-*trans*-10-dodecadien-1-ol, nor the mixtures between two substances. In the mixtures, the *trans*-8-*trans*-10-dodecadien-1-ol seems acting even as an inhibitor for the attraction of *A. gamma* L. moths.

The total catches of *A. gamma* L. moth obtained in the two experiments indicated a good attractiveness of *cis*-7-DDA. That this attraction

Table 1

The number of *Laspeyresia pomonella* L. and *Autographa gamma* L. moths caught in a sprayed orchard (Cluj-Napoca, June 27 – August 10, 1978)

Substances and doses tested	*L. pomonella* L. moths caught	*A. gamma* L. moths caught		
		Total catches	% of the maximum catches	Males caught
trans-8-*trans*-10-dodecadien-1-ol (1 mg)	18	0	0.0	0
trans-8-*trans*-10-dodecadien-1-ol (1 mg) + *cis*-7-dodecenyl-acetate (0.25 mg)	16	22	59.4	22
trans-8-*trans*-10-dodecadien-1-ol (1 mg) + *cis*-7-dodecenyl-acetate (0.5 mg)	13	24	64.8	24
trans-8-*trans*-10-dodecadien-1-ol (1 mg) + *cis*-7-dodecenyl-acetate (1 mg)	14	30	75.6	30
cis-7-dodecenyl-acetate, (0.5 mg)	0	25	67.0	25
cis-7-dodecenyl-acetate (1 mg)	0	37	100.0	37
cis-7-dodecenyl-acetate (2 mg)	0	32	86.4	32
cis-8-dodecenyl-acetate (1 mg)	0	0	0.0	0
cis-9-dodecenyl-acetate (1 mg)	0	0	0.0	0

Table 2

The number of *Autographa gamma* L. moths caught in a sprayed red clover field (Cluj-Napoca, June 29 – September 6, 1978)

Substances and doses tested	Total catches	% of the maximum catch	Males caught
trans-8-*trans*-10-dodecadien-1-ol-(1 mg) + *cis* 7-dodecenyl-acetate-(0.5 mg)	62	59.0	62
trans-8-*trans*-10-dodecadien-1-ol (1mg) +*cis*-7- dodecenyl-acetate (1 mg)	78	74.2	78
cis-7-dodecenyl-acetate (0.5 mg)	97	92.3	97
cis-7-dodecenyl-acetate (1 mg)	105	100.0	105
cis-7-dodecenyl-acetate (2 mg)	80	83.9	88

is undoubtedly a sex-attraction was demonstrated by the fact that all the caught moths, sexed by genitalia preparations, were males.

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Octavian Udriște, *Gena ancestrală și originea cancerului* (Le gène ancestral et l'origine du cancer). Editura științifică și enciclopedică, Bucarest, 1978, 215 pages.

Le cancer a causé et provoque encore, non seulement de nombreuses victimes et de très amples recherches, mais aussi d'hypothèses et des conceptions nouvelles, qui enrichissent la biologie et qui constituent des points de départ pour des voies nouvelles de recherches. Le livre de Monsieur O. Udriște entre dans cette dernière catégorie.

L'auteur a formulé sa conception il y a plus de 15 ans. L'idée centrale de sa pensée est que dans l'évolution de la Vie, on a commencé par l'obtention de l'énergie nécessaire aux manifestations vitales par des réactions anaérobies, car l'oxygène manquait dans l'ambiance des premiers organismes. Le phénomène qui dirigeait ces premières manifestations de la Vie était la glycolyse anaérobie. C'est seulement beaucoup plus tard (deux milliards d'années, selon l'auteur) que l'apparition de l'oxygène dans le milieu de la Vie a permis aux organismes de remplacer les réactions anaérobies par des réactions aérobies, donc par des phénomènes oxydatifs, beaucoup plus économiques et d'un rendement énergétique plus grand. Mais dans le génome situé dans les acides nucléiques (ADN et ARN) on trouve encore quelques gènes ancestraux qui étaient les déterminants des réactions anaérobies. Dans certaines conditions les facteurs extérieurs à la cellule peuvent arrêter l'activité des gènes aérobies et laisser donc libre l'activité des gènes anaérobies. Par ce phénomène l'apparition du cancer est déclenchée.

Le livre de O. Udriște expose dans un premier chapitre les considérations qui l'ont conduit à cette double origine des réactions énergétiques, anaérobie d'abord, aérobie plus tard. Dans les deux chapitres suivants il nous expose la vérification de cette conception, en analysant l'énergétique du développement ontogénétique de l'homme et des animaux ; elle commence toujours par des réactions anaérobies et c'est plus tard que ces réactions, d'un faible rendement, sont remplacées par des réactions oxydatives, beaucoup plus rapides et plus économiques pour l'organisme.

Le quatrième chapitre, le plus étendu et le mieux argumenté par les recherches de l'auteur et par les informations bibliographiques, est consacré à la genèse du cancer. Les cellules cancéreuses se caractérisent par une simplification très poussée de leur organisation, par la perte de leur spécialisation et par la substitution des réactions oxydatives par des processus anaérobies. Ce changement est dû sans doute aux modifications qu'on constate dans la structure de l'ADN, où on a trouvé de nombreuses anomalies chromosomiales, ce qui confère à cette cellule une autonomie nouvelle, qui lui donne la possibilité de s'infiltrer dans tous les autres tissus spécialisés des organes et de les remplacer en provoquant leur destruction. C'est le cancer !

C'est donc aux gènes ancestraux, qui se trouvaient dans chaque génome de nos cellules normales, qu'on doit ce comportement aberrant qui aboutit au cancer. L'hypothèse de O. Udriște affirme que toutes les cellules normales de notre corps ont dans leur ADN, les causes primordiales qui peuvent déclencher le remplacement des réactions aérobies par des réactions anaérobies, qui peuvent donc déterminer l'apparition du cancer dans n'importe quel tissu du corps.

Les gènes aérobies (est-ce qu'on peut parler des gènes aérobies ou anaérobies ?) peuvent être inhibés par certaines substances chimiques (cancérigènes), par la présence de certains virus (qui placent leur génome anaérobie à la place de celui aérobie de la cellule normale), par des radiations ionisantes, par des enzymes aberrantes, par des antigènes néoplasiques, etc. Plus de 100 pages sont consacrées à ce problème central de la théorie de O. Udriște, de la substitution des activités des gènes aérobies par des gènes anaérobies ancestraux.

Le livre de docteur O. Udriște apporte une perspective nouvelle sur la genèse du cancer. Elle est une hypothèse roumaine et par cela je le recommande à l'attention des oncologues, des généticiens, des médecins, des biologistes, à tous ceux qui s'occupent directement ou indirectement de cette maladie terrible, dont on ne connaît pas complètement les causes complexes. Nous avons, selon l'hypothèse du docteur O. Udriște, en nous même la cause qui peut déclencher le remplacement des réactions aérobies par des réactions anaérobies. Il faut maintenant trouver les moyens d'empêcher ce remplacement et de maintenir dans toutes les cellules de notre corps la prédominance des réactions oxydatives, donc des processus aérobies. C'est une voie nouvelle d'éviter le cancer, si la théorie de O. Udriște s'avère juste. L'avenir nous confirmera ou infirmera ce point de vue.

E. A. Pora

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.

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

La bibliographie, les tableaux et l'explication des figures seront dactylographiés sur pages séparées et les diagrammes exécutés à l'encre de Chine noire, sur papier calque.

Les tableaux et les illustrations seront numérotés avec des chiffres arabes. La répétition des mêmes données dans le texte, les tableaux et les graphiques sera évitée. Les références bibliographiques, citées par ordre alphabétique des auteurs, comporteront le nom de l'auteur, l'initiale du prénom, l'année, le titre de la revue, abrégé conformément aux usances internationales, le tome, le numéro, la page. Les travaux seront accompagnés d'un court résumé de maximum 10 lignes. Les textes des travaux ne doivent pas dépasser 7 pages dactylographiées (y compris les tableaux, la bibliographie et l'explication des figures). Le responsabilité concernant le contenu des articles revient exclusivement aux auteurs.

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