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REVUE ROUMAINE DE BIOLOGIE

SOMMAIRE

CUBANOCUMA GUTZUI GEN. ET SP. N. (CUMACEA, NANNASTACIDAE) FROM THE TROPICAL WESTERN ATLANTIC

BY

MIHAI BĂCESCU and ZARUI MURADIAN

The authors describe a new nannastacid of Cuba's littoral waters, *Cubanocuma gutzui* gen. et sp. n. Both sexes are figured.

The material used in this paper was collected from the Cuban waters by one of the authors (Mihai Băcescu, 1968), by Marian Traian Gomoiu (1969) and by Modest Guțu (1973), namely :

— In front of Havana, at 8 m depth, 5 XII 1968, M. Băcescu, 2♀♀ marsupiphore, 1♂ P (only carapace).

— In front of Batabano, at 3 m depth, muddy sand with *Thalassia*, sample taken by sounding, 10.III.1969, M. T. Gomoiu, 1♂ ad.

— In front of Havana, from spongiae, 15.III.1969, M. T. Gomoiu, 1♂ ad.

— 21°33′N — 79°21′W, Ana Maria Gulf, dredging at 6 m depth, muddy sand with *Thalassia*, t° 28,6°C, 10 IV 1973, M. Guțu, 4♀♀ marsupiphore.

— 21°33′N — 79°41′W, Ana Maria Gulf, dredging at 7.5 m depth, rough sand with *Thalassia*, t° 27,3°C, 10 IV 1973, M. Guțu, 1 ♀ P.

— 21°25′N — 79°07′W, Ana Maria Gulf, sample taken by sounding at 12.5 m depth, muddy sand with coral scraps, t° 27,6°C, 11 IV 1973, M. Guțu, 1 ♂ P.

Cubanocuma gen. n.

Nannastacid with at first sight appearance resembling the genus *Campylaspis*, with same type of maxillipeds I, II, III. Carapace deep, with high share for the total body length, partially covering the free thoracic segments on their edge.

Eye lobe particularly prominent, chiefly in ♂, frontopseudorostral line short, nearly transversal.

Male antenna with segment 5 of peduncle slightly longer than 4 and numerous lanceolate phanera also present on the segments of the short flagellum, not extending beyond carapace.

Exopodite formula is 4 in males and 3 in females.

Cubanocuma gutzui [1] sp. nov.

Description of adult male. Anterior half of dorsal field of carapace distinctly delimited by a *carina* the ends of which reach beneath the pseudo-rostral lobes (Fig. 1). Lateral and posterior parts of carapace with an almost vertical projection, so that the carapace appears very high, 0.4 mm, as against the 0.65 mm of its total length. The postero-lateral corner appears as a right angle, whilst the antero-lateral corner is rounded; antennal notch rather weakly marked. Dorsal part of carapace showing a series of tubercles more numerous in preadult specimens, even on its edges. Pseudo-
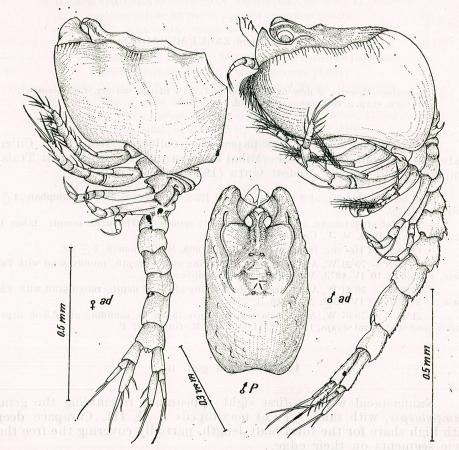

Fig. 1. — *Cubanocuma gutzui* gen. et sp. n. ♂ ad., in lateral view, and ♂ P, carapace in dorsal view. ♀ ad., in lateral view.

rostrum very short, not exceeding the frons, whilst the eye lobe strongly raises above it, measuring 1/8 of carapace length and approximately 1/6 of its height.

Thoracic segments laterally concealed — the first two completely, the third partially — by the postero-lateral fold of carapace. First two

---

thoracic segments have medio-dorsal crests; the length of the combined free thoracic segments measures merely 0.25 mm. Pleon of 0.6 mm; the antennal notch is lacking because the antennal flagellum does not extend beyond the carapace length.

Antennule (Fig. 2*A*). The width of the first segment of the basis is about 2/3 of its length. Main flagellum with 2 aesthethascae. Segments 4 and 5 of the basis of antenna (Fig. 2 *B*) are subequal, the last being slightly longer, almost concealed by numerous transversal series of lanceolate sensitive elements fixed by a narrow peduncle. The same type of expansions are to be found — by two — on each of the 15 segments of the flagellum, but much narrower than those of the basis. Maxilla and maxilliped I as in Fig. 3 *A* and *B*. Maxilliped II (Fig. 3 *C*) with 4 spines on the dactylopodite, concealed by the propodal spine which is much longer. Maxilliped III (Fig. 2 *C*) has a stout basis, slightly shorter than the combined remaining segments. Meropodite not excessively wide measures 1/2 of the basis length. Setae of the dorso-distal angle of basis, short. Peraeopod I similar to that of the female (Fig. 3 *F*) of nearly the same length as maxilliped III and the same ratio between the basis and the rest of the appendage. Basis of peraeopod II (Fig. 2 *D*) slightly larger than 1/3 of its total length. Carpopodite and dactylopodite are subequal in length; the long apical seta of dactylopodite is much longer than the latter. Peraeopod III (Fig. 2 *E*) bears a well developed exopodite, which in the preadult specimen (with already formed antenna, not merely beginning to show) is but rudimentary (*r*, Fig. 2 *F*). Peraeopods IV and V in the adult specimen, as in Fig. 2 *G* and *H*. Uropods (Fig. 2 *I*) measuring about 0.26 mm in length have the following ratios: peduncle only by 0.01 mm longer than the pleotelson and by 0.05 mm longer than the uropodal rami ± equal. On the inner edge of the peduncle and of the uropodal endopodite, 9—10 strong phanera are to be found. Of the apical spines the longer one extends beyond the endopodite. The exopodite has a much longer apical spine, broken in our specimen.

Length: 1.76 mm.

Colour: bright red.

Description of the adult female. Carapace similar to that of the male (Fig. 1) being however deeper, and the eye lobe, although prominent, does not reach the size of the male one. Free thoracic segments without medio-dorsal *carina*, almost telescoped, partially concealed on the edges by the carapace; their length reaches only 0.2 mm. Pleon also short; together with the free thoracic segments it is as long as the carapace.

Antenna as in Fig. 3 *D*. Maxilliped III (Fig. 3 *E*) with basis much shorter than the rest, as compared with the maxilliped of the male. Peraeopod II (Fig. 3 *G*) with basis much shorter than 1/3 of its total length. Peraeopod III and V as in Figs. 3 *H* and *I*. Uropods (Fig. 3 *J*) proportionally somewhat smaller and stouter than in males. Uropodal peduncle bearing neither spines nor setae.
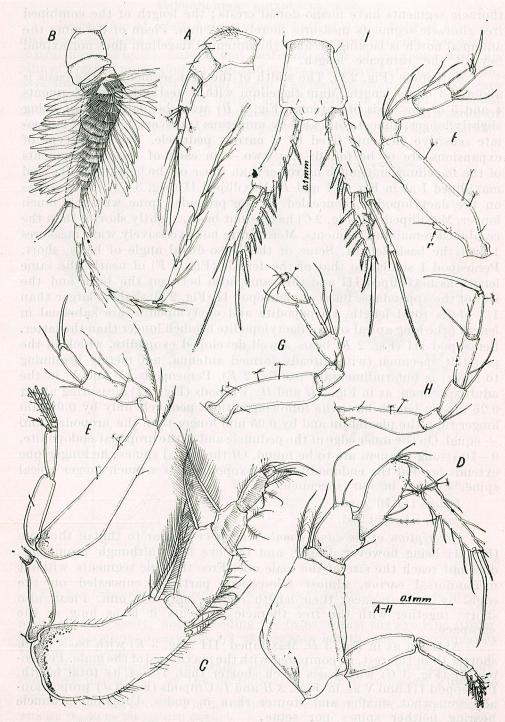
Fig. 2. — *Cubanocuma gutzui* gen. et sp. n. ♂ ad. *A*, antennule ; *B*, antenna ; *C*, maxilliped III ; *D*, peraeopod II ; *E*, peraeopod III ; *F*, peraeopod III in ♂ *P − r*, its rudimentary exopodite ; *G*, peraeopod IV ; *H*, peraeopod V ; *I*, uropods.
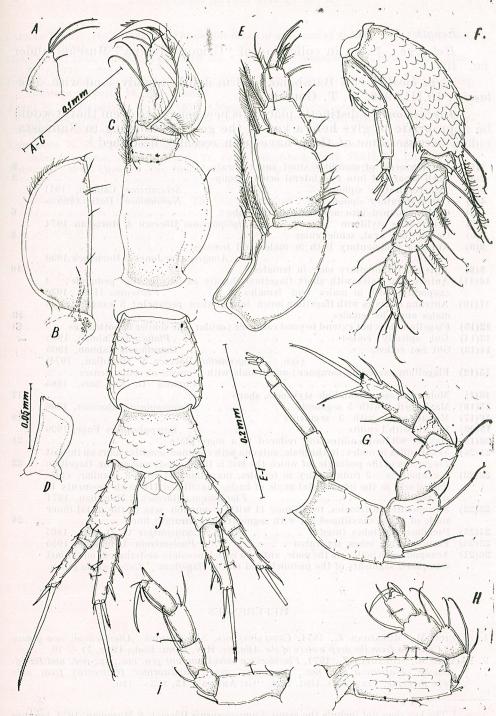
Fig. 3. — *Cubanocuma gutzui* gen. et sp. n. *A − C*, ♂ ad. *A*, maxilla ; *B*, maxilliped I ; *C*, maxilliped II. *D − J*, ♀ ad. *D*, antenna ; *E*, maxilliped III ; *F*, peraeopod I ; *G*, peraeopod II ; *H*, peraeopod III ; *I*, peraeopod V ; *J*, uropods.

Length : 1.6 mm.

Holotype : ♂ ad., in collection of "Grigore Antipa" Museum under no. 405.

Type locality : off Batabano, at 3 m depth, muddy sand with *Thalassia*; 10.III.1969, M. T. Gomoiu.

Discussion. To distinctly place this new genus, we deem that it would be appropriate to give here a key of the genera belonging to Nannastacidae, because some of them have been recently described [1].

1(16)	Molar process of mandible stout and truncate	2
2(5)	Oceli separated into two lateral ocular groups	3
3(4)	Two respiratory siphons . . . .*Schizotrema* Calman, 1911	
4(3)	One respiratory siphon . . . .*Nannastacus* Bate, 1865	
5(2)	Ocelli grouped into a median ocular lobe	6
6(7)	Ocular lobe styliform . . . . *Styloptocuma* Băcescu & Muradian 1974	
7(6)	Ocular lobe oval, semicircular	8
8(9)	Antenna rudimentary both in males and females	
	. . . .*Almyracuma* Jones & Burbanck,1959	
9(8)	Antenna rudimentary only in females	10
10(11)	Antenna in males with short flagellum, hardly as long as the peduncle; 4 exopodites both in males and females . . . .*Picrocuma* Hale, 1936	
11(10)	Antenna in males with flagellum much longer than peduncle; 5 exopodites in males and 3 in females	12
12(15)	Flagellum does not extend beyond carapace; ocular lobe similar in both sexes	13
13(14)	Gut spirally coiled . . . .*Platycuma* Calman, 1905	
14(13)	Gut not coiled . . . .*Cumellopsis* Calman, 1905	
	(syn.: *Bathycumella* Băcescu & Muradian, 1974)	
15(12)	Flagellum exceeding carapace; adult male with a crown of ocular lenses . . .	
	. . . .*Cumella* G. O. Sars, 1865	
16(1)	Molar process of mandible styliform, sharp	17
17(18)	Maxilliped I with 5 segments . . . .*Procampylaspis* Bonnier, 1896	
18(17)	Maxilliped I with 3 segments	19
19(20)	Maxilla with 1 endite . . . .*Campylaspides* Fage, 1929	
20(19)	Maxilla without endites and reduced to a mere plate	21
21(26)	5 exopodites in males; in the male, antenna with common sensitive hairs on the last 2 segments of the peduncle (of which the last is the longest) and on the flagellum . .	22
22(23)	3 exopodites + 2 rudimentary in females, maxilliped II with a peculiar, club-shaped seta in the inner distal angle of basis, maxilliped III with segments in zig-zag . . . . *Floridocuma* Băcescu & Muradian, 1974	
23(22)	3 exopodites in females, maxilliped II with a common seta in the distal inner angle of basis, maxilliped III with segments in a straight line	24
24(25)	Pseudorostral lobes fused . . . .*Campylaspis* G. O. Sars, 1865	
25(24)	Pseudorostral lobes separated . . . .*Pavlovskeola* Lomakina, 1955	
26(21)	4 exopodites in males; in the male, antenna with lanceolate aestethascae on the last subequal 2 segments of the peduncle and on the flagellum .. *Cubanocuma* gen. n.	

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[1] The key does not include the genus *Campylaspenis* Băcescu & Muradian, 1974, because the presence of a penial organ — a unique instance in the Order Cumacea — makes it impossible to be classified.

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DEUX ESPÈCES NOUVELLES DU GENRE *CHEILOSIA* MEIGEN (DIPTERA, SYRPHIDAE)

PAR

VL. BRĂDESCU

In the present work two news species named *Cheilosia dacica* sp. n. and *Cheilosia toniuci* sp. n. (Diptera, Syrphidae) are described. The author made a comparative study of the species *Cheilosia montana* Egg. and *Cheilosia gigantea* (Zett.) with which these news species have resembling characters.

Le matériel qui forme l'objet de la présente communication a été collecté dans le Parc National de Retezat, pendant des recherches diptérologiques effectuées dans la période 1974—1975.

Cheilosia dacica sp. n.

Holotype ♂; Roumanie, Carpates Méridionales, Massif du Retezat, clairière Gemenele (1 930—2 000 m) de la réserve scientifique Gemenele —Tăul Negru, environ 6 km ESE de Gura Zlata; 25.VII.1974.

L'espèce appartient au groupe C conformément à la classification de Sack concernant ce genre. Moyennant cette clef, nous arrivons à *Cheilosia montana* Egg. Pour souligner la différence évidente entre ces espèces, nous présentons un tableau comparatif :

Cheilosia dacica sp. n.	*Cheilosia montana* Egg.
♂	♂
Face luisante, couverte d'un toment cendré très faible.	Face intensément couverte d'un toment cendré.
3e article antennaire subcirculaire.	3e article antennaire presque carré.
Chète antennaire évidemment pubescente.	Chète antennaire avec une pubescence microscopique (×40).
Thorax à pilosité jaune blanchâtre.	Thorax à pilosité jaune cendré sur la moitié antérieure, noire sur la moitié postérieure.
Ailes légèrement brunies, plus intensément vers le bord antérieur.	Ailes transparentes, un peu jaunâtres.
Pattes entièrement noir-brun.	Pattes colorées brun et jaune foncé.
Abdomen allongé, à pilosité jaune blanchâtre.	Abdomen elliptique, à pilosité rougeâtre.
Longueur : 9 mm.	Longueur : 10 — 11 mm.

Description

Tête. Yeux à pilosité épaisse, brun clair, de longueur modérée. Front noir, plus ou moins luisant, faiblement sillonné, à pilosité brun noirâtre, égale à la longueur du 3ᵉ article antennaire. Vertex à pilosité brun clair sur la partie antérieure, le reste jaunâtre. Péristome légèrement enfoncé dans la zone sous-antennaire, jusqu'au calus facial ; la face luisante, très faiblement couverte d'une pruinosité jaune blanchâtre, plus évidente dans la zone stricte sous-antennaire. Le calus facial, modérément proéminent, occupe à peu près 1/3 de la largeur de la face. Vu de face, le calus facial présente d'un côté et de l'autre de la proéminence centrale deux fossettes verticales, de sorte que le calus semble sectionné longitudinalement en trois segments quasi égaux. Le calus est séparé de l'épistome par une creux faible. À la partie ventrale le profil de la face présente un aspect pointu, par le prolongement accentué du péristome (fig. 1). Gènes égales à la largeur du 2ᵉ article antennaire, à poils brun jaunâtre, à peu près égaux à la longueur de la pilosité des yeux. Les deux articles antennaires basaux noirs luisants ; le 3ᵉ brun foncé, subcirculaire. Chète antennaire pubescente.

Thorax. Noire olivâtre, luisant, légèrement tomenté de noir à la partie antérieure. Pilosité jaune blanchâtre, de longueur modérée. Scutellum à pilosité de la même couleur, plus longue sur le bord et sans macrochètes marginales. Ailes légèrement brunies, plus intensément vers le bord costal ; t-m d'une allure aiguisée en face de r 4 + 5, mais placée en angle droit sur cette nervure (fig. 2). Cuillerons blanchâtres, avec des marges jaunâtres. Balanciers jaunes brunis, à renflement bruni. Longueur de l'aile : 8,75 mm. Pattes brun noirâtre ; f 1 et f 2 à plusieurs poils longs noirs ; à la partie basale quelques poils blanchâtres ; f 3 avec des poils longs plus rares.
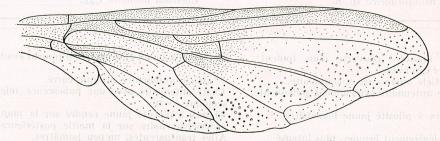
Fig. 1.— *Cheilosia dacica* sp. n. ♂ ; tête (original).

Fig. 2. — *Cheilosia dacica* sp. n. ♂ ; aile (original).

Abdomen allongé, noir mat, tomenté de noir sur les tergites 2 et 3 et sur la partie antérieure centrale du tergite 4, à pilosité jaune blanchâtre, plus longue et plus épaisse sur les bords, surtout chez le deuxième tergite. La partie ventrale luisante.

Longueur totale du corps : 9 mm.

Nous dénommons la nouvelle espèce *Cheilosia dacica*, hommage à nos ancêtres, les Daces.

Cheilosia toniuci sp. n.

Holotype ♂ ; Roumanie, Carpates Méridionales, Massif du Retezat, clairière Rotunda (1 100 m), vallée de la rivière Lăpuşnicul Mare, environ 6 km ESE de Gura Apei ou du confluent du Lăpuşnicul Mic (Branul) avec Lăpuşnicul Mare ; 27.VII.1975.

L'espèce appartient au groupe D conformément à la classification de Sack, concernant ce genre. Moyennant cette clef, nous arrivons à *Cheilosia gigantea* (Zett.). Pour souligner la différence évidente entre ces espèces, nous présentons un tableau comparatif :

Cheilosia toniuci sp.n.	*Cheilosia gigantea* (Zett.)
♂	♂
Thorax à pilosité noire.	Thorax : sur la moitié antérieure, parmi les poils noirs sont mêlés aussi des poils jaune cendré.
Ailes incolores, légèrement irisées.	Ailes brunies ; à la base et vers le bord antérieur plus intensément.
Abdomen à pilosité jaune blanchâtre.	Abdomen banderolé, dû à la disposition des poils noirs et jaunes brunis.
Longueur : 6,75 mm.	Longueur : 9 — 13,5 mm.

Description

Tête. Yeux à pilosité épaisse, courte, brun clair, à la partie supérieure plus foncé. Front noir, luisant, avec deux bandes étroites blanchâtres tomentées, situées auprès des yeux ; fossette médiane longitudinale légèrement sillonnée ; pilosité du front brune, un peu plus longue que la longueur de 3ᵉ article antennaire. Vertex à pilosité noire. Péristome évident enfoncé dans la zone sous-antennaire, jusqu'au calus facial. La face luisante, très faiblement couverte d'une pruinosité blanchâtre, seulement dans la zone strictement sous-antennaire. Le calus facial, proéminent, occupe à peu près 1/3 de la largeur de la face. Entre le calus facial et l'épistome un creux évident. À la partie ventrale, la face présente, en profil, un aspect pelotonné (fig. 3). Gènes étroites, avec des poils jaune blanchâtre, un peu plus longs que la pilosité des yeux. Antennes brun foncé, légèrement tomentées de brun clair ; le 3ᵉ article quadrangulaire arrondi, un peu plus long que large. Chète antennaire pubescente.

Fig. 3. — *Cheilosia toniuci* sp.n. ♂ ; tête (original).

Thorax. Noir luisant, pilosité noire, longue, plus longue vers le scutellum. Scutellum noir luisant, à pilosité noire, vers le bord des poils noirs plus développés et six macrochètes marginales longues. Sur les parties latérales du thorax, des poils noirs et quelques macrochètes suralaires et post-

alaires. Ailes incolores, légèrement irisées ; t-m d'une allure aiguisée en face de r 4+5, mais placée en angle droit sur cette nervure (fig. 4). Cuillerons jaune blanchâtre bordés d'orange. Balanciers jaunes, à renflement brun clair. Longueur de l'aile : 6 mm. Pattes : f noires brunies ; les genoux, les parties basales et apicales des t et, en partie, les métatarses
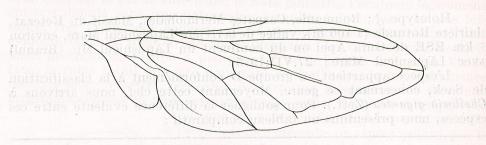

Fig. 4. — *Cheilosia toniuci* sp. n. ♂ ; aile (original).

des p 1 et p 2 jaunes brunis, sans limites tranchantes ; f 1 avec des poils longs noirs, jaune blanchâtre vers la base, f 2 avec des poils longs jaune blanchâtre.

Abdomen allongé, noir mat, tomenté de noir sur les tergites 2 et 3 et sur la partie antérieure centrale du tergite 4 ; à pilosité jaune blanchâtre plus longue et plus épaisse sur les bords, surtout chez le deuxième tergite. À la marge postérieure des tergites 3 et 4 des poils plus longs, noirs. Sur l'hypopyge prédominent les poils blancs. La partie ventrale luisante.

Longueur totale du corps : 6,75 mm.

Nous dénommons la nouvelle espèce *Cheilosia toniuci*, hommage au secrétaire scientifique de la Commission pour la protection des Monuments Naturels, Nicolae Toniuc, enthousiaste animateur des recherches scientifiques dans le Parc National de Retezat.

Les types sont déposés au Muséum d'Histoire Naturelle « Grigore Antipa » de Bucarest.

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GROWTH OF DIFFERENT INSTARS OF *PORCELLIO LAEVIS* (LATREILLE) (ISOPODA, PORCELLIONIDAE)

BY

G. A. NAIR

The growth rates in the natural populations of different terrestrial Indian isopods have not yet been studied in detail and a beginning in this direction was made by Menon et al. [3] [4], who studied the growth pattern of *Porcellionides pruinosus* (Brandt) and *Cubaris robusta* (Collinge), the two most abundant terrestrial isopods found in Punjab. A good deal of information is available about the various aspects of growth rates of different terrestrial and marine isopods. Needham [5] [6], Paris and Pitelka [7], Holdich [2], Sutton [9] and Radu and Tomescu [8] have made useful contributions in this field.

In the present study, observations on the growth of different instars of the common terrestrial isopod, *Porcellio laevis* (Latreille), found in plenty in and around Delhi and New Delhi were made, as a part of study of the population dynamics of the same. These animals were maintained in the laboratory for more than one to one and a half year, and observations were made on their moulting, breeding habits, age and growth pattern. Culture method described by Heeley [1] for terrestrial isopods with slight modifications was adopted for rearing *Porcellio laevis* in the laboratory. They thrived well under constant care. The newly liberated young ones from the brood pouches of the gravid females were immediately reared separately and were studied with respect to moulting time and rate of growth in relation to body length and body width (Tables 1 and 2). The body length was measured from the anterior edge of the frontal lobe of the head to the tip of the telson, while the body width was measured across the second thoracic segment in each case. All the new born instar group showed a body length of 1.5 mm. The first moult took place within 24 hours after liberation from the brood pouch. Three moults occurred during the first two weeks after liberation from the brood pouch. This was a period of rapid growth and the body length had almost doubled to 3 mm. After the third moult the sexes could be distinguished by the appearance of secondary sexual characters. Moulting intervals became longer in subsequent instars. There was a steady increase in body length until the animal became 7 mm to 8 mm. Some individuals, however, continued to grow until they were 9 mm in length.

A general division of the different instar groups on the basis of their reproductive conditions could be made at this stage. The first two instar groups (body length ranged from 1.5 mm to 3 mm) could be grouped as immatures where sexual differentiation had not taken place ; the third

Table 1

Approximate moulting intervals in days between different instars of *P. laevis* raised in the laboratory

Moults	Range of days for different moults	Average age in days
1	Within 24 hours to 36 hours	1
2	5 to 7 days	6
3	11 to 17 days	14
4	28 to 36 days	33
5	51 to 57 days	54
6	85 to 89 days	87
7	125 to 139 days	133
8	151 to 155 days	153
9	209 to 219 days	215
10	302 to 325 days	316

Table 2

Mean body lengths and body widths of different instars of *P. laevis* with standard deviation and standard error

Total number of animals observed	Instar number	Mean body length in mm	Mean body width in mm	Standard deviation	Standard error
45	1	1.5	0.6	0.096	0.014
42	2	2.0	1.0	—	—
33	3	3.0	1.1	0.110	0.031
28	4	4.0	1.5	0.220	0.052
24	5	5.1	2.0	0.310	0.083
20	6	6.4	2.5	0.490	0.155
20	7	7.2	3.1	0.260	0.082
19	8	8.2	3.4	0.170	0.051
18	9	9.0	3.6	0.290	0.100
14	10	10.2	4.0	0.210	0.100

and fourth instar groups (body length ranged from 3 mm to 5 mm) as sexually mature but non-reproductive ones; the fifth, sixth and seventh instar groups (body length ranged from 5 mm to 8 mm) as the sexually mature and the early reproductive ones and the eighth, ninth and tenth instar groups (body length ranged from 8 mm to 11 mm) as the sexually mature and late reproductive ones.

RATE OF GROWTH

The growth curves (Fig. 1 *A, B*) showing the logarithmic plot of length and geometric growth, constant against time, were based on laboratory individuals that lived for five months or more. These animals were isolated soon after they were liberated from the brood pouch and were measured after each moulting, beginning with the emergence from the marsupium. To fit the points of these curves, measurements of mean body lengths (Y) of different instars were converted into their natural logarithms ($\log_e Y$). The regression of these logarithms on different instars (t) was then calculated and the slope of that regression line was K_g, the geometric rate of increase. These measurements are given in table 3. From the logarithmic plot of length against time, it was found that the resulting line was near to rectilinear. The least squares fit for these points resulted in the equation

$$\log_e Y = 0.412 + 0.210 \ t$$

where $\log_e Y$ was the logarithmic measurement of body length for a given instar t. There was, however, a slight suspicion of a curvilinear relation in figure 1 *A* and to check this, the values of K_g, the geometric rate of increase for each growth period was calculated and graphed in figure 1 *B*. This confirmed the suspicion that the growth function was not quite expo-

Fig. 1.— *A*, Growth curve based on natural log of mean body length; *B*, graph showing geometric rate of increase in body length; *C*, graph showing changes in α, the coefficient of allometry from one instar to another: growth of body length relative to body width.

Table 3

Logarithmic rate ($\log_e Y$) and geometric rate (K_g) of increase in body lengths of different instars of *P. laevis*

Instar (t)	Mean body length in mm	$\log_e Y$	K_g
1	1.5	0.405	—
2	2.0	0.693	0.288
3	3.0	1.099	0.406
4	4.0	1.411	0.312
5	5.1	1.629	0.218
6	6.4	1.856	0.277
7	7.2	1.974	0.118
8	8.2	2.104	0.130
9	9.0	2.197	0.093
10	10.2	2.322	0.125

nential, for despite the fluctuation in K_g with time, there was an evident decreasing trend from the first to the last instar.

From the above observation, it was safe to assume that the growth of *P. laevis* in nature could not possibly have a constant value if observations were made over a sufficiently longer period of development. Thus the only adequate representation growth by a single function would be one in which the growth rate decreased with time in such a way to make the curve reach some maximum point.

The changes in α, the coefficient of allometry, from one instar to another based on the growth of the body length relative to body width had been plotted and given in figure 1 *C*. While it was clear that the two dimensions were not related by a single allometric relation from the scatter diagram, the pattern of the deviation from the simple allometric growth was best seen by the graph of α, over time. Here the body width did not change proportionately in the initial instar groups (one to five), but afterwards a uniform proportion in the relative growth of body length on width took place.

From the observations cited above, it is now possible to come to some conclusions regarding the growth pattern of different instars of *P. laevis*. The first moult of all the young ones occurred within one day of their liberation from the maternal brood pouch. The second and third moults took place at an approximately one week interval, while the subsequent moults occurred at intervals of 2 to 13 weeks so that a total of 9 or 10 moults occurred within the first eleven months of life. The growth

of young proceeded at a rate of approximately 0.094 mm per day in length up to the age of nearly two months. After that for the remaining nine months the growth proceeded at a rate of approximately 0.019 mm per day. From this it was clear that the initial instars showed a faster rate of growth compared to later instars. The body width of *P. laevis* also increased in direct proportion to the body length from the fourth instar onwards.

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OCULAR ANOMALIES IN *OPHION LUTEUS* L. AND *ENICOSPILUS MERDARIUS* GRAV. (HYM. ICHNEUMONIDAE)

BY

IONEL P. PETCU

The author mentions and describes for the first time the ocular anomalies in two species of Ophioninae. The anomalies refer to the total lack or to the reduction to half of the left ocellus, to the modification of the position, size, or number of the ocelli.

In the speciality literature there are mentioned many teratological cases in Hymenoptera, located at different organs or parts of the body.

Balazuc [1] sets in order these data, by classifying them into several categories, among which there are also the ocular anomalies. On the other hand, Paetzold and Vater [3] and Petcu [4] synthesize the possible causes which bring about these anomalies in some primary and secondary parasites. From the available literature it results that in Ichneumonidae there were noticed ocular anomalies in one single species, *Pimpla detrita* [2].

In the present paper we mention other types of ocular anomalies in two species of Ophioninae : *Ophion luteus* L. and *Enicospilus merdarius* Grav.

Without making references to the causes which determine these curious anomalies, we notice that in the regions where the two species were collected chemical treatments for the pest control had been administered, which, as it is known, affect not only the host but also its natural parasites.

It is worth mentioning that such forms of ocular anomalies were noticed in species of other families of Hymenoptera, but in Ichneumonidae, respectively in Ophioninae, they are noticed for the first time.

Further we shall describe the ocular anomalies met in the two species of Ophioninae.

Ophion luteus L., ♀ (Fig. 1 *A*, *B*, *C*, *D*)

1 ♀, captured at 22.VII.1972, Obcioara—Suceava (leg. A. Murgoci).

The left eye is missing (Fig. 1 *A*, *B*). In the region of the eye there are hairs uniformly distributed, as in the other regions of the head. The perimeter of the missing eye presents very thin suture lines which converge in a point with a more or less central position (Fig. 1 *C*). The absence
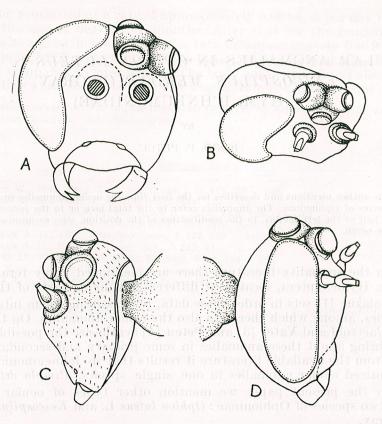
Fig. 1. — *Ophion luteus* L.: *A*, the head, front view (original);
B, the head, dorsal view (original); *C*, the head, right lateral view
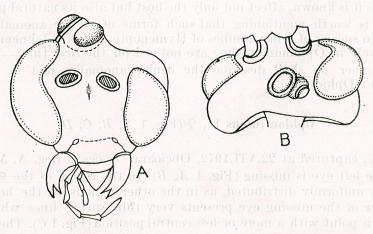(original); *D*, the head, left lateral view (original).

Fig. 2. — *Enicospilus merdarius* Grav.: *A*, the head, front view
(original); *B*, the head, dorsal view (original).

of the left eye determined modifications of the position and size of the left posterior ocellus, which is bigger, slightly pedunculate, directed towards the missing eye, partially compensating the role of the latter (Fig. 1 *A*, *C*). The absence of the left eye also determined a partial deformation of the cephalic capsule (Fig. 1 *A*, *B*).

Enicospilus merdarius Grav., ♀ (Fig. 2 *A*, *B*)

1 ♀, captured at 13.VII.1973 in artificial light, on the territory of the Agricultural Station Podu Iloaiei, Iași (leg. eng. dr. T. Săpunaru).

The left eye and the ocelli present modifications in position, size and number.

The left eye is reduced to about a half (Fig. 2 *A*, *B*).

The anterior ocellus is missing. The position and the size of the posterior ocelli are different in comparison with the normal one : the right posterior ocellus is smaller, removed towards the left eye and situated on an evident prominence. The left posterior ocellus is bigger, removed forewards, with a median position, the field of view towards the left, partially compensating the function of the reduced eye. These ocular modifications somehow ensure a more or less normal visual orientation, a fact expressed by the flight during night, when, in fact, the above-mentioned exemplar was captured.

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MUTATIONS CONVERGENTES ET DIVERGENTES CHEZ LES LÉPIDOPTÈRES

PAR

EUGEN V. NICULESCU

The author introduces in this paper two notions new to biology, namely the convergent and respectively divergent mutations.

The resemblance, as far as the habitat is concerned, of two species belonging to the same genus or to different genera is admitted to be due to some non fortuitous, i. e. oriented mutations, the author denominates convergent mutations. Besides these latter, there also exist fortuitous, divergent, mutations which produced the so varied habitat to be found in numerous "species groups" belonging to the same genus. The habitat resemblance of some species belonging to different genera living in faunistic regions occurring far from each other (South America, Equatorial Africa and the Indo-Australian region) is explained by the fact the similar climatic conditions characteristic of these regions produced homologous genes and subsequently homologous mutations, a phenomenon which led to the appearance of habitat-resembling characters occurring during the subspeciation stage.

Les notions de *mutations convergentes* et *divergentes* sont le résultat de nos recherches sur l'habitus et les genitalia chez les Lépidoptères.

Les biologistes sont presque unanimes à admettre que les mutations sont toujours fortuites. De nos observations il résulte que toutes les mutations ne sont pas fortuites ; un grand nombre de celles-ci ne sont pas soumises au hasard, elles sont «orientées» dans la même direction. Dans un travail, sous presse, nous avons donné des exemples de mutations orientées dans *l'armure génitale*, à savoir dans la série orthogénétique *Papilio hoppo — P. polytes*.

Dans le présent travail nous allons faire un exposé sur les mutations fortuites et non fortuites qui ont produit les caractères morphologiques concernant le *graphisme* chez les *Nymphalidae* et *Papilionidae*.

Pour que l'exposé soit aisément compris nous allons prendre comme exemple trois espèces de *Nymphalis* bien connues par les lépidoptéristes : *N. polychlores*, *N. xanthomelas* et *N. antiopa*.

En examinant l'habitus chez les deux premières espèces, nous sommes frappés par la grande ressemblance entre ces espèces, phénomène que nous avons nommé [4] *convergence interspécifique*. C'est de toute évidence que les mutations qui ont produit ces caractères de l'habitus ne peuvent être fortuites ; ici nous sommes en présence d'un cas du *mutation orientée*, car le graphisme (et le coloris) des deux espèces tend, en direction rectiligne, à un facies convergent, motif pour lequel nous avons donné au phénomène le nom de *mutations convergentes*. Donc *les mutations convergentes sont des mutations non fortuites*. Si nous regardons maintenant

le dessin et le coloris de l'espèce *N. antiopa* nous constatons qu'il diffère profondément et de manière *divergente* de celui des deux premières espèces. Cette divergence au sein d'un genre a été nommée par nous [4] *divergence intragénérique*. Il est clair que *N. antiopa* s'est écarté du type de dessin polychloros existant chez *N. polychloros* et *N. xanthomelas*. Il est aussi clair qu'un tel dessin « aberrant » à l'intérieur du genre est le résultat d'une *mutation fortuite*, une mutation qui a produit, comme l'affirmait N. Timofeef - Ressovsky [6] une *« déviation »* du type initial du caractère dont il s'agit. Donc *les mutations divergentes sont des mutations fortuites.* Ces deux phénomènes se passent au niveau spécifique.

Nous constatons un phénomène de la même nature, toujours intragénérique, mais d'une grande ampleur, chez les genres euryvalents de *Papilioninae, Nymphalidae, Hesperiidae*. Nous allons présenter le phénomène chez un seul genre, à savoir le genre *Eurytides*. Dans ce genre la *divergence intragénérique* est très accentuée par suite d'un grand nombre de *mutations divergentes fortuites*. Ainsi ont paru, dans ce genre, de nombreux « groupes d'espèces ». Le premier groupe est la remarquable série orthogénétique — *Eurytides stenodesmus* — *Eurytides marcellus*, à facies podaliriforme, dont nous parlerons plus loin. Le deuxième groupe d'espèces, séparé du premier par une grande discordance intragénérique, est le groupe *Eurytides belesis, E. ariarathes, E. euryleon,* etc. dont les ailes sont noirâtres avec des taches rouges sur la face supérieure des ailes postérieures. Chez le troisième groupe *(E. dolicaon, E. iphitas, E. orabilis, E. columbus)* les ailes ont le fond jaune ou orange avec de grandes taches noires confluentes dans la région apicale, sur le bord externe et dans la cellule des ailes antérieures. Nous signalons aussi le groupe *E. marchandi, E. dioxippus, E. lacandones, E. leucaspis,* avec des bandes larges sur le bord costal des ailes antérieures et sur le bord externe des quatre ailes [1]. Chez tous ces groupes d'espèces le graphisme constitue une « déviation » du type initial, ce qui s'est produit par des mutations fortuites. De tels exemples sont très nombreux chez les Lépidoptères et nous les trouvons non seulement chez les *Papilionidae* mais aussi dans les familles des *Nymphalidae, Pieridae, Lycaenidae* et surtout *Riodinidae* et *Hesperiidae.*

Très intéressant est aussi le phénomène de *convergence intergénérique* exprimé chez les *Papilioninae* par une belle série orthogénétique où les mutations convergentes, non fortuites, montrent une évolution dans des directions déterminées et précises, d'où le hasard est éliminé. La série commence par *Eurytides stenodesmus* et se termine avec *Graphium androcles* (fig. 1). *E. stenodesmus* a les ailes blanchâtres avec quelques bandes noires cunéiformes disposées obliquement, et une bordure noire très réduite au bord externe des ailes antérieures. Chez *Graphium androcles* la moitié externe des ailes antérieures est complètement noire par la fusion des

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[1] Nous attirons l'attention de nouveau sur le fait que l'habitus ne constitue pas toujours un bon critère générique [3]. D'après l'habitus nous serions enclins à grouper ces espèces en 4 genres distincts, tant sont différents ces caractères. Mais l'armure génitale montre aussi dans le cas présent, qu'elle fournit de bons caractères génériques [3]. Toutes ces espèces du genre *Eurytides,* quel que soit l'habitus, ont un même « plan de structure » [2] visible surtout dans le superuncus (un petit lobe membraneux), l'uncus trifide et la harpe très compliquée.

bandes noires de cette région. Entre le stenodesmus et l'androcles se trouvent un grand nombre d'espèces intermédiaires dont les bandes noires cunéiformes sont de plus en plus larges, jusqu'à une fusion complète dans la moitié externe. Les espèces intermédiaires sont les suivantes : *Eurytides*
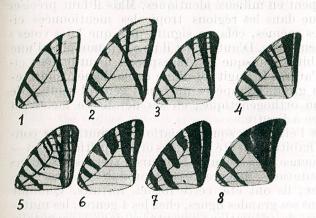

Fig. 1. — Aile antérieure chez *Eurytides stenodesmus* (1), *E. telesilaus* (2), *E. autosilaus* (3), *Iphiclides podalirius* (4), *Graphium eurous* (5), *Graphium antiphates* (6), *Protographium leosthenes* (7), *Graphium androcles* (8).

telesilaus, E. protesilaus, E. epidaus, E. earis, E. molops, E. nigricornis, E. travassosi, E. autosilaus, E. marcellus, Graphium agetes, G. glycerion, G. euphrates, G. eurous, G. aristeus, Iphiclides podalirius et *Protographium leosthenes.*

Chez toutes ces espèces appartenant à 4 genres, le facies est podaliriforme, résultat des mutations orientées orthogénétiquement. Puisque les genitalia chez les 4 genres sont le résultat des mutations fortuites, il faut admettre que les mutations qui ont produit l'habitus et les structures génitales n'ont pas été synchrones. Cette affirmation confirme celle faite dans un autre travail [1] en 1960, selon laquelle la spéciation se produit en deux étapes ; l'une de sous-spéciation quand ont lieu des modifications de l'habitus sous l'influence directe des facteurs externes et la deuxième de spéciation proprement dite quand a lieu brusquement une réorganisation de l'armure génitale, cette dernière à niveau spécifique et dans certains cas à niveau générique.

Examinant l'habitus chez le groupe d'espèces d'*Eurytides* du type podaliriforme de la région néotropicale, chez *Graphium eurous* de la Chine tropicale, *G. antiphates* de Thaïlande, *G. androcles* de Célèbes et *Protographium leosthenes* d'Australie, nous avons l'explication des mutations convergentes, phénomène qui se trouve directement en rapport avec les facteurs du milieu externe tels que les radiations naturelles, la température et l'humidité — les mêmes dans toutes ces régions. Le facies convergent de la série orthogénétique *E. stenodesmus* — *G. androcles* est dû, *probablement, à certaines mutations homologues produites par des gènes homologues. Les mêmes facteurs externes ont produit, dans tous les cas, les mêmes mutations homologues, sélectionnées par la sélection naturelle dans le même sens, orthogénétique, dans toutes les régions mentionnées.*

Si les choses en sont là, nous voulons souligner que nous ne sommes pas d'accord avec B. Stugren [5] qui affirme que « c'est une illusion de

croire qu'en conditions identiques de milieu l'évolution adoptera des voies identiques ». L'auteur explique sa thèse en affirmant que la constitution héréditaire a une structure flexible et que les constellations des gènes, soumises à certains facteurs accidentels, ne seront pas les mêmes chez deux populations qui se développent en milieux identiques. Mais il faut préciser que lorsque nous disons que dans les régions tropicales mentionnées ci-dessus les effets ont été les mêmes, cela ne signifie pas que les « voies » ont été aussi absolument identiques. D'autre part il ne s'agit non plus d'une identité absolue dans l'habitus, puisque les 18 espèces, mentionnées ci-dessus, diffèrent l'une de l'autre. Il s'agit ici d'une même ligne évolutive-générale qui a imprimé au groupe d'espèces un facies général semblable (podaliriforme) en direction orthogénétique ; en ses détails ce facies est varié et diffère d'une espèce à l'autre.

Par conséquent, dans l'étape de sous-spéciation les mutations con-vergentes, qui ont produit l'habitus, ont été orientées orthogénétiquement — comme résultat de l'influence des mêmes facteurs externes, et dans l'étape de spéciation, les mutations, divergentes ont produit les caractères subgénériques et génériques ; ils ont été, cette fois-ci, fortuits. Quoique l'habitus se soit conservé dans ses grandes lignes, chez les 4 genres, les mu-tions ont produit, cette fois, des déviations accentuées dans les genitalia et ainsi sont apparus les 4 genres possédant 4 plans de structure nettement distincts.

Nous espérons que les nouvelles notions de *mutations convergentes orientées* et *mutations divergentes fortuites* vont enrichir la théorie synthé-tique de l'évolution avec quelques nouveaux éléments.

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THE PRESENCE OF LYSOSOMES IN THE OOGENESIS OF CARASSIUS AURATUS GIBELIO

BY

M. I. VARO, R. MEŞTER, D. SCRIPCARIU and L. PECINGINE

The use of combined histoenzymological, electrophoretical and spectrophoto-metrical methods in the identification of lysosomal enzymes enables us to con-sider the vitelline platelets as a lysosomal structure. Oocytic lysosomes occur as small granules, first under the plasmalemma, but as the clear vacuoles arrange themselves at the oocyte periphery, they are spreading under the form of vitel-line platelets in the entire cytoplasm. At that moment the vitelline platelet is formed by the emergence of a phosphoprotein crystalloid in a vesicle with acid phosphomonoesterase, arylsulphatase, arylesterase and beta-glucuronidase.

The presence of lysosomes in animal oocytes made the object of few papers ; Denchar [5] identified histochemically a high amount of cathepsin in vitelline platelets of *Xenopus*, and Rebhun [14] found in eggs of *Spisula* multivesicle bodies of Golgian origin named beta gra-nules by Dalcq [2], Dalcq and Pasteels [3] and Pasteels [12]. In these, basic anilin stains accumulate, determining an intensive metachromasia of these granules. Pasteels [12] detected in *Barnea candida* an acid phos-phomonoesterasic activity strictly located at the Golgi apparatus level. In the same bivalve mollusc, no primary lysosomes were found in oocytes, yet in some vitelline platelets a "constellation of small dotty phosphata-sic granules uniformly spread in platelets"[1] were detected. The author assumes that acid phosphatasic activity is specific to platelets and does not occur by a possible lysosomal contribution. Subsequently the plate-lets are digested, appearing as multivesicle bodies. Summarizing the issue of oocytic lysosomes, Brachet [1] supposes that vitelline platelets could be considered as very changed lysosomes because they contain phos-phoproteinphosphatase, acid phosphatase and cathepsin.

In the present paper the activity of some lysosomal enzymes in the vitelline platelets of crucians (*Carassius auratus gibelio*) oocytes is studied.

MATERIAL AND METHODS

Histochemistry. The ovaries of freshly fished specimens of *Carassius auratus gibelio* were fixed in 2% glutaraldehyde in cacodylate buffer solution 0.05 M, pH 7.4, for one hour at low temperature (4°C). The pieces washed several times with cacodylate buffer solution 0.05 M, pH 7.4, were maintained overnight in the same buffer solution at cold. The pieces were cut

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[1] Quotation from [12].

with a cryotom, at a mean thickness of 10 μ. To identify the acid phosphatase, the sections were placed in Weissenfels medium, for 30 min, at 37°C. Lead phosphate deposits were made visible with ammonium polysulfite. In the mean time, the coupling method with azo-stains was used, namely: for the identification of non-specific acid phosphomonoesterase, AS-MX, AS-BI or AS-CL naphthol phosphate was used coupled with Fast Blue B, Fast Red ITR or Red Violet LB; for the histochemical identification of non-specific esterases AS-D, AS-LC or AS-BI naphthol acetate coupled with Fast Blue BB or Fast Garnet GBC was used; peptidases were identified by L-leucyl-4-methoxy-2-naphthylamide and Fast Blue B. The latter two enzymes were histochemically identified only on fresh preparations.

Electrophoresis. Fresh ovaries were macerated with trypsin and pronase 0.05 % in buffer solution tris-HCl 0.1 M, pH 7.2, for 3 min. The free oocytes washed 3 times with the same buffer solution were homogenized in a Potter homogenizer, in distilled water, at a dilution of 1 : 10, g/v. For an accurate protein extraction the homogenate was kept one hour at low temperature and then centrifuged at cold at 8000 r.p.m., for 10 min. The supernatant obtained after centrifugation was used for a quantitative and electrophoretic determination of some enzymatic activities. By differential centrifugation the oocytic vitelline platelets were isolated, from which a series of proteins were extracted by means of a buffer solution of acetate-acetic acid 0.2 M, pH 5.0.

For electrophoretical identification of the proteins and the activity of some enzymes, the disc-electrophoresis method on polyacrylamide gel, according to Davis' technique [4] was used. Proteins were rendered evident with Amido Black 10 B solution; the acid phosphatase was electrophoretically identified by placing the gels in an incubation medium, similarly to the histochemical techniques.

The activity of the acid phosphatase was quantitatively determined according to Linhardt and Walter's technique [11], using beta-glycerophosphate as substrate. The released orthophosphate obtained by the enzymatic reaction was determined according to Fiske and Subarrow's method [10].

The proteins of the oocytic supernatant and of the vitelline platelets extract were determined spectrophotometrically at 280 nm.

RESULTS

Histoenzymological study. Young oocytes show a very reduced localization of the acid phosphomonoesterase. The enzyme appears as small granules under the plasmalemma and at the nucleoli surface; they can be detected by lead technique, as well as by association with diazonium salts, particularly on very young ovaries where the weak growth of oocytes was ended. In a previous paper [15] we pointed out in such ovaries, a parallelism between RNA distribution and a series of basic proteins in cytoplasm and nucleoli.

As soon as the clear vacuoles — initially situated perinuclearly and then in the entire cytoplasm — appear, the cortical proteic vitelline globules are readily visible due to their high content in acid phosphomonoesterase.

During subsequent vitellogenesis, the globules spread in the cytoplasm among the cortical clear vacuoles, after which they begin to change into vitelline platelets. At this stage, the clear vacuoles migrating under the plasmalemma become cortical and the vitelline globules give an intensely positive reaction for the acid phosphomonoesterase (Plate I, *A*, *B*).

There is a moment when the vitelline globules are spread in the whole cytoplasm among the mucopolysaccharide clear vacuoles and the amount of acid phosphatase at their level remains high. After this stage they begin to change into true vitelline platelets, with an inner concentration of phosphovitin and lipovitins and a positive enzymatic reaction in the plate-
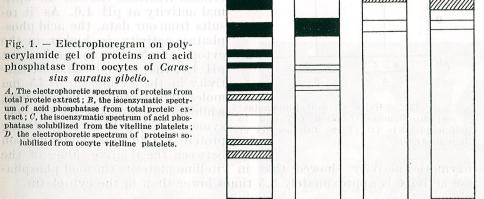
lets matrix (Plate I, *C*). Over this period, the cortical vitelline globules at the oocyte periphery disappear.

The same location was found for non-specific esterase, giving intense positive reactions, except for the nucleoli in the young oocytes. At the beginning, very small granules appear under the plasmalemma, whilst the reaction with diazonium salts shows a rich enzymatic activity in one or more juxtanuclear zones (Plate I, *D*, Plate II, *B*).

Noteworthy is the fact that both acid phosphatase and non-specific esterase have the same intracellular location, in vitelline globules as well as in platelets.

Arylsulphatases show a somewhat different location. They do not appear juxtanuclearly and the vitelline globules and platelets show an intensely positive reaction (Plate II, *C*). Leucylaminopeptidases have an oocytic intracellular location similar to that of sulphatases (Plate II, *D*).

Electrophoretic study. To elucidate the nature of acid phosphatase in cytoplasm and oocytic vitelline platelets, an electrophoretic study was carried out on the two cellular fractions separated by differential centrifugation (Fig. 1). The electrophoretic spectrum of the proteins from the

Fig. 1. — Electrophoregram on polyacrylamide gel of proteins and acid phosphatase from oocytes of *Carassius auratus gibelio*.

A, The electrophoretic spectrum of proteins from total proteic extract; *B*, the isoenzymatic spectrum of acid phosphatase from total proteic extract; *C*, the isoenzymatic spectrum of acid phosphatase solubilized from the vitelline platelets; *D*, the electrophoretic spectrum of proteins solubilized from oocyte vitelline platelets.

supernatant of the oocytic proteic extract (cytoplasmic or postmitochondrial fraction) showed 12 proteic bands with different electrophoretic mobilities. A comparison between the protein electrophoregram of cytoplasmic fractions and vitelline proteins revealed that the latter have only 4 bands, of which 3 with slow migration and a fourth one in the lower portion of the gel.

The electrophoretic spectrum of acid phosphatase in the oocytic cytoplasm fractions shows 3 isoenzymatic fractions, of which a major one with a high enzymatic activity. The electrophoretic spectrum of the

acid phosphatase from the vitelline platelets appears as very particular (Fig. 1). Three isoenzymatic fractions were electrophoretically identified, of which two are specific to vitelline proteins. The isoenzyme with a high electrophoretic mobility is also present in the cytoplasmic fraction, appearing like a contamination of the latter.

Electrophoretic data demonstrate almost with certainty that the two oocytic acid phosphatases — in the cytoplasm and in vitellus — differ in their behaviour in the electrophoretic field.

Quantitative study. The activity of acid phosphomonoesterase in the cytoplasm fraction and in the proteic extract of vitelline platelets was also followed up (Fig. 2). The study of acid phosphatase activity depending on pH revealed that the enzyme in both cellular fractions showed an optimal activity at pH 4.0. As it results from our data, the acid phosphatase activity is higher in the cytoplasm than in the vitellus : at pH 4.0, the acid phosphatase activity in the cytoplasm is 75 mμ moles P$_i$/mg protein per 30 min, while in vitelline platelets this enzyme activity is 28 mμ moles P$_i$/mg protein per 30 min. A comparison between the relative values of the enzymatic activity showed that in vitelline platelets the acid phosphatase activity is approximately 2.5 times lower than in the cytoplasm.
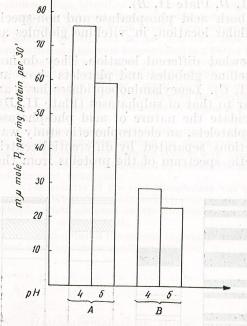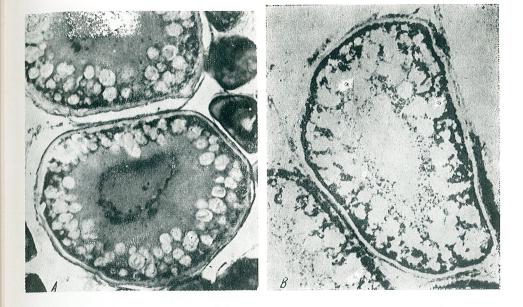
Fig. 2. — The activity of acid phsophatase in post-mitochondrial fraction (A) and in vitelline platelets (B) cf the fish oocytes at different pH.

DISCUSSION

The histochemical investigation of fishes oocytes made the object of numerous studies, yet the problem of the enzymatic and particularly the acid phosphatase activity is still poorly elucidated [5] — [9] [15] — [18]. The studies of Pasteels [12] on *Barnea* showed that in vitelline platelets which have acid phosphatase, a change takes place transforming them into multivesicled bodies. The idea of their permanent reshuffling is supported by the studies of Donato et al. [6] — [8], while Dalcq [2] [3] noticed a relationship between vitelline platelets and multivesicled bodies (named beta granules), suggesting that platelets turn into a lysosomal type structure. Studying vitellogenesis in *Lebistes reticulatus*,
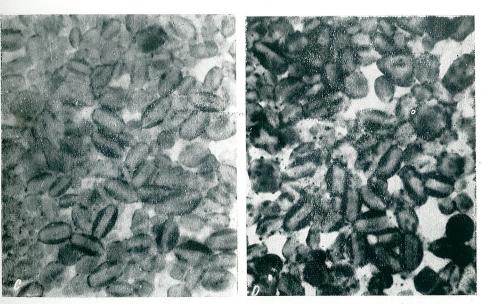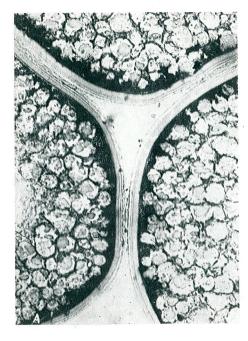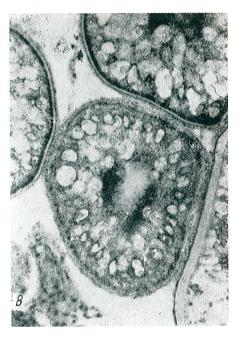
Plate I. — *A*, Oocytes with polysaccharide cortical vacuoles in which acid phosphomonoesterase is located perinuclearly and in cortical granules. Azo-coupling method.
B, Oocytes with polysaccharide cortical vacuoles spread in the cell; among them granules appear which give a positive reaction with Pb, for the acid phosphatase.
C, The vitelline platelets in which the acid phosphatase appears in matrix by histochemical reaction. Azo-coupling method.
D, In the matrix of the vitelline platelets a positive reaction for non-specific esterase was visualized. Azo-coupling method.
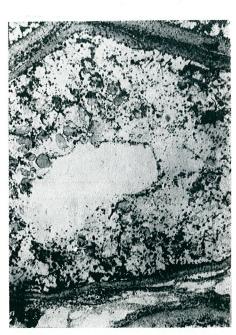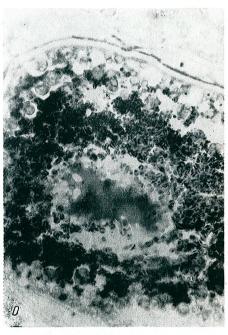
Plate II. — A, Non-specific esterase in cortical granules, among clear vacuoles. Azo-coupling method.
B, Oocytes cortical granules with non-specific esterase. Azo-coupling method.
C, Oocyte cortical granules with arylsulphatase. Azo-coupling method.
D, Oocyte cortical granules with aminopeptidase. Azo-coupling method.

Droller and Roth [9] noticed the formation of dark granular corpuscles in the Golgian system, which fuse into a vitellus drop delimited by a membrane. Such granules with a high arylsulphatase content were also described by Varo [15] [16].

As to the origin of vitelline platelets the opinions are controversial : they may be formed by the Golgi apparatus the vesicle of which fuse into crenellated corpuscles ; on the other hand, vitelline platelets may occur from endoplasmic reticulum, which turn into large vesicles fusing with dark crenellated corpuscles. To this vitellus with double origin, exogenous material can be added by pinocytic processes of some plasmatic proteins. Such proteins were found in the oocytes of *Carassius auratus* and *Cyprinus carpio* [17] .

During our investigations we observed the formation of some small granules with a high acid phosphomonoesterasic and arylesterasic activity in the subcortical area, which grow as the oocyte develops and invade the entire cytoplasm through the mucopolysaccharide clear vacuoles. The acid phosphatase of vitelline platelets differs from the perinuclear one, because it appears with the formation of mucopolysaccharide clear vacuoles. At the same time a protein segregation takes place (phosphovitin and lipovitins), under the form of crystalline concentrations in the vitelline platelets matrix, which contains an acid phosphatase, non-specific esterase, leucylaminopeptidase and arylsulphatase.

The presence of these enzymes makes us view the vitelline platelets in *Carassius auratus* as a particular form of lysosomes. This opinion is also supported by Denchar's observations [5], who found in the vitellus homogenates a cathepsinic activity characteristic of lysosomal formations. Moreover, the transformation of the vitellus into multivesicle bodies pleads for the same idea. Such cellular cytoplasmic structures in which a series of proteins crystallize, being then involved in the intracellular digestion are well known with leucocytes, where the eosinophil granules participate in the phagocytosis with lysosomal role.

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OSMOTICITY DEPENDENCE OF THE ACID-INDUCED CHANGES IN THE MITOCHONDRIAL ULTRASTRUCTURE

BY

MARIA-LUIZA CĂLUGĂREANU

The objective of the present study has been to investigate the kinetics of the ultrastructural response of the mitochondrial compartments which develops in the presence of a discontinuum gradient of HCl, in media of variable osmoticity. The progressive protonation of liver mitochondria under isoosmotic conditions (0.3 M sucrose) results in a stepwise swelling (pH : 6.5 — 3.5), which is followed by gradual lysis of the outer membranes, that attains maximal intensity at pH 1.5. Lower pH-s (1.0) bring about histological fixation of specimens.

Increased osmoticity of the suspending medium diminishes both the amplitude and the time course of the acid effect, while hypoosmotic media (H₂O dist.) induce a two-step response. This consisted of a sudden reversal of the hypotonic swelling and the subsequent distension of the mitochondrial bag (down to pH 3.5).

Recent studies of the ion transport through the mitochondrial membranes have shown the ability of these organelles to concentrate or to secrete various ions (Na^+, K^+, Ca^{++}, Mg^{++}, Sr^{++}, H^+). It is now recognized that changes of the proton gradient on the two mitochondrial membrane surfaces induce deep modifications both in the mitochondrion structure [7] and in its functions [12]. Thus, a moderate increase of the H^+ concentration in the suspending medium, which inhibits the intramitochondrial NADH oxidation, diminishes respiration [2], while conformational changes of the mitochondrial membrane have been induced by excess H^+ ions [13].

On the other hand, Fleischer et al. [4] have shown that excess protons are capable of releasing a negatively charged protein, from the outer membranes of several species of mitochondrion, which seems to account for the maintenance of the *in vivo* membrane configuration [1] [9].

Other studies on the interaction between mitochondrial membranes and external H^+ ions have recently been reported [6] [7] [11]. These authors have demonstrated that the inner mitochondrial membrane can be structurally dissected by means of strong or weak acids, which proved to be instrumental in releasing the matrical compartment.

In an attempt to correlate these data with the ultrastructural modifications, we have tried to evaluate by means of transmission electron-microscopy, the kinetics of the response of the various mitochondrial compartments, as induced by a discontinuum gradient of HCl in media with variable osmoticity.

MATERIAL AND METHODS

Monkey (*Cercopithecus aethyops*) liver mitochondria (MLM) were obtained by the procedure already described [8], which is based on the Schneider technique, supplemented by a correction meant to eliminate the extraparticulate watery phase from the pellet.

The isolated mitochondria were incubated in a discontinuum pH gradient performed in iso-, hyper- or hypotonic media (0.3 M; 1.5 M sucrose and distilled water respectively) at a ratio of 2.4 mg of mitochondrial protein per ml. The reproducibility of the results was ensured by maintaining rigorously the same time (17 min) of incubation in the acid solution.

Fixation was done in 2.5 % glutaraldehyde and postfixation in OsO_4. The ultrathin sections obtained, after embedding in Vestopal W, on a Porter-Blum ultramicrotome, were doubly stained with uranyl acetate and lead citrate, and examined on a Hitachi H11 electronmicroscope.

RESULTS

1. DEPENDENCE OF THE ULTRASTRUCTURAL RESPONSE OF MLM ON INCREASING EXTERNAL H^+ CONCENTRATION IN AN ISOTONIC MEDIUM

The sequence of the ultrastructural changes of liver mitochondria, which develop during acid titration in 0.3 M sucrose, are reproduced in plate I.

The incubation conditions corresponded to state I described by Chance and Williams [3], when ADP and substrate were missing from the incubation medium.

It is seen that mitochondria incubated in 0.3 M sucrose, final pH 6.5 (Fig. 1) and 6.0 (Fig. 2) exhibit incipient swelling consisting of a slight enlargement of the outer compartment, a decrease of the electronoptic density, extension of the matrical area and distension of the intracristal spaces. At this pH value, the outer and inner membrane enclosure is still apparently undamaged, and "condensed" forms, described by Hackenbrock [10] as actively respiring forms in 0.25 M sucrose, are still visible.

The fact that at this stage of protonation, the mitochondrial swelling mostly implies the enlargement of the sucrose accessible space, while the inner compartment keeps constant size, points to the protectory role of the outer membrane to acid-induced changes at this pH level.

At pH = 5 (Fig. 3) a disjunction of the mitochondrial reaction to swelling appears : there are to be seen non-swollen mitochondria with intact outer and inner membranes, as well as swollen mitochondria which present an expansion of the matrix area with an irregularly aggregated content, which may be considered, by correlating the ultrastructural aspect with other physical data [5], as the beginning of the matrical protein release from the swollen mitochondria. Figure 4 (pH = 4.0) shows the appearance of matrical vesiculations and the fade-out of the crystal configuration.

Lower pH-s (3.5 and 3.0) (Figs. 5 and 6, respectively) bring about the beginning of the outer membrane lysis, whose dissociated fragments form a sort of emptied bags, with an electron-opaque adhering material, which may be considered as part of the matrical protein released from the membranous bags.

A marked lysis of the outer and inner membrane occurs down to pH = 2.5 (Fig. 7). The outer membrane forms emptied bags, which were already present at pH = 3.5 (Fig. 5), while the inner membrane turns to vesicles, which are deprived of any adhering electron-opaque material.

At pH = 1.5 (Fig. 8) the total lysis of the mitochondrion is recorded, and the same two types of membranous vesicles are evident.

At pH = 1.0 (Fig. 9) the histological fixation of the swollen mitochondria occurs, the apparent integrity of the membranous system and of the matrix is conserved, due to superprecipitation of the material filling up the inner compartment. At this pH value a decrease of the matrical protein release and of the water uptake is recorded [7] [8].

2. ACID-DEPENDENCE OF THE CONFIGURATION CHANGES IN A HYPERTONIC MEDIUM

If we examine the changes induced by acidification of the external medium under hyperosmotic conditions, the whole process is similar, but higher sucrose concentrations result in a marked decrease of the acid effect (Plate II).

In samples obtained by incubation of mitochondria in 1.5 M sucrose at pH 6.5 (Fig. 10) and 5.0 (Fig. 11) the presence of three types of organelles is observed : condensed, relatively orthodox and swollen forms, respectively. The existence of these forms cannot be evidenced by absorption measurements [6], which furnish an average value.

The condensed forms, which characterize the active, respiratory state, have a decreased matrical volume and consequently an increased optical density of the matrix; the integrity of the outer and inner membrane is conserved and increased matrical vesiculations occur.

The continuity of the membranous system is maintained also when considering the other two forms : orthodox and swollen, whose matrix is normal and expanded, respectively.

At pH = 4 (Fig. 12) the swollen forms prevail. The swelling observed at this H^+ ion concentration is chiefly due to the matrical vesiculation, which is due to the expansion of the intracristal spaces. Therefore the outer mitochondrial compartment increases firstly, suggesting that passive penetration of H^+ ions in or across the inner membrane increases sharply at this acid value.

At pH = 3.5 (Fig. 13) the mitochondrial swelling is considerably amplified; the integrity of the membranous system is apparently maintained and the matrix is characterized by a low electronoptic density.

The appearance of swelling suggests that the hypertonic sucrose decreases the lytic effect of H^+ ions on the outer membrane and potentiates the permeabilization of the inner membrane to added H^+ ions, allowing a larger amplitude swelling of the inner compartment as compared to 0.3 M sucrose, which virtually causes the swelling of the outer compartment (matrical vesiculations).

The pH values down to 3.0 (Fig. 14) and 2.5 (Fig. 15) induced a large amplitude swelling, and expansion of the matrix. The electron-opaque material which is described under isoosmotic conditions is no longer visible. The structural continuity of the membranous system is still intact.

3. ULTRASTRUCTURAL RESPONSE OF MLM TO ACID TITRATION UNDER HYPOTONIC CONDITIONS

As seen in plate III, the acid disruption of the mitochondrial membranes proceeds in a quite different manner in a sucrose-free medium.

When MLM are titrated with standard HCl in dist. water, a sudden reversal of the water-induced swelling takes place, over the pH region between 6.5 and 5.2 (Fig. 16).

In contrast to water-swollen MLM (pH = 6.9), a strong contraction of the outer membrane occurs upon lowering pH over the energized acid range (7.0 — 5.2) [2], which is accompanied by a marked condensation of the matrical area (Fig. 17). This contraction takes only place in mitochondria with a virtual respiratory activity, a fact which suggests that the response to acid changes in this pH region is an active process, which requires the integrity of the energy-dependent mechanisms of the swelling — contraction cycle.

By lowering pH below 5.3 (Fig. 18), which results in the suppression of the energized reactions, the contraction is replaced by the distension of the membranous enclosure. On the other hand (Fig. 19), these hypotonic conditions amplify the sensitivity of the mitochondrial membranes to added H^+ ions and the quantitative disruption of both membranes occurs at a pH of some 3.5. At this pH value both the outer membrane and the cristae of the internal membrane undergo a rapid disintegration, accompanied by the release of the mitochondrial matrix. It is worth noting that the characteristic density pattern of the mitochondrial content has completely disappeared, and the cicatrization of the membranous remnants occurs as in the sucrose-rich media (Fig. 20).

Under hypotonic conditions the disruption of the membranous system takes place in relation to the acid concentration; it is evident that lower pH values (1.0) have no more an inhibitory effect on the acid disintegration (Fig. 21).

DISCUSSION

It is reasonable to assume that the physical mechanism involved in the disruption of the mitochondrial membranes, by increasing proton concentration in the suspending medium does not occur as a direct effect of the caustic action of the higher acid concentrations. This conclusion results from the different responses in the presence or absence of sucrose.

The electronmicroscopic images suggest that the acid effect is inhibited by solutes, such as sucrose, which do not penetrate the inner mitochondrial membrane; by increasing the tonicity of the external medium, this type of substances would act by modifying the osmotic
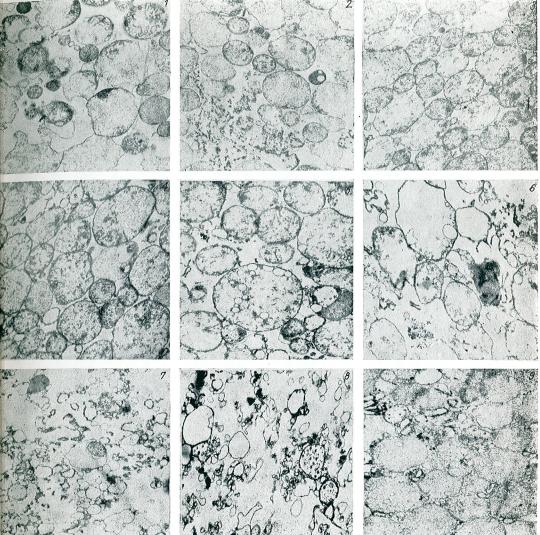
PLATE I. — Electron micrographs of the liver mitochondria incubated in 0.3 M sucrose in a discontinuum pH gradient. (Details in the text).
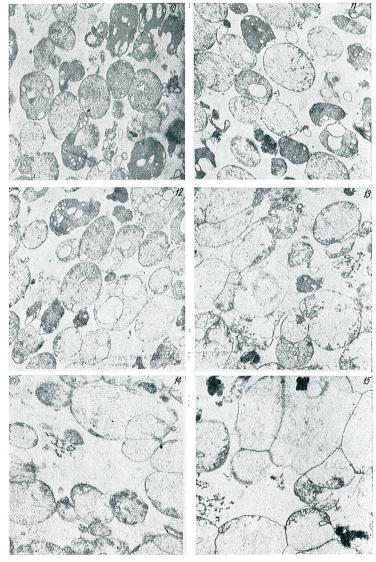
PLATE II. — Electron micrographs of the liver mitochondria incubated in 1.5 M sucrose in a discontinuum pH gradient. (Details in the text).
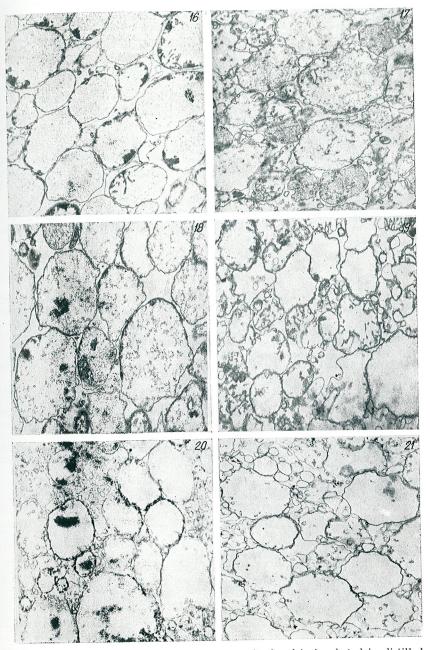
PLATE III. — Electron micrographs of the mitochondria incubated in distilled water in a discontinuum pH gradient. (Details in the text).

pressure of the outer phase, and this fact could probably result in reducing the proton influx through the mitochondrial membrane.

The fact that the osmotic pressure of the external phase significantly affects the time course of the acid effect, suggests that the passive transport mechanisms of the mitochondrial membrane participate, at least partly, in this process. The biphasic mitochondrial response to higher or lower acid concentrations ranging above or below pH = 5.2, is another argument for involving these mechanisms in the proton transport through the outer and inner membrane.

By comparing the shrinking effect induced over the energized pH region (pH 5.2 — 6.8), when a normal respiration is possible, with the distension process which occurs under this pH value (in the absence of intramitochondrial NADH oxidation), it results that the mitochondrial contraction — swelling cycle induced by excess H^+ ions is critically dependent on the functional preservation of the energy linked reactions in the inner membrane.

This latter type of mitochondrial response has a special importance, since it precedes the acid dissociation of the outer and inner membrane in view of the quantitative release of the matrical content.

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— Control group, injected with saline solution (filtered and sterilized natural sea water, containing 1% gelatine "Merck");
— Group injected with saline solution + insulin;
— Control group, injected with saline solution + glucose;
— Group injected with saline solution + glucose + insulin.

The compounds were injected into the hepatopancreas *via* ligament, giving either 50 microliter (for mussels of middle size) or 100 microliter solutions (for mussels of large size) by means of a microsyringe, having an adjustable needle N° 20.

Recrystallized ox insulin ("Calbiochem", San Diego, Calif., Lot 201248, grade B, 26.1 IU/mg) was injected in a dose of 2.5 IU/100 g b.w., while glucose ("UCB", Belgium) in a quantity of 50 mg/100 g b.w. (without shell and interpallial water).

Six hours after injection, the shells of the animals were opened following unilateral sectioning of the posterior adductor muscle, the interpallial water was blotted by filter paper, and haemolymph was collected in a china capsule.

In other experiments, the mussels (mean length = 4.88 cm) were injected with saline solution (without gelatine), or with saline solution containing 12.1 mg alloxan ("Austranal") per 100 g b.w., and after 12 or 24 hours the haemolymph was collected using the above-mentioned procedure.

The determination of glucose was made from 100 μ liters haemolymph using the glucose-oxidase-peroxidase method of Krebs et al. [12]. The trials were measured at 545 nm, using a spectrophotometer VSU-2G (Carl Zeiss, Jena).

The data (expressed as mg glucose/100 ml haemolymph) were calculated and compared statistically according to Student's *t* test, P values of 0.05, or less, being considered statistically significant.

RESULTS

The data referring to the effect of insulin upon the quantity of glucose in haemolymph, depending on the size of *Mytilus galloprovincialis* are summarized in figure 1, while the per cent modifications of glycemia as compared to the control values are presented in figure 2.
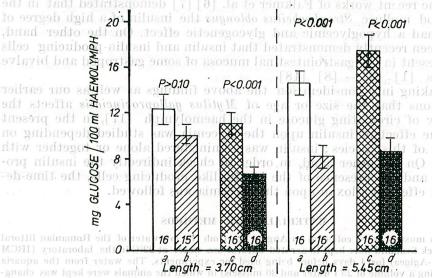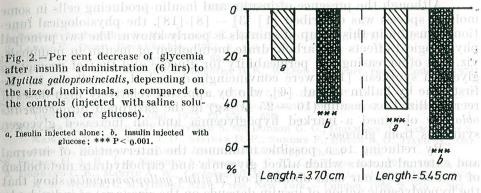
Fig. 1. — Effect of insulin (6 hrs after administration) upon the quantity of glucose in haemolymph of *Mytilus galloprovincialis*, depending on the size (length in cm).

a, Control, injected with saline solution; *b*, injected with insulin (2.5 IU/100 g b.w.); *c*, control, injected with glucose (50 mg/100 g b.w.); *d*, injected simultaneously with glucose (50 mg/100 g b.w.) and insulin (2.5 IU/100 g b.w.). Figures in columns represent the number of experiments.

One can see that in the mussels of middle size, 6 hours after insulin administration the glycemia shows a slight decreasing tendency from the mean value of 12.16 ± 1.31 to 9.71 ± 0.80 mg/100 ml (decreasing by 20.15%, P > 0.100). On the contrary, the concomitant administration of glucose and insulin to mussels of this size group induces a significant decrease of glycemia (40.02%), the quantity of glucose in the haemolymph diminishing from 10.97 ± 0.84 to 6.58 ± 0.57 mg/100 ml (P < 0.001).

The data show that in mussels of large size, 6 hours after insulin administration the glycemia decreases by 43.95%, i.e. from 14.54 ± 1.03 to 8.15 ± 0.89 mg/100 ml (P < 0.001). In the case of simultaneous administration of insulin and glucose to mussels of large size it may be observed a greater diminution of glycemia than in the case of hormone

Fig. 2.—Per cent decrease of glycemia after insulin administration (6 hrs) to *Mytilus galloprovincialis*, depending on the size of individuals, as compared to the controls (injected with saline solution or glucose).

a, Insulin injected alone; *b*, insulin injected with glucose; *** P < 0.001.
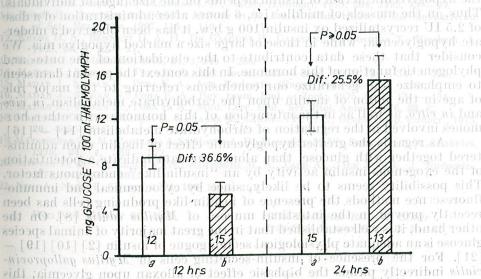
Fig. 3. — Effect of alloxan (12.1 mg/100 g b. w.) upon the quantity of glucose in haemolymph of *Mytilus galloprovincialis*, depending on the time following administration (12 and 24 hrs, respectively).

a, Controls, injected with saline solution; *b*, injected with alloxan. (Mean length of animals = 4.88 cm.) Figures in columns represent the number of experiments.

administration alone : the level of haemolymphatic glucose decreased by 50.63% as compared to the corresponding control values, i.e. from 17.44 ± 1.38 to 8.61 ± 1.22 mg/100 ml haemolymph (P < 0.001).

As regards the effect of alloxan upon glycemia, depending on the time, the data from figure 3 show that 12 hours after its administration this beta-cytotoxic compound causes a diminution of glycemia by 36.68% (i.e. from 8.66 ± 1.94 to 5.23 ± 1.13 mg/100 ml, P = 0.05), while at 24 hours it elicits a slight hyperglycemia ; the glucose concentration of haemolymph increased from 12.18 ± 1.31 to 15.28 ± 2.35 mg/100 ml (P ≥ 0.05).

DISCUSSION

Although the presence of insulin and insulin-producing cells in some mollusc species was described [1] [3] — [8] [18], the physiological function of insulin in this group of animals is poorly known. The two principal physiological effects on carbohydrate metabolism of insulin in mammals, viz. that of increasing cell permeability for glucose and that of promoting glycogen synthesis [13], were convincingly described in molluscs for the first time by Falkmer et al. [6], who by applying relatively high doses of recrystallized ox insulin (10 — 25 IU/kg) in the gastroped *Strophocheilus oblongus* obtained a marked hypoglycemia and an increased glycogen synthesis from glucose.

By reducing to a possible minimum the intervention of internal and external factors which affect glycemia and carbohydrate metabolism of molluscs [9], our data obtained on *Mytilus galloprovincialis* show that the hypoglycemic action of insulin depends on the size (age) of individuals. Thus, in the mussels of middle size, 6 hours after administration of a dose of 2.5 IU recrystallized ox insulin/100 g b.w. it has been observed a moderate hypoglycemia, while in those of large size a marked hypoglycemia. We consider that these data contribute to the elucidation of the onto- and phylogenetic function of this hormone. In this context the present data seem to emphasize and generalize our conclusions referring to the major role of age in the action of insulin upon the carbohydrate metabolism *in vivo* and *in vitro*, as well as in the interaction of this hormone with other hormones involved in the regulation of carbohydrate metabolism [14] — [16].

As regards the greater hypoglycemic effect of insulin when administered together with glucose, than alone, our data indicate a potentiation of the exogenous insulin activity by an "insulin-like" endogenous factor. This possibility seems to be likely, since by cytochemical and immunofluorescence methods the presence of insulin-like producing cells has been recently proved in the intestinal mucosa of *Mytilus edulis* [8]. On the other hand, it is well established that in the great majority of animal species glucose is an adequate physiological secretagogue of insulin [2] [10] [19] — [21]. For the presence of insulin-secreting cells in *Mytilus galloprovincialis* indirectly pleads the biphasic effect of alloxan upon glycemia, this effect being characteristic of the beta-cytotoxic and diabetogenic action of alloxan in mammals [11]. Our histological and cytological investigations referring to the presence of insulin-like producing cells and their possible modifications under the action of alloxan in this species are in progress.

CONCLUSIONS

1. Recrystallized ox insulin in *Mytilus galloprovincialis* of middle size has a moderate hypoglycemic action, while in those of large size the hypoglycemic effect of the hormone is significant.

2. In both size groups of mussels insulin administered simultaneously with glucose induces a greater hypoglycemia than administered alone. This fact suggests the potentiation of the hypoglycemic effect of exogenous insulin by an endogenous "insulin-like" factor, as well as the presence of insulin-like producing cells in this species.

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SEASONAL VARIATIONS OF FATTY ACID BIOSYNTHESIS AND RELATED ENZYME ACTIVITIES IN LIVER OF FROG (*RANA ESCULENTA* L.)

BY

ANCA CRISTEA and EUGEN A. PORA

Incorporation of D-(U-^{14}C)-glucose and (2-^{14}C)-acetate into fatty acids of frog (*Rana esculenta* L.) liver slices and the activity of some NADPH-generating enzymes in relation to lipogenesis have been studied. The results show that incorporation of acetate is much higher than that of glucose in all the seasons. There were also significant seasonal differences in the incorporation of both precursors. The activity of some NADPH-generating enzymes does not seem to be a rate limiting step in fatty acid biosynthesis.

The incorporation of various precursors into fatty acids as well as the activity of NADPH-generating systems in different mammalian species are experimentally well documented [2] [5] [7]—[11]. Both parameters are, however, less studied in lower vertebrates. In a previous paper [4] we showed that *in vivo* fatty acid biosynthesis in the liver of *Rana esculenta* L. is subjected to seasonal variations. In the present paper we present results concerning the incorporation of D-(U-^{14}C)-glucose and (2-^{14}C)-acetate into fatty acids in liver slices, as well as the activity of some NADP-dependent dehydrogenases in the liver during a complete yearly cycle.

MATERIALS AND METHODS

Chemicals. The coenzymes were obtained from either Boehringer Mannheim or Sigma Chem. Co.D-(U-^{14}C) -glucose was from NEN and (2-^{14}C)-acetate from the Radioisotopes Factory, Leningrad, USSR.

Animals. Male frogs *Rana esculenta* L. were used. The animals for winter series of experiments were caught in October and maintained under appropriate conditions at $4 — 6°C$ until December—January; the animals for spring, summer and autumn were caught at the beginning of May, middle of July, and middle of October, respectively, and immediately submitted to experiments.

ASSAY OF FATTY ACID SYNTHESIS FROM D-(U-^{14}C)-GLUCOSE AND (2-^{14}C)-ACETATE BY LIVER SLICES

The liver of animals was exposed like in a previous work [4] and perfused *in situ* with approx. 10 ml of cold Krebs-Ringer bicarbonate buffer, pH 7.4. Liver was sliced and rinsed in Krebs-Ringer bicarbonate buffer, pH 7.4, and about 300 mg of slices were transferred into Erlenmeyer flasks (25 ml) at a final volume of 5.0 ml Krebs-Ringer bicarbonate buffer, pH 7.4 (previously gased with $CO_2 : O_2(5 : 95)$), containing the substrates at the following concentrations : $3 — 4$ μCi D-(U-^{14}C)-glucose and 5 mM glucose ; $3 — 4$ μCi (2-^{14}C)-acetate and 5 mM Na-acetate. The vessels were gased for 5 min with a mixture of $CO_2: O_2$ (5 : 95), then

closed with rubber stoppers. Incubation was performed at 29°C for 90 min in an atmosphere of CO_2 : O_2 (5 : 95) in a shaking water bath. At the end of incubation, the slices were separated from the medium by filtration, rinsed in Krebs-Ringer bicarbonate buffer, and transferred into a hydrolyzing flask, containing 5 ml of 5% methanolic KOH, 1 ml distilled water and 0.5 ml of 1% methanolic palmitic acid (as carrier). The content was saponified on a boiling water bath for 3 hours. The resulted mixture was extracted three times with 5 — 10 ml portions of petroleum ether (30 — 60°) and the extracted fractions were removed. The remained content was acidified at pH 1 — 2 and extracted four times with 10 ml portions of petroleum ether. The combined fractions were evaporated at room temperature. After evaporation the fatty acids were dissolved in scintillation solution as previously described [4]. The radioactivity was measured using a Betaszint spectrometer.

ASSAY OF NADP-DEPENDENT DEHYDROGENASES IN RELATION TO LIPOGENESIS

The enzyme activities were determined in supernatant of 10% liver homogenate, prepared as previously described [4] except for the homogenizing medium, which was 154 mM KCl.

All enzyme activities were determined according to [3], following the absorbancy at 340 nm in a USU—2 recording spectrophotometer.

RESULTS

As seen in table 1, acetate incorporation into fatty acids is approximately 12-fold higher than that of glucose in all the seasons.

Table 1

Incorporation of D-(U-14C)-glucose and (2-14C)- acetate into fatty acids in liver slices of *Rana esculenta* L.

Season	Glucose (n moles/100 g body wt/h)		Acetate (n moles/100 g body wt/h)	
Spring	1.4±0.05	(6)*	15.6± 1.8	(10)*
Summer	5.7±0.4	(6)	70.8± 2.2	(9)
Autumn	9.6±1.6	(9)*	135.8±17.8	(10)*
Winter	3.7±0.3	(6)*	44.8± 5.6	(10)*

The values in the table are mean±S.E.M. with the number of animals in parentheses. * shows a statistically significant difference at P<0.001 when compared to the control.

Table 2

Activities of NADPH-generating enzymes in the liver of *Rana esculenta* L.

Season	G6PDH + 6PGDH µ/g wet wt		MDH µ/g wet wt		ICDH µ/g wet wt	
Spring	3.09±0.02	(9)*	0.47±0.01	(9)*	1.59±0.06	(9*)
Summer	3.88±0.13	(9)	0.60±0.03	(9)	4.05±0.01	(9)
Autumn	6.23±0.08	(7)*	0.81±0.01	(7)*	6.02±0.01	(7)*
Winter	3.40±0.09	(9)*	1.04±0.03	(9)*	2.90±0.06	(8)*

The values in the table are mean±S.E.M. with the number of animals in parentheses.* same as for table 1.

Enzyme activities (Table 2) show that there are significant variations with the seasons. These activities are much higher than the necessity for fatty acid biosynthesis in all the seasons.

Finally, one can see in figures 1 and 2 that there is a close relationship between fatty acid biosynthesis and the activity of NADP-dependent dehydrogenases.

DISCUSSION

Our data show that there are significant variations in fatty acid biosynthesis connected with the seasons. Taking the summer value as a term of comparison, one can see that the maximum velocity of the process takes place during autumn, while the minimum one during spring. In all the seasons the acetate incorporation into fatty acids is about 12-fold higher than that of glucose, suggesting that formation of acetate from glucose, but not its conversion into fatty acids is the rate limiting of fatty acid biosynthesis.

On the other hand, one can see that the activity of the NADPH-generating enzymes is much in excess as compared to the needs for NADPH of fatty acid biosynthesis from either glucose or acetate and that it does not seem to be rate limiting for fatty acid synthesis. Some conclusions were reached by Baranska and Wlodawer [1] on the basis of measurement of G6P- and malate-DH in the liver of frogs acclimated to 7° and 20°C.

Our data show that there is a close correlation between fatty acid biosynthesis and the activity of NADPH-generating enzymes. The minimum and maximum points of both parameters are closely related, which suggests that the measured enzymes ensure reducing equivalents for fatty acid biosynthesis and also for some other reductive biosynthesis.

The shape of the curve representing the intensity of fatty acid biosynthesis shows an enhancement of this process during summer and autumn, suggesting its involvement in ensuring a reserve of energy for the cold seasons. Our data are in agreement with those of Pasanen and Koskela [6], showing that the highest content of lipids in liver is detected during the months of summer and autumn, while the lowest, during the months of winter and spring.

CONCLUSIONS

1. Acetate incorporation into fatty acids is about 12-fold higher than that of glucose in all the seasons, suggesting that formation of acetate from glucose is rate limiting for fatty acid biosynthesis.

2. The activity of the NADPH-generating enzymes does not seem to be rate limiting for fatty acid synthesis, but there is a close relationship between the biosynthesis of fatty acids and their activities.
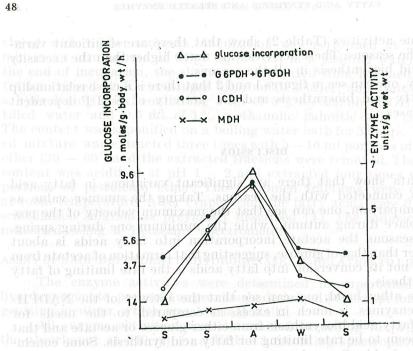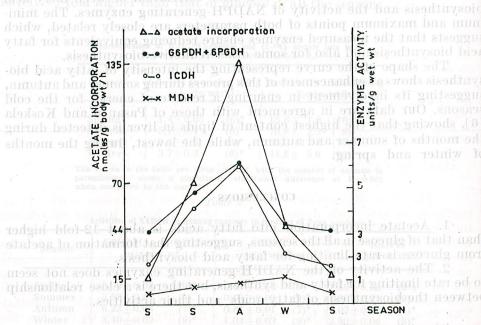
Fig. 1. — Glucose incorporation into fatty acids and enzymes activities in the liver of *Rana esculenta* L.

Fig. 2. — Acetate incorporation into fatty acids and enzymes activities in the liver of *Rana esculenta* L.

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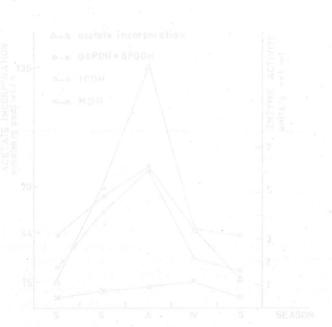
EFFECT OF CYCLOPHOSPHAMIDE ON THYMUS AND BURSA FABRICII

BY

RODICA GIURGEA and VIRGIL TOMA

Cyclophosphamide in intramuscular doses of 3 mg/kg induces in 5-day Studler-Cornish chickens an involution of the T and BF : the weight of the glands and their contents of proteins, RNA and DNA decrease, their glycogen content increases. The serum gamma-globulin is reduced. The involution is earlier in the BF ; in the T it develops gradually during the treatment.

The cyclophosphamide (CPA) is considered as an immunosuppressive antimytotic agent since it blocks the lymphocytes multiplication by interfering with the DNA synthesis [8]. Both the thymus (T) and the bursa Fabricii (BF) are central organs in the system of humoral immunity in birds, having a high content of nucleic acids [3] [12]. Since the immunobiologic capacity of birds develops gradually after hatching we have studied the effect of CPA on the T and BF in juvenile chicken [4].

Hybrid, tetralinear Studler-Cornish chicken of five days were given intraperitoneally CPA in a dose of 3 mg/kg b.w./day, four days, as freshly prepared aqueous solution (cyclophosphamide was from VEB Jenopharm, GDR). At 24 hrs after inoculation the animals were sacrificed. Control group received only the solvent, in the same volume as inoculated animals. The total proteins (TP) [6], RNA and DNA [11] as well as the glycogen (G) content [7] were assayed in both T and BF. The weight of the organs was also determined. Ascorbic acid (AsA) in adrenals [5] and the content of serum gamma-globulins [13] were assayed, too.

The data in figures 1 and 2 show that CPA has a significant involuting effect on both T and BF. This was associated with an increase of glycogen content in both glands, similar to what happens in the thymus of rats subjected to a hydrocortisone stress [9].

One can also observe that CPA exhibits a selective effect in the sense that BF reacts more drastically to the drug at the beginning of the treatment while T reacts in a dose-response manner. Such a behaviour might be ascribed to the different immunologic capacity of the central lymphatic organs [4]. Some other effects of CPA, like depletion of B lymphocytes from bursal follicles as well as the absence of germinal and plasma cells in the spleen and other peripheral lymphoid tissues [10], are well known.

It is to be pointed out that in our experiments the content of serum gamma-globulin was significantly lowered (after the last CPA injection by 9%, at p < 0.05). An effect of CPA was seen also on the AsA content in adrenals, which was lowered by the period of the third injection of
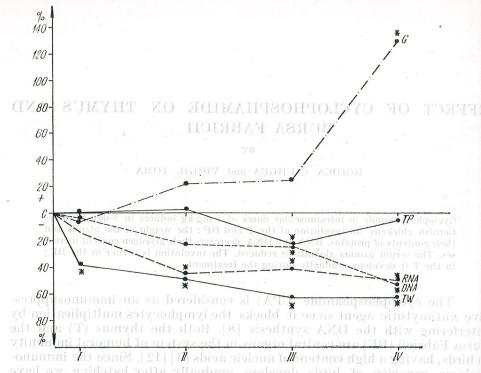
Fig. 1. — Modifications in the T of chicken 24 hrs after each injection with CPA in repeated doses (I — IV) (per cent differences as against the controls).

* = Statistically significant modifications.
↓ = Statistically significant AsA differences.

Fig. 2. — Modifications in the BF of chicken 24 hrs after each injection with CPA in repeated doses (I — IV) (per cent differences as against the controls).
For * and ↓ same legend as in figure 1.

CPA, showing a hypersecretion of cortical steroids. In their turn, these are also immunosuppressive agents [1] [2].

The conclusion is drawn that the pronounced involution of both T and BF during the treatment with CPA is a consequence of the mechanisms by which CPA affects the immunobiologic reactions in birds.

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INFLUENCE OF THYMECTOMY ON THE LANGERHANS ISLETS
AS A FUNCTION OF DEVELOPMENTAL STAGES

BY

V. TOMA, MARIA CRIVII and C. CRĂCIUN

Thymectomy modifies the islets coefficient (Q_i) of the Langerhans islets in rats, depending on the age at which intervention is performed. If the thymus is neonatally removed, the Q_i is most markedly decreased; this modification is less pronounced in young mature animals and is practically absent in thymusless adults.
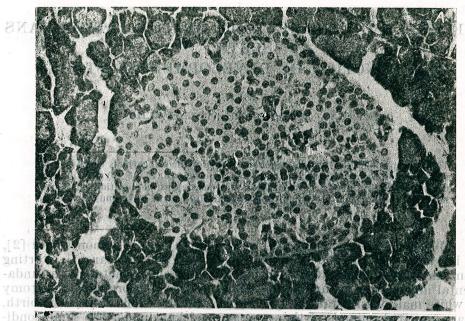
While the immunobiological role of the thymus is demonstrated [2], its influences on the endocrine equilibrium are still unclear [5]. Starting from the well demonstrated fact that the ontogenetic factor has a fundamental importance in thymus physiology [3], we performed thymectomy in white male Wistar rats at 1, 60 — 65 and 340 — 360 days after birth. Sham-operated animals of the same age and kept under the same conditions served as controls. One month after operation, 18-hour fasted animals were killed by decapitation and the pancreas was fixed in Bouin's mixture and coloured according to Gömöri's procedure as modified by Rona. Simultaneously we checked the degree of the thymectomy completeness. Twenty to 25 Langerhans islets were microscopically examined and the islets coefficient (Q_i) was calculated according to Scharf et al. [4]:

$$Q_i = \frac{\text{Number of B cells}}{\text{Number of A cells}}$$

In the control groups the Q_i dit not show any significant differences depending upon the age of animals. The B cells clearly predominated over the A cells. After thymectomy the Q_i had suffered considerable modifications, depending upon the age at which the thymectomy was performed. The neonatal thymectomy induced the most pronounced decrease of Q_i value, by increasing the number of A cells. In the second group of thymectomized animals, i.e. at 60 — 65 days, the A cells neogenesis was diminished, while in the group operated at 340 — 360 days, the Q_i values were almost similar to those in the control animals.

The data presented here are relevant for explaining hyperglycaemia, the decreased responsiveness in "wasting disease" in rats thymectomized at an early stage of ontogenesis [1].
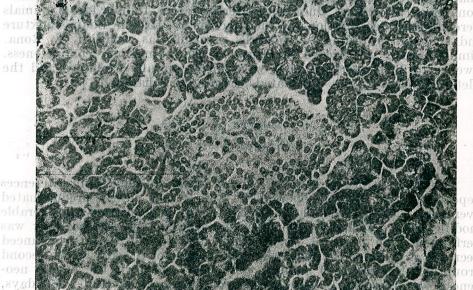
Fig. 1. — *A*, Langerhans islet of a control rat. Gömöri-Rona staining (× 25); *B*, Langerhans islets of a neonatally thymectomized rat. Gömöri-Rona staining (× 25).

Table 1

Variations of Q_i in rats 30 days after thymectomy performed at different ages

Animal group * (ages)	Control	Thymectomized at...days		
		1	60—65	340—360
Q_i	$\frac{4}{1}$	$\frac{1}{2}$	$\frac{2}{1}$	$\frac{3}{1}$

* Each group of 8 animals.

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CIRCADIAN RHYTHMS OF RNA, DNA AND PROTEINS IN THE RAT THYMUS. EFFECTS OF NEONATAL PINEALECTOMY

BY

GRAZZIELA YVONNE NICOLAU and CORNELIA BOTA

Studies performed on 45-day-old white Wistar male rats have demonstrated a circadian rhythm in the variation of the thymic concentration of RNA and DNA. The study was carried out for 72 hrs and the variation tested every 6 hrs. The highest levels were recorded in the nocturnal period (00^{00} hrs) and the lowest, in the diurnal period (6^{00} hrs). The variation in the thymic proteins seem to lack any circadian rhythm. Neonatal pinealectomy abolished the circadian rhythm of the thymic nucleic acids and reduced the amplitude of the circadian variations in the thymic proteins. The 45-day-old neonatally pinealectomized rats showed a decrease in the thymic RNA and DNA concentration and an increase in the thymic protein level.

Circadian rhythmic variation in the endocrine system was established by many studies, at the level of secretion, metabolization and hormonal elimination. Circadian rhythms were reported in the rat adrenal [9] and rat thyroid [8] RNA concentrations with the highest levels recorded at 6^{00} (beginning of darkness under our experimental conditions), and in the rabbit which had maximum day time levels at 6^{00} for the adrenal RNA circadian rhythm [11] and at 12^{00} for the thyroid RNA rhythm [13]. In the rat adrenal, the circadian rhythm of RNA synthesis is in the same phase with the rhythm of corticosterone secretion * and the rhythm of thyroid RNA synthesis coincides with the plasmatic PBI rhythm, both in the rat and the rabbit, as we have reported elsewhere [12]. In a later paper we reported the disappearance of the circadian variations in the RNA concentrations of rats pinealectomized at an adult age, noticed 15 days following surgery [14].

Circadian rhythms in the nucleic acids and proteins were recorded for the rat pineal gland, whose patterns were slightly differing in relation to sex, and the correlation of these rhythms with the variation of endocrinologically active pineal indoles was discussed [15].

As concerns the thymus, Golicov [3] reports seasonal rhythms in the intact, adrenalectomized and hypophysectomized rat thymus weight and correlated these variations by the functional status of the hypophyso-adrenal system. A circadian rhythm in the mitotic activity with maximum

values in the early morning hours was also found in organs of a lymphoid type with similar cellular structure — thymus and the lymphatic ganglia [1] [5] [6]. Guseynov et al. [4] reveal an increase in the DNA supramolecular content in the rat thymus at the beginning of the nocturnal period reaching a maximum at 20^{00}, the moment at which the authors found also the most important decrease in the elastoviscosity of the supramolecular DNA. Maximum synthesis of DNA in the rat lymphatic ganglia occurs in the evening hours [6].

In the present work our objective was to study the circadian variations in the RNA, DNA and proteins concentration in the rat thymus and the possible intervention of the pineal in maintaining and synchronizing the circadian biorhythmicity at the level of the thymus.

EXPERIMENTAL PROCEDURES

The study was made on white Whistar male rats aged 45 days and weighing 54 — 63 g, kept at a constant temperature of about 24°C. Post-partum, up to the age of 21 days the animals were fed naturally but thereafter they were given heterogeneous food ad libitum. The light regimen consisted in normal alternation of a 12-hr light period with a 12-hr dark period. The light period began in our experiment at 6^{00}.

The experiment was made on 2 lots consisting of 48 animals each : one lot of intact and one lot of neonatally pinealectomized animals. From both lots, groups of 4 animals were daily sacrificed by decapitation at 6^{00}, 12^{00}, 18^{00} and 00^{00} for 3 days, their thymus glands removed, cleaned and instantly frozen by carbonic snow and stored at $-15°C$ until the time of assay.

Pinealectomy was performed according to the surgical technique described by Simionescu and Scherzer [17] adapted to fit the newborns in relation to the bone development of the neurocranium.

The nucleic acids and thymic proteins were extracted through Schneider's technique [16] and the respective concentrations in the obtained extract were measured by Mejbaum's spectrophotometric method for RNA [10], by Dische's method for DNA [2] and Lowry et al.'s method for proteins [7]. Statistical significance was evaluated with the Student's ''t'' test.

RESULTS AND DISCUSSIONS

A. In the intact animals, the RNA concentrations in the thymus along the 72 hours of the experimental period show a circadian rhythm with minimum levels at 6^{00} and maximum levels at 00^{00} (Fig. 1). In the same interval, the DNA concentration in the thymus has a similar evolution with minimum levels at 6^{00} and maximum levels at 00^{00} (Fig. 2). No rhythmic pattern was found in the circadian evolution of the thymic proteins, though there was a variation during the 72 hrs from 137.30 ± 4.27 to 174.21 ± 2.56 mg/g tissue (Fig. 3).

B. Neonatal pinealectomy induced changes in the circadian rhythmicity of the nucleic acids and in the variation of the thymic proteins.

In the neonatally pinealectomized rats, the circadian curve of the RNA concentration in the thymus shows statistically nonsignificant variations meaning a disappearance of the circadian rhythm of the thymic RNA mainly by a reduced variational amplitude. At the same time the thymic RNA concentration in the pinealectomized animals diminishes, as can be seen from the variation in the 72 hrs mean concentration value : 8.52 ± 0.12 mg/g tissue in pinealectomized as against 10.64 ± 0.35 mg/g

Fig. 1. — Circadian rhythm of RNA in the thymus of the 45-day-old rat and abolition of this rhythm in neonatally pinealectomized rats of the same age ; mean values, n = 4.
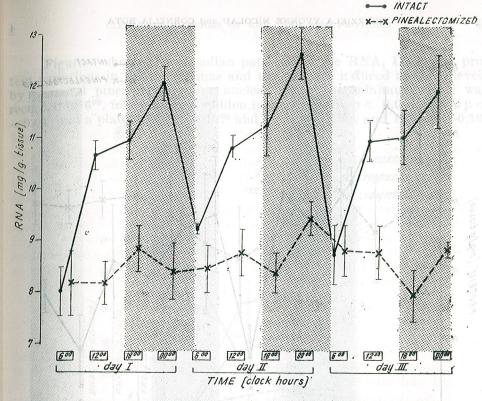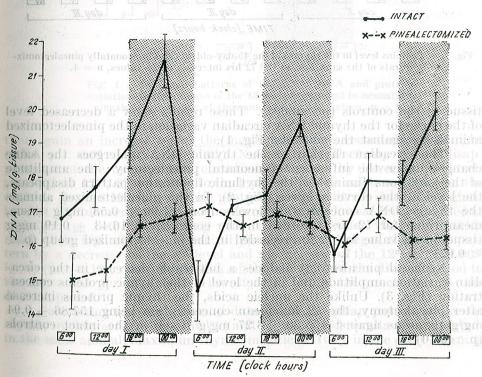

Fig. 2. — Circadian rhythm of DNA in the thymus of the 45-day-old rats and abolition of this rhythm in neonatally pinealectomized rats of the same age ; mean values, n = 4.
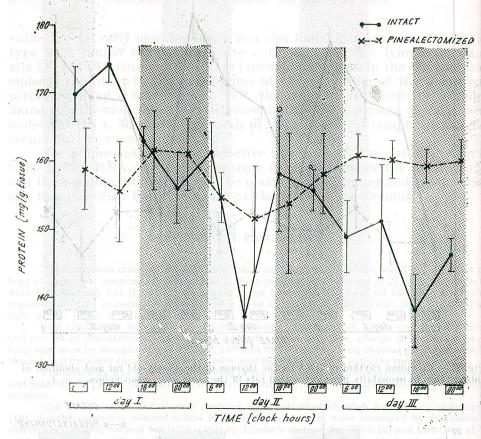
Fig. 3. — Proteins level in the thymus of the 45-day-old rats and in neonatally pinealectomized rats of the same age, in the 72 hrs interval; mean values, n = 4.

Figure 4 shows the circadian patterns of the RNA, DNA and protein variations in the rat thymus and the changes induced at these levels by neonatal pinealectomy. For nucleic acids the minimum level was recorded at 6^{00}, followed by a sudden increase (RNA $p < 0.001$, DNA $p < 0.01$) and a plateau between 12^{00} and 6^{00} (both RNA and DNA $p > 0.10$)
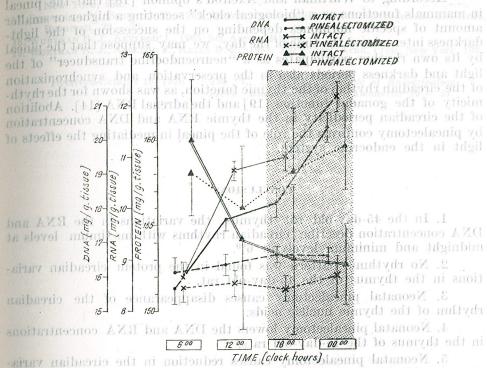
Fig. 4. — Circadian patterns of the DNA, RNA and proteins variations in the thymus of the 45-day-old rats and in neonatally pinealectomized rats of the same age; mean values, n = 12.

tissue in the controls ($p < 0.001$). These results show a decreased level of the curve for the thymic RNA circadian variation in the pinealectomized animals as against the controls (Fig. 1).

The circadian rhythm of the thymic DNA undergoes the same changes under the influence of neonatal pinealectomy: the amplitude of the variations diminishes, the rhythmic fluctuation pattern disappears, the level of the curve is lower (Fig. 2). In the pinealectomized animals the thymic DNA concentration lowers from 17.99 ± 0.53 mg/g tissue, mean value for 72 hrs recorded in the controls, to 16.43 ± 0.19 mg/g tissue, mean value for 72 hrs recorded in the pinealectomized group ($p < 0.02$).

Neonatal pinealectomy induces a noticeable decrease in the circadian variation amplitude and in the level of the thymic proteins concentration (Fig. 3). Unlike the nucleic acids, the thymic proteins increase after pinealectomy, the 72 hrs mean concentration being 157.86 ± 0.94 mg/g tissue as against 155.80 ± 3.27 mg/g tissue in the intact controls ($p > 0.10$).

then again an increase up to the maximum values recorded at midnight (RNA $p < 0.01$, DNA $p < 0.001$). Neonatal pinealectomy causes disappearance of the nucleic acids circadian variations by lowering the levels recorded at 12^{00}, 6^{00} and 00^{00} to the level of 6^{00}, the minimum level in the controls (ps > 0.10). The thymic proteins in the controls show the highest level at 6^{00} followed by continuous decrease to the lowest level recorded at midnight (ps > 0.10) coinciding with the moment when the nucleic acids reach their highest level. Neonatal pinealectomy changes the curve pattern by a decrease of the 6^{00} level and an increase of the 12^{00}, 6^{00} and 00^{00} levels (ps > 0.10), moving the peak of the curve from the diurnal period to the nocturnal period and the lowest level from the nocturnal to the diurnal period.

Correlating our experimental data on the circadian pattern of the rat thymic nucleic acids with Alov [1] and Hussar's [5] results who reported, in the same animal, maximum thymic mitotic activity in the early morning

hours, it results that DNA and RNA syntheses are metabolic activities separated in time by the maximum phase of the mitoses. A similar conclusion was reached at by Loguchev and Pivovarova [6] as concerns DNA synthesis and the mitotic index in the rat lymphatic ganglia.

According to Wurtman and Axelrod's opinion [18] that the pineal in mammals functions as a "biological clock" secreting a higher or smaller amount of specific hormones depending on the succession of the light-darkness interval and the time of the day, we may suppose that the pineal by its own rhythm participates as a "neuroendocrine transducer" of the light and darkness periods also in the preservation and synchronization of the circadian rhythm of the thymic function, as was shown for the rhythmicity of the gonadal function [19] and the adrenal RNA [14]. Abolition of the circadian periodicity in the thymic RNA and DNA concentration by pinealectomy confirms the role of the pineal in mediating the effects of light in the endocrine system.

CONCLUSIONS

1. In the 45-day-old rat thymus, the variations in the RNA and DNA concentration describe circadian rhythms with maximum levels at midnight and minimum levels at 6^{00}.

2. No rhythmic pattern was found in the protein circadian variations in the thymus of the 45-day-old rat.

3. Neonatal pinealectomy causes disappearance of the circadian rhythm of the thymic nucleic acids.

4. Neonatal pinealectomy lowers the DNA and RNA concentrations in the thymus of the 45-day-old rats.

5. Neonatal pinealectomy causes reduction in the circadian variations amplitude of thymic proteins.

6. Neonatal pinealectomy increases protein concentration in the thymus of the 45-day-old rats.

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A CELL MIGRATION INHIBITORY FACTOR PRESENT IN THE RAT TUMOR ASCITES FLUIDS AND TUMOR CELL CULTURE SUPERNATANTS

BY

LIANA ŞANDRU and G. ŞANDRU

A migration inhibitory factor has been demonstrated in the fluid of B_1R syngenic ascites tumor as well as in the culture supernatant of the same tumor cells by using the blood clot fragment and spleen explant migration methods. This factor is resistant to freezing-thawing and is not dialyzable. Different from the classic MIF, it is partially degradable by heating, could not be blocked by L. Fucose and adsorbed on type 0 human erythrocytes. It does not present a nonspecific cytotoxic effect evidenced by Cr^{51} release technique.

Apart from the migration inhibitory factor (MIF) secreted by sensitized lymphocytes stimulated by antigen [2] and by nonsensitized lymphocytes stimulated by mitogens [7] or by mixed leukocyte cultures [1], migration inhibitory factors have been found to be released also by cells from the established lymphoid or nonlymphoid lines of normal or tumoral origin [5] [6] [9].

In this paper we shall present our preliminary results demonstrating the presence of a migration inhibitory factor in the rat tumor ascites fluids as well as in the tumor cell culture supernatants.

MATERIAL AND METHOD

Tumors : B_1R ascites tumor, a spontaneous mammary tumor in R rats, converted into ascitic form and maintained syngenically.

Tumor ascites fluid preparation. Tumor ascites fluid harvested from tumor-bearing rats has been centrifuged at 6,000 g for 15 minutes and the supernatant was used for detecting the migration inhibitory activity. Aliquots of tumor ascites fluid have been processed before migration inhibitory assay determinations, as follows :

a) inactivation at 56°C for 45 minutes ;

b) freezing-thawing : tumor ascites fluid has been submitted to 10 cycles of alternate freezing in alcohol-dry ice mixture and thawing in tap water at 37°C ;

c) dialyzation : aliquots of 4 ml of tumor ascites fluid have been dialyzed against 100 volumes PBS for four days by changing the buffer daily ;

d) adsorption : v : v upon spleen cells (rat, mouse) and human erythrocytes of 0 type ; in some experiments, rat spleen cells were trypsinized with 0.2 % trypsin for 15 min, at room temperature. Aliquots of tumor ascites fluid were mixed with cells and incubated for 1 h at 37°C. After incubation, the ascites fluid-cell suspension was centrifuged at 3,000 g for 15 minutes and the supernatant tested for the migration inhibitory activity.

Cell culture supernatant preparation. After harvesting in Hanks solution with antibiotics, ascites cells were washed 3 times, at 150 g, 6 minutes and resuspended in TC 199 medium enriched with 20 % fetal calf serum, antibiotics and fungizone. 10^6 cells were cultured for 24 — 96 h at 37°C in 5 % CO_2 and water-saturated atmosphere and supernatants were harvested by centrifugation at 600 g 15 minutes.

The cell migration from the blood clot fragment method was performed as previously described [11]. Two milliliters blood were settled on a 3.2 cm Ø glass dish, and after 20 minutes fragments were cut by a trocar of 1.2 mm inner diameter. After 4 washings and 1 h at 4°C, clot fragments were incubated in control (TC medium + 20% normal rat serum) and assay medium (TC medium + 20% tumor ascites fluid) in the migration chambers for 16 — 18 h at 37°C. In some experiments, L-Fucose 0.1 M diluted 1/3 was added to culture medium.

The spleen explant method was performed by using a slightly modified Svejcar and Johanovsky's method, as already described [10]. From identical spleen slices obtained by using a slice maker, fragments were cut by a 1.2 mm Ø trocar. After washing, spleen fragments were cultured 24 h in control and assay medium.

Microscopical examination of the migration areas and quantitative determinations. The migration areas were microscopically examined, photographed and projected on nr. 1 Whatman paper for quantitative determinations on weight basis; the arithmetic means, standard deviations (SD) and migration index (MI) were computed. MI was calculated as percentage of control migration :

MI = area X. 100/control area.

The cytotoxicity assay was performed by using a Cr^{51} labeling method [3]. 10^6 labeled spleen cells suspended in control and assay medium were incubated for 24 h at 37°C. Then the Cr^{51} release in the supernatants was measured.

RESULTS AND DISCUSSIONS

In agreement with other papers demonstrating the presence of the migration inhibitory factor (MIF) in other circumstances than those of an immunologic nature [5] [6] [9], a migration inhibitory activity has been detected by us in the rat tumor ascites fluid as well as in the supernatants of the same tumor cell cultures.

The rat B_1R tumor ascites fluid exhibited *in vitro* a strong inhibitory activity upon cell migration from blood clor fragments. The inhibitory action was resistant to dialyzation and freezing-thawing but, contrarily to the classic MIF, it was partially destroyed by inactivation at 56°C and could not be blocked by L-Fucose [8] (Table 1).

Table 1

In vitro inhibition of cell migration from blood clot fragments exerted by the fluid of rat B_1R ascites tumor

Assay medium enriched with :	Migration mean ± SD	MI
NRS (Control)[a]	370.42±12.13	
AF[b]	26.26± 2.19	7.08
Dialyzed AF[c]	29.72± 2.82	8.02
Freezed-thawed AF[d]	60.31± 3.90	16.28
Heat inactivated AF[e]	172.80±17.07	46.65
AF + L-Fucose[f]	32.97 ± 3.74	8.90

[a]NRS : 20% heat inactivated normal R rat serum ; [b]AF : 20% ascites fluid of B_1R tumor ; [c]AF previously dialyzed against 100 v PBS ; [d]AF previously submitted to freezing-thawing ; [e]AF inactivated at 56°C ; [f]L-Fucose 0.1 M diluted 1/3 added to assay medium. Migration mean values ±1 SD (standard deviation) were obtained from 6 weighings (mg) of migration area projection on Whatman paper ; migration index (MI) was calculated as percentage of control migration.

The migration inhibitory activity of the tumor ascites fluid could be adsorbed on syngenic rat spleen cells and only partially on trypsinized syngenic spleen cells or mouse spleen cells.

In contrast with the classic MIF [4], no adsorption was found by using type 0 human erythrocytes (Table 2).

Table 2

Adsorption of B_1R ascites fluid migration inhibitory factor on rat and mouse spleen cells and human type 0 erythrocytes tested by the blood clot fragment technique

Assay medium enriched with :	Migration mean ± SD	MI
NRS (Control)[a]	374.20±15.95	
AF adsorbed on rat spleen cells[b]	339.40± 9.48	90.70
AF adsorbed on trypsinized rat spleen cells[c]	247.38±10.07	66.11
AF adsorbed on mouse spleen cells[d]	175.96± 8.57	47.02
AF adsorbed on type 0 human erythrocytes[e]	26.12± 0.48	6.99
Ascites fluid	26.33±2.29	7.03

[a]NRS: 20% heat inactivated normal rat serum ; [b]20% ascites fluid previously adsorbed v:v on syngenic rat spleen cells ; [c]20% ascites fluid previously adsorbed v:v on trypsinized rat spleen cells ; [d]20% ascites fluid previously adsorbed on mouse spleen cells ; [e]20% ascites fluid previously adsorbed on human type 0 erythrocytes.

By using the Cr^{51} labeling technique the ascites fluid did not reveal a cytotoxic effect upon the target spleen cells.

Supernatants of B_1R ascites tumor cell cultures inhibited but moderately the cell migration *in vitro* (MI between 70 and 90) ; sometimes they induced only a cell clumping without decreasing the size of the migration areas (Fig. 1).
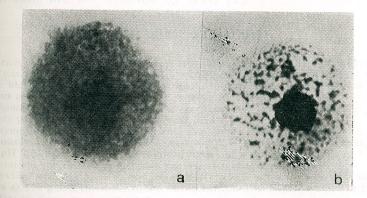

Fig. 1. — A spleen cell migration area from the explant in the presence of normal rat serum (control) *(a)* and in the presence of B_1R tumor cell culture supernatant *(b)*.

In conclusion, evidence presented here demonstrated by means of two migration methods a cell migration inhibitory factor present in the fluid of rat tumor ascites and culture supernatants of the same tumor cells. Our findings are in agreement with other papers suggesting that MIF could be secreted by the proliferating cells, sensitized cells stimulated by antigen to proliferate representing only a fraction of all dividing cells [5].

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MICROELECTROPHORETIC MOBILITY CHANGE DETECTION OF ANTITUMOUR SENSITIZED THYMUS CELLS

BY

AUREL I. POPESCU, TRAIAN ANDRIAN and MARIA NAUM

The influence of tumour-bearing rat serum (TS), normal serum (NS) and antithymocyte serum (ATS) on the microelectrophoretic mobility of the thymus lymphocytes harvested from normal rats (NTL) and from tumour-bearing rats (TTL) was studied. Wistar rats with xenotransplanted h 18 R tumours were used. A different influence of ATS on NTL and TTL microelectrophoretic mobilities was observed.

Though some antigens are undoubtedly intracellular, it is likely that antigenic properties of the surface may determine the susceptibility of the cells to immune conditions, whether mediated by humoral or by cellular mechanisms [5].

For particles of the size of living cells suspended in salt solutions, the mobility in an electrical field depends mainly upon the nature of the surface and too little upon the size, shape and orientation of the particles.

If immune reactions involved charged groups in the cell surface, then they might produce an alteration in the microelectrophoretic mobilities of these cells after their contact with specific antisera.

Measurement of the microelectrophoretic mobility of the cells treated with antibodies and complement may provide information concerning the nature of immune reactions at the cell surface (5). Thus, human erythrocyte incubation with ABO antisera results in a reduction in the mobility of the cells. On the same line of evidence ATS acts differently on the T and B lymphocytes as follows : T cells manifest a decreased mobility while B cells are not affected by ATS [2].

MATERIAL AND METHOD

Serum preparation. NS was prepared from Wistar rat blood harvested by cardiac puncture. TS was prepared from the blood of h 18 R tumour-bearing rats (7). ATS was prepared from the blood of repeatedly immunized goat. Immunization was performed using Wistar rat thymus cells, that were three times s.c. injected to a goat.

Thymus lymphocyte preparation. For each average microelectrophoretic mobility five Wistar rats were used in order to prepare NTL and TTL.

Thymus equal amounts from five rats were mixed and homogenized with microelectrophoretic medium consisting of 0.160 M sucrose, 0.012 M Na_2HPO_4, 0.003 M NaH_2PO_4, 0.044 M NaCl at pH 7.0, having an ionic strength of 0.83 and a relative viscosity of 1.14 [9]. After homogenization, the cellular suspension was successively passed through three stainless steel filters provided with 450 μ, 250 μ and, respectively, 150 μ orifices. The filtrate was centrifuged 5 minutes at 175 g and the supernatant was discarded. Then, three washes in the microelectrophoretic medium were performed.

In order to study the NS, TS and ATS effects on the microelectrophoretic mobilities of thymus lymphocytes, these latter were incubated 30 minutes at 37°C with NS, TS and ATS in a 1 : 100 dilution adjusting the cell concentration to 2×10^7 cells/ml.

After incubation the cells were washed three times with the microelectrophoretic medium and finally resuspended in the same medium to a 10^7 cells/ml concentration. These latter suspensions are proper for measurements [10].

Microelectrophoretic measurements. The mobility measurements were performed using a microelectrophoresis apparatus provided with a cylindric-type migration chamber [6]. The applied electrical field was 2.46 V/cm and the current intensity 0.65 mA.

The average mobility (u) was calculated according to the following formula [1] :

$$ u = \frac{\sum_{k=1}^{n} (V_{rk} + V_{1k})}{2NE} , $$

were V_{rk}, V_{1k} are the velocities of the kth particle to the right and to the left side, respectively ; N is the number of the cells (N > 30) and E is the electrical field strength.

For a sufficiently homogeneous cell population, the average mobility for a single determination, calculated using the average transit times, does not differ significantly from the average of the individual mobilities, if transit times in both directions are not much different. As in our experiment these times were significantly different, we averaged the individual mobilities, as recommended for such cases [3].

RESULTS

The results of the microelectrophoretic measurements are given in table 1.

Table 1

Electrophoretic mobilities of thymus lymphocytes harvested from normal rats (NTL) and from tumour-bearing rats (TTL) incubated with normal serum (NS) and anti-thymocyte serum (ATS)

Incubation conditions	Mobilities ± S.E. (μ cm V^{-1} s^{-1})	Incubation conditions	Mobilities ± S.E. (μ cm V^{-1} s^{-1})
NTL only	1.41±0.05	TTL only	1.38±0.06
NTL + NS	1.50±0.06	TTL+NS	1.48±0.04
NTL + ATS	1.27±0.06	TTL+ATS	1.06±0.05

As seen in this latter, the average mobility of NS-treated NTL is not significantly modified as compared with the mobility of untreated NTL (P > 0.05). On the other hand the mobility of ATS-incubated NTL is significantly decreased (P < 0.01).

A similar behavior is observed in the case of TTL which have the same average mobility as NTL. However, in this case the mobility of ATS-incubated TTL is significantly decreased (P < 0.01) as compared with the corresponding mobility of NTL.

DISCUSSION

All mammalian cells that have so far been examined have been found to carry a net negative charge under physiological conditions. It was demonstrated that sialic acid groups play an important part in determining the surface charge of the mammalian cells [4] [8].

The electrical properties of the cell surface depend not only on the magnitude of the net surface charge, but also on the relative contributions that different types of charged groups make to the total charge.

Thus, the effect of added materials (e.g. different kind of sera) on the microelectrophoretic behaviour of the cells may be due to the modifications of the relative contributions of the charged groups that preexist in the cell surface membrane, as well as to the electrical properties of the added material itself (5).

It seems that ATS masked some of the electrical charged groups on the surface on NTL and TTL differently, since their mobilities are differently affected by ATS. This implies different configurations and/or compositions of the charged groups in the two cases.

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KINETICS OF RADIONUCLIDES UPTAKE BY FISH IN A CLOSED AQUARIUM SYSTEM

BY

ILCA MĂRGINEANU and ION CHIOSILĂ

This paper builds up the mathematical frame for the analysis of our experimental data concerning ^{85}Sr incorporation in gold-fish, in a closed aquarium system, after an initial contamination of water. The time dependences of the specific activities in fish and water are derived, and their consequences are discussed. The experimental curves giving the time course of radioactivity in fish and water are compared with the theoretical ones and on this basis the water volume from which ^{85}Sr is retained by the fish is estimated to be about $0.3-0.5$ cm^3/g$\acute{\text{i}}$ day.

The knowledge of the kinetics of radioactive compounds uptake in organisms and organs deserves a wide interest [4] [5] [7] [9] [10]. However, the biological parameters underlying the accumulation and the fixation of radionuclides in living organisms, are not as yet well known.

In this paper we build up the mathematical analysis of our experimental data concerning ^{85}Sr incorporation in fish, in a closed aquarium, following a sudden contamination of water. Such a system is representative for an acute contamination, like that occurring in accidental industrial releases.

The theoretical model suitable for our experimental test possibilities is the following. Water volume V_w and fish volume V_f form a closed bicompartmental system, that is the overall radioactivity in both these compartments decreases only due to the physical decay with a radioactivity constant λ. Each compartment is treated as homogeneous with respect to radioactivity distribution.

We make the following obvious assumptions : the time variation of specific radioactivity in fish $d\Lambda_{sf}/dt$ is due to water intake and biological turnover including the physical decay. If W is the rate of water intake in the mass unit of fish, then $d\Lambda_{sf}/dt$ increases with $\alpha W \Lambda_{sw}$, where α is a microscopical concentration factor. The parameter α is the fraction actually kept for metabolic purposes from the whole radioactivity intake in the time unit. Both α and W are metabolism dependent factors, and we are treating them as time independent parameters. As we have no means to separate α and W, the accessible quantity being only their product $I = \alpha W$, we will treat this product as a single factor.

The physical decay contribution to Λ_{sf} decreasing in the time unit is : $-\lambda \Lambda_{sf}$ and the metabolic contribution is : $-\beta \Lambda_{sf}$. β is the biological

loss coefficient which is defined as : $\beta = 0.693/T_b$, where T_b is the biological half-life of the radionuclide in the organism.

Taking into account all the contributions to Λ_{sf} variation, we have the following differential equation :

$$\frac{\mathrm{d}\Lambda_{sf}}{\mathrm{d}t} = I\Lambda_{sw} - (\beta + \lambda)\,\Lambda_{sf} \tag{1}$$

The solution of this linear non-homogeneous equation is :

$$\Lambda_{sf} = \left[I\Lambda_{sw}^0 \Big/ \left(I\frac{V_f}{V_w} + \beta \right) \right] \exp\,(-\lambda t) \left\{ 1 - \exp\left[-\left(I\frac{V_f}{V_w} + \beta \right) t \right] \right\} \tag{2}$$

where subscript "s" stands for "specific", Λ_{sw}^0 is the specific radioactivity introduced at 0 moment in water and V_f and V_w are the fish and water volumes.

We also obtained the analytical expressions of $t_{\max}$, the time lag for reaching the maximum of Λ_{sf}, the corresponding maximal value of Λ_{sf}, $\Lambda_{sf}^{\max}$, and the expressions of specific activity in water Λ_{sw} and of the con-
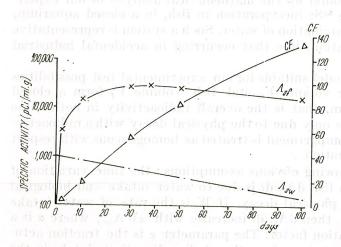
Fig. 1. — The theoretical time dependences of specific radioactivity in fish (Λ_{sf}), water (Λ_{sw}) and of the concentration factor CF.

centration factor CF, defined as $\Lambda_{sf}/\Lambda_{sw}$ at each moment. These expressions show that $t_{\max}$ does not depend on the initial water activity Λ_{sw}^0 and CF depends only on the biological values of the radionuclide metabolism, being independent on and $_{sw}^0$. The saturation value of CF, asymptotically approached, is $CF_\infty = I/\beta$.

The theoretical time course of Λ_{sf}, Λ_{sw} and CF are shown in figure 1 for the following arbitrary values of the parameters : $\Lambda_{sw}^0 = 1{,}000$ p Ci/ml, $I = 2$ ml/g. day, $T_b = 70$ days, $T_f = 35$ days, $V_f/V_w = 10^{-3}$.
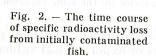
MATERIAL AND METHOD

We tested the above description under the following conditions. Five adult individuals of gold-fish (*Carassius auratus gibelio*) of about 30 grams each were introduced into a 30-l closed aquarium filled with tap water. The fishes were fed with non-contaminated food. During the experiment, the fish weight was constant. At zero moment of the experiment, we put into the aquarium the following activities of ^{85}Sr $(T_f = 65$ days): 150; 500 and 1,200 pCi/ml, as ^{85}SrCl$_2$ (Amersham). We followed up the time course of Λ_{sw} and Λ_{sf}. From these we directly obtained CF. For the determination of β, several ^{85}SrCl$_2$-contaminated fishes were introduced into non-contaminated water. The water from the aquarium was daily changed. The effective half-time T_{ef} thus obtained allows to compute T_b.

The water temperature was $22 \pm 2°C$ in all experiments. The activity was measured in a whole-body system with a Philips monochannel analyzer and calculated against a plexiglass fish ghost filled with 30 ml of water. The detailed experimental device was described elsewhere [2]. Each result is the average of 5 measurements.

RESULTS

From the data in figure 2, we obtain $T_{ef} = 36$ days and, consequently, $T_b = 81$ days.

Fig. 2. — The time course of specific radioactivity loss from initially contaminated fish.
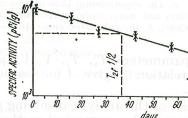
Figure 3 clearly shows that the shape of the experimental curves bears a close resemblance with that given by the theoretical representation in figure 1. The concentration factor is very slightly dependent on the initially added activity. Owing to some independent biological reasons, the measurements have been ceased before reaching $t_{\max}$. Accordingly, we have not checked the expected independence of $t_{\max}$ on Λ_{sw}^0.

DISCUSSIONS AND CONCLUSIONS

The only unknown parameter appearing in the equation (2) is $I = \alpha W$, that is the water volume from which ^{85}Sr is actually retained by the unit mass of fish, per time unit. We have tried to estimate this parameter by comparing the experimental data with the theoretical curves.

The value of $CF_\infty = I/\beta$ suggests the simplest way to obtain I. Since, as previously mentioned, we could no obtain CF_∞, we used (2) rewritten as :

$$\frac{10^{-3}\,\Lambda_{sf}\,(t)}{\Lambda_{sw}^0}\,\exp{(0.01066\,t)}\cdot(5 + 8.56/I) = 1 -$$

$$- \exp{(-0.00856\,t)}\,\exp{(-5 \times 10^{-3}\,I \times t)} \qquad (3)$$

Fig. 3. — a, The experimental time dependences of specific activity in fish (continuous line) and water (interrupted line) for $\Lambda_{sw} = 150$ (O); 500 ($\triangle$) and 120 ($\times$) pCi/ml. b, the calculated concentration factor for the same experiment.

where the parameters T_b, T_f, V_f/V_w are replaced by their numerical values. The relation (3) gives I for a series of experimental values Λ_{sf} and a given Λ_{sw}^0.

Instead of the analytical solving of (3), which is at least cumbersome we have used a numerical method which consists in representing the two sides of the equation as functions of I, and taking as the solution, the abscissa of their intercept. The following mean results have been obtained, for each Λ_{sw}^0 :

Λ_{sw}^0 : 150	500	1,200	pCi/ml
I : 0.37 $\pm$ 0.01	0.45 $\pm$ 0.02	0.82 $\pm$ 0.07	cm³/g · day

The slight increase of I with Λ_{sw}^0 seems to suggest that our initial assumption of independence between I and Λ_{sw} is but an approximation. Since the W value is influenced only by metabolic parameters, being independent of water radioactivity, the increase of I with Λ_{sw}^0 is due only to α contribution. Most probably, it could be explained by an increased external contamination of the scales for increasing Λ_{sw}^0, even if the adsorption contribution to the scales activity is not yet clearly known [1],

Assuming that for the smallest Λ_{sw}^0 (150 pCi/ml) the value of I ($\approx$ 0.4 cm³/g.day) is the real one, for $\Lambda_{sw}^0 = 1200$ pCi/ml, the specific activity due to external contamination of the scales is 2000 $\pm$ 350 pCi/ml. This represents the difference between the experimental value of Λ_{sf} and the corresponding theoretical value calculated from the slope of a linearized dependence between Λ_{sf} and Λ_{sw}, eq. (2).

If the value of W is known from independent investigations, one can obtain the microscopical concentration factor α. With $W = 6000$ ml/day for 30 g of fish, obtained by Pentreath [6] for marine teleosts, it results a rather plausible $\alpha = 0.2\%$. This value has to be considered a mere orientative one, in view of the differences that could appear at the species of the same order, living under diferent conditions.

The above description is one of the simplest, as it deals only with a two-compartmental system. It can nevertheless offer the basis for studying the three- or multi-compartmental systems, in which one can consider several fish species, some plants and the water. The model allows the investigation of the metabolism influencing factors on the radionuclide concentration. Such factors are temperature, oxygen concentration, the action of competitive ions, a.s.o. The model could also be extended to the description of the radionuclide uptake in specific organs, considering for each of them a specific metabolism.

From a practical point of view, our study could be relevant in radioecology, for example in water decontamination through radionuclide concentration by some organisms. Such procedures are actually used [3] [8], but mostly on empirical ground.

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"SECLUSION THROUGH CALCIUM" — A NEW THEORY ON THE FUNCTIONALITY OF THE MEMBRANE

BY

CORNELIU STANCIU

"Seclusion through calcium" represents a regulating mechanism of the material transfer at the level of membrane pores. It is of a physical-biochemical nature, representing an important means of saving energy. Elucidated as a result of the available data, the "seclusion through calcium" mechanism provides the explanation of all the membrane phenomena and according to these, of many physiological processes in the cell.

From our earlier researches with respect to the action of the *rhopic* factor on the transfer of the passive material at the level of the membrane pores, as well as on the amplitude of the electropotentials of the muscular fibre [21] [27]—[30], we drew several conclusions which, corroborated with literature data, led us to the formulation of a new theory explaining the functionality of the periplasmatic membrane.

The main experimental premises from which we started, besides our own results, are *the existence of the membrane pores* [6] [14] [26] [31] and *the ionizing effects of Ca^{2+} and K^+* upon the membrane phenomena [10] [12] [17] [24] [29].

The "seclusion through calcium" represents a physical-biochemical phenomenon of metal-proteic complexing of the ion with the biomolecules which surround the outer borders of the membrane pores, whose first consequence is the partial obturation (a reduction in diameter) or the total one (the reduction of the pore density), *without any consumption of metabolic energy*. The result of this process is a change in the relationship existing between the passive and the active transport, first of all for the mineral ions (Na^+ and K^+) and second, for the small molecules. The quality of being a ligand is a characteristic of the macromolecule depending on the existence and preservation of its *specific features*, that is, on that one which is characterized by a certain activation degree of the active points, a certain spatial conformation, or a certain conformational transition, a certain general reactivity state based on the energies of the interactional forces that they possess, a certain manifestation form and intensity of the cooperational phenomena, allosterism or histeresis [1] [5] [6] [18] [26]. The modification of these parameters leads to the reduction or stopping of seclusion. We shall enumerate the factors that are capable, among others, of producing such modifications.

The electric state of rest of the membrane, that is the electropositivity of its outer surface, constitutes a factor favouring seclusion, probably by discovering and exciting the active points of the biopolymers, at the level of which the interactional forces become maximal. Depolarization, that is the outer electronegativity, stops seclusion and sets free Ca^{2+} from the complex. There are numerous researches proving the discharge of the ion into the extracellular liquid as a result of depolarization by electric excitation of the cells [32] [33] or of the sarcoplasmatic cisternal system [34], in a depolarized state these formations lacking the capacity to accumulate it [25].

The pH value constitutes a limiting factor of seclusion, i.e. the rise, up to a certain limit, of the concentration of hydrogen ions on the outer face favours it, while its rise beyond this value or a marked drop inhibits it. In this respect, Mitchel [16] and Chance and Mela [3] conclude that in a state of rest, *the active transportation* of the ions through the membrane is coupled with an electrochemical current consisting in the continuous discharge of protons and the penetration of electrons into the interior. The pH value on the outer face in the state of rest favours seclusion, because under these conditions the quantity of ionized calcium increases, while the proteins show a basic character. Through the accumulation of protons on the inner face (whith nigericine or by calcium pulsation), Pressman et al. [22] and Caswell [2] obtained a massive discharge of K^+ from the intracellular space.

The value of the ionic relations (rhopia), first of all of the K^+/Ca^{2+} relationship, exerts an influence upon seclusion on the basis of the antagonism between the ions, which manifests itself within these latter's unity of action upon the biopolymers [19] [20]. The K^+ ion inhibits seclusion, while Ca^{2+} is directly participating in this process, and as a result, it will also depend on the concentration achieved on the outer face of the membrane. We emphasize the fact that the antagonism between the two ions does not refer to the competition for the complexing process, as the K^+ ion has a different spatial arrangement of the orbitals than Ca^{2+}. That is why at a normal outer concentration of the Ca^{2+} ion and at an increased one of K^+, the liquid that bathes the muscular fibres does not become poorer in K^+, on the contrary, it becomes richer (producing an inhibition of seclusion, a part of the intrafibrillary K^+ comes out, too) [30]. Indirect proofs with respect to the role of the value of the K^+/Ca^{2+} relationship in the protein functionality of the outer face of the membrane may be found in the papers of Pora [19] [20], Engel et al. [9], Merklen et al. [15], Reiter [23] and Kohlhard et al. [11]. In a direct way, we proved the inhibitory role of K upon seclusion in researches dealing with the action of the rhopic factor upon the permeability of the membrane for water, urea, natrium and toluilen red, as well as upon the distribution of the Ca^{2+} and K^+ ions and of the electrical potentials [21] [27] — [30].

The hormones and the chemical mediators present a more generalized action. Those hormones which favour the passive transfer act as inhibitors of seclusion. For instance, the neurohypophyseal hormones, insulin and adrenaline, raise the passive permeability for ions and water by extending the diameter of the pores, as stated by some authors. From the group of

mediators, those belonging to the excitational type are strongly inhibiting seclusion, as in the case of acetylcholine. Krnjevic [13] and Coombs et al. [4] have proved the increase of passive permeability for ions under its action. Many other researches bring additional proofs, but we shall deal only with those of Nachmansohn [17] and of Eccles [8], the only ones which are close to our conception in this problem. Thus, Nachmansohn believes that the action of acetylcholine on the membrane proteins results in a conformation change accompanied by movements of the charges, but it might also be a "discharge of the calcium ions bound to the protein, that might determine a rearrangement of the polyelectrolytes in the membrane". On the other hand, Eccles considers that the action of this mediator is such that it removes the "lids" of the membrane pores. The authors do not propose other explanations of the phenomenon under consideration. The action of the mediators belonging to the inhibitory type (GABA) might be explained, within the same context, by the stimulation of seclusion.

The first and in fact the most important implication of the "seclusion through calcium" may be found in the membrane electric phenomena. For exemplifying, we shall analyze, based upon the theory of "seclusion through calcium", the way in which the muscle fibre is engaged and disengaged.

In its general acceptation, the term "depolarization" defines in fact two processes which develop successively at the level of the free portion of the fibre membrane, as a means of engaging the fibre for giving a specific answer. On the one hand, there is the problem of *the electric depolarization* through the rapid transfer of electrons and protons, and on the other hand, the problem of *the Na^+ and K^+ ion distribution*, according to the direction of the concentration gradients. If the first process leads to the rapid change in the electric state of the membrane, the second plays only the role of keeping it up. At the level of the postsynaptic membrane, the action of the chemical mediator substitutes the electric depolarization, i.e. it determines, in a way similar to the latter, the cancelling of seclusion by reducing the interactional forces in the metal-protein complex, leading to the same consequences upon the passive transfer of ions.

The chemical mediator discharged at the level of the neuromuscular junction will mask the interactional forces of the active points in the biopolymers that surround the pores of the postsynaptic membrane, substituting the Ca^{2+} ions coordinated in the rest phase. As a result, the pores of this portion of membrane will be widely open, and the Na^+ and K^+ ions will suddenly migrate, wiping out their own concentration gradients, reversing and maintaining the electric charge of the latter. The potential drop appearing between the limited postsynaptic zone and the rest of the sarcolemma determines an *electric depolarization* (through the migration of electrons and protons) that is generalized on the entire fibrillary surface. The electronegativity of the outer surface of the sarcolemma will reduce the interactional forces of the active points on the biopolymers that surround the pores, determining the discharge into the extrafibrillary space of the Ca^{2+} ions coordinated into a complex in the rest period. The discharged pores will allow the movement of the K^+ and Na^+ ions, wiping out the gradients and ensuring in this way the maintenance of depolari-

zation during a time interval long enough to couple electro-mechanically the contraction. At the level of the "T" system triads the sarcolemma depolarization is *electrically* induced also at the level of the intrafibrillary cisternal system, which we consider — based on all the experimental data offered by the available specialty literature — *a simple calcium reservoir* having the capacity to stock it by active transportation. Once depolarized, the calcium reservoir will set free this sarcoplasm ion, releasing the enzymatic reactions discharge energy, and therefore the contraction.

Given the thermodynamic significance — through finality — of the distribution of ions in the depolarization phase, the coupled active pump will be wholly used in the asymmetrical redistribution process of the K^+ and Na^+ ions, in this way the fibre passing on to the repolarization phase. As the K^+ ions are actively carried towards the interior and those of Na^+ towards the exterior, we also witness a proton — electron translocation (accumulation of hydrogen ions on the outer face), a progressive growth of the electropositivity of the membrane outer face being ensured. Under these conditions, the biopolymers that surround the pores will regain the specific features of the state of rest, among which that of being a ligand in the process of calcium complexing. The physiological consequence is a reduction in the diameter and density of the pores (seclusion) and by this, a reduction of the rate of passive transfer of the K^+ and Na^+ ions. These processes have a certain degree of inertia owing to which "the seclusion through calcium" will progress for a certain time interval beyond the value of the initial state of rest. The surpassing of this value will lead to a growth, beyond certain limit, of the concentration of the hydrogen ions on the outer face of the membrane, to the change in the values of the ionic relations, to the growth in the potential difference on the two faces of the membrane, etc., all these being factors capable of correspondingly modifying the specific features of the biopolymers, among which the quality of being a ligand in the complexing process with Ca^{2+}. In other words, under these conditions the stability of the metal-protein complex diminishes, slightly reducing seclusion to the value of rest. With respect to the same mechanism, acting however at the level of the motor neurons, we gave an explanation to the existence [30] of miniature potentials [7] [8] which may be interpreted today as physiological ways of functionally tuning the excitability of the two interconnected formations — the neuron and the muscle fibre.

As concerns energy, the mechanism of the "seclusion through calcium" proves to be extremely efficient, both with respect to the electric processes and to the passive transportation of other small-sized particles. The progressive seclusion of the cell, correlated with the values of the activity of the coupled active pump, exempts the cell from the effort necessary to counterbalance the passive migration in a reverse direction of the K^+ and Na^+ ions, which is the greater, the more numerous are the actively carried ions (owing to the growth of the gradients).

With respect to the passive transfer of the small molecules, whose concentration on one side or the other of the membrane determines movements that are not always corresponding to the metabolic needs of the moment, the absence of an economic and rapid seclusion mechanism

would expose the cell either to unfavourable chemical actions, or to a great consumption of energy.

In the light of the mechanism of "seclusion through calcium" the most varied and important membrane processes get an explanation, both at the level of the cell and at the sub- and supracellular ones, opening new and large prospects to practical applications in the bio-medical field.

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THE pH ACTION ON THE MEMBRANE DEPOLARIZATION BY K+ IN THE PRESENCE OF DDT

BY

ŞT. AGRIGOROAEI and V. CRĂCIUN

The experiments were performed on single frog muscle fibres by using the intracellular glass microelectrode technique. At pH = 7.2 (carbonate buffer) in the presence of DDT (5.10^{-4} M) a small increase of the amplitude and length of K+ depolarization (30 mM) took place. After washing with normal Ringer solution the depolarization persisted some 10 minutes, then the repolarization took place very slowly. At pH = 6 this effect was less evident. On the basis of these results the authors consider that the effects of DDT on the membrane rely on the insecticide interaction with the external membrane layer, and not with the internal one.

The investigation of the insecticide action on the excitable systems has developed in the last time because these chemicals became, by their massive use in pest control, a part of the environmental medium, occurring in all the living forms, from Arctica to Antarctica. There exist now at least a billion pounds of DDT in the biosphere [5].

Moreover, the so-called "biological magnification" phenomenon has been evidenced, that is the increase of insecticide concentration in the biological systems starting from very low initial concentrations in the medium. Through accumulation in the lipids of the organisms, the insecticides can be concentrated 80,000 to 1,000,000 times. The danger arises especially when the organism consumes his own lipids as an energy source : the lipid mobilization results in a DDT redistribution to other tissues and leads to the acute poisoning.

For this reason, the action of DDT on the biological membranes should be explained by considering the liposolubility of this drug and implicitly the possibilities of its interaction with the membrane phospholipids. It is well known that DDT strongly binds to the membrane and cannot be removed even after repeated and extensive washing, while its action develops with time [3] [6].

Although some authors hypothetically admitted a DDT interaction with the membrane proteins [7] [8] [12], recent studies demonstrated the possibility of DDT to bind the membrane phospholipids and to form charge transfer compounds [13]. They showed that the DDT binding to the membrane proteins is uncertain.

In fact, the numerous effects of DDT on the bioelectrical phenomena described in the literature cannot be understood without allowing for some interactions with the phospholipids, admitting these latter play a basic part in the bioelectrical phenomena.

Based on the pattern of specific ionic channels with "gates" and taking into consideration the effects of DDT upon the Na^+ - and K^+-conductances during the action potential, some authors admitted that the site of the DDT action is on the inner surface of the membrane [9 — 11]. The same authors and others, too, [4] showed that DDT has no effect on the resting potential, although from their data a small membrane depolarization can be deduced.

Our experiments pointed out that such an effect is real and that it is more evident if the Ringer solution is slightly acid. The studies were performed on single muscle fibres of the frog, by means of the intracellular glass microelectrode technique, using an electronic "Tönnies" device for amplification and recording. The DDT concentration in the Ringer solution was $5.10^{-4}M$ and was obtained by dissolving beforehand the insecticide in ethanol and then adding it to the Ringer solution, so that the final ethanol concentration does not outrun 1%.

In an external Ringer solution with DDT at pH = 7.2 (carbonate buffer) we found, after 5 minutes, a slow membrane depolarization which maintained itself, with some oscillations, throughout the observation period (1h). The average amplitude of this depolarization was 3.7 mV, which represented 3.8% of the normal value of the resting potential.

At pH = 6 (phosphate buffer) in the presence of DDT we found a membrane depolarization of 6.88 mV amplitude (7.45 % of the normal value).

The intimate mechanism of this DDT effect remains to be deepened in the future, although we mention that its pH-dependence may be considered in correlation with the observation of Tinsley and co-workers [13] that the insecticide interaction with the membrane phospholipids is influenced by the medium in which the phospholipid molecules are situated. As shown by us in other paper [1], an acid external Ringer causes a partial removing of Na^+ from the protection sphere of the liophobic laminar sols of the external membrane layer by replacing it with H^+, and thus decreasing their degree of stability.

On the other hand, the experiments at pH = 6 were performed under such conditions that the intracellular pH remained unchanged (phosphate buffer) and hence the internal membrane layer remained unchanged too. Therefore one may admit that the depolarizing effect of DDT, dependent upon the external pH variation, is localized at the level of the external membrane layer and is based on the interaction of the insecticide with the phospholipids.

More important aspects resulted from the experiments in which we followed the DDT effect on the membrane depolarization by high external K^+. The presence of DDT in the high K^+-Ringer (30 mM K^+, by equimolar substitution of Na^+) (Fig. 1) resulted in a small increase of the amplitude and length of the K^+-depolarization. The fact was more obvious that, after washing with normal Ringer, the depolarization persisted some 10 minutes, repolarization taking place very slowly afterwards. This means that DDT, still present in the membrane, became more effective on the globular structures of the external membrane layer which

appears from K^+-depolarization. In spite of the normal Ringer washing, the DDT remained in the membrane and prevented the Ca^{++}-binding, and, thus, the membrane repolarization.
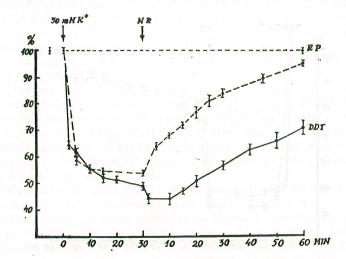
Fig. 1. — The membrane K^+-induced depolarization and repolarization in the presence of DDT at pH = 7.2. *NR*, normal Ringer; *RP*, resting potential.

This effect was also found at an external pH = 6 (Fig. 2), but it was less evident.

In another paper [2] we have shown that at an acid external pH the globular micellae contain more phospholipid molecules and, thus, the external layer of the membrane is more open than at a normal pH. Although DDT prevents, to a certain extent, the binding of the external Ca^{++} to the membrane, the transitional action of Ca^{++} cannot be prevented to the same extent as at normal pH.

If the membrane K^+-depolarization in the presence of DDT takes place at an acid pH after which the repolarization is allowed to develop at a normal pH, the rate of repolarization appears greater than in the case the entire phenomenon occurs at a normal pH, and smaller than in the case in which it occurs entirely at an acid pH (Fig. 3). This fact may be explained if we take into consideration that the repolarization started under conditions of a greater membrane opening, imposed by the acid pH, while the normal pH imposed a decrease of this opening.

Our experimental data outlined above led to two important conclusions :

— no matter the pH at which the K^+-depolarization in the presence of DDT occurred, the more characteristic DDT effect was observed when the membrane was brought back to a normal Ringer solution ;

— under these conditions the membrane repolarization was strongly influenced by the extracellular pH.

These conclusions allowed us to reassess the explanation of the DDT effect on the action potential described in the literature. It is known that this effect differs according to the tissue studied. In the DDT-poisoned lobster axon a very prolonged falling phase of the action potential, like
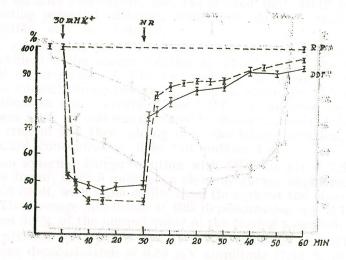

Fig. 2. — The membrane K⁺-induced depolarization and repolarization in the presence of DDT at pH = 6.
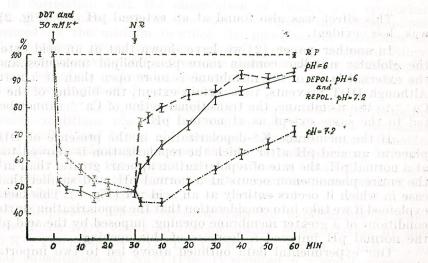

Fig. 3. — The membrane K⁺-induced depolarization in the presence of DDT at pH = 6 and repolarization at pH = 7.2.

a cardiac action potential, was found [9 — 11]. By the voltage-clamp method the authors found that DDT slows the turning-off process of the Na⁺-conductance and inhibits the turning-on process of the K⁺-conduc-

tance. In the myelinated nerve fibres [3] and in the striate muscle fibres [4] a slowing down of the initial phase of decay of the action potential and a marked increase of the negative after-potential was found.

In none of these cases the rising phase of action potential caused by the Na⁺ entering into the fibres was not visibly influenced by DDT. We consider this fact as an evidence against the opinion that the insecticide action would occur at the level of the internal membrane layer [11]. The phase transition produced at this level by the external Na⁺ after stimulation, which in fact results in an active membrane depolarization, proceeds just like in the absence of DDT.

The slowing down of the first part of the falling phase of the action potential indicates, on the other hand, that DDT acts at the level of the external membrane layer by delaying the reversing action of the internal K⁺ upon the electric charge of this layer (the phase transition from laminar to globular micellae). This fact is in good agreement with our observation according to which DDT delays and prolongs the passive depolarization by highK⁺ at normal pH.

The dramatic slowing down of the second part of the falling phase of the action potential indicates that DDT inhibits very strongly the inverse phase transition from globular to laminar micellae in the external layer by preventing the Ca⁺⁺-binding from the external solution. This phenomenon is concordant with our finding which points out that DDT inhibits very strongly the passive membrane repolarization.

Therefore, by explaining these phenomena on the basis of the "2-MSI" concept we have all the reasons to assert that the effects of DDT on the action potential are based on the interactions of the insecticide with the external membrane layer and not with the internal one.

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CYTOGENETIC EFFECTS OF SOME PESTICIDES ON RODENTS

BY

DOINA DUMA, P. RAICU, M. HAMAR and A. TUŢĂ

The analysis of the cytogenetic effects of zinc phosphide and Nuvacron in *Arvicola terrestris* and *Mesocricetus auratus* emphasized considerable differences between the two pesticides. It was found out that zinc phosphide does not influence the mitotic index, although it causes strong chromosomal aberrations (pycnotic metaphases, chromosome despiraling, telomeric associations, deletions, etc.). Nuvacron caused a decrease in the mitotic index 48 hrs after the treatment, but it did not affect the morphology of the chromosomes.

The pesticides commonly used in fighting rodents are very little known genetically. Recent research on pesticides shows them to possess teratogenic and cancerogenic effects and a rather highly frequent ability to induce mutations, both in the species against which they are used in the other vegetable and animal species within the respective ecosystem [3] [9] [10] [12].

Therefore we have thought it useful to study the cytogenetic effects of some pesticides such as zinc phosphide and Nuvacron on some species of rodents.

MATERIAL AND METHODS

The authors have supervised the effects of zinc phosphide in several batches of at least 10 animals belonging to the species *Arvicola terrestris*. One batch was made up of animals that had been captured a few hours after treatment in agricultural farms near the towns of Braşov and Sibiu. The rest contained animals which had been administered the substance through food in the laboratory. The effects of zinc phosphide were also studied on two batches of 10 individuals of *Mesocricetus auratus*.

For studying the cytogenetic effects of zinc phosphide, the animals were given subletal doses of 2 mg/100 g body weight and 1 mg/100 g body weight. The animals were killed at 24, 48 and 72 hrs intervals after feeding them with raticide, and the meiotic index and the mitotic chromosomes aspect was examined on smears made from bone marrow, a technique slightly modified by us according to Ford and Hamerton (4).

The procedure used was similar when studying the cytogenetic effects of Nuvacron, though using this time animals from the species *Mesocricetus auratus*. Batches of control animals that had not been fed on pesticides were studied cytogenetically too.

RESULTS AND DISCUSSION

As compared to the controls, no significant change in the mitotic index of *Arvicola terrestris* has been noted as a result of zinc phosphide influence. Thus the control mitotic index was 14.5⁰/₀₀ and that of the animals treated : 13.8⁰/₀₀ at 24 hrs, 12.9⁰/₀₀ at 48 hrs and 14.8 at 72 hrs.

It has been noted that the mitotic index of *Mesocricetus auratus* was not affected by zinc phosphide. Thus the control mitotic index was 17.95⁰/₀₀ and that of the treated animals 19.72⁰/₀₀ at 24 hrs, 16.78⁰/₀₀ at 48 hrs and 18.56⁰/₀₀ at 72 hrs (Table 1).

Table 1

The mitotic index (0/00) of *Arvicola terrestris* and *Mesocricetus auratus* after administering the pesticides

Species	Treatments	Recovery time		
		24 hrs	48 hrs	72 hrs
Arvicola terrestris	Control	14.5	—	—
	Zinc phosphide 2mg/100 g b.	13.8	12.9	14.9
Mesocricetus auratus	Control	17.9	—	—
	Zinc phosphide 1 mg/100 g b.	19.7	16.7	18.5
Mesocricetus auratus	Control	14.1	—	—
	Nuvacron 1.05 mg/100 g b.	13.8	7.9	29.7

Yet zinc phosphide strongly affected the morphology of the chromosomes. Numerous chromosomal anomalies were found at mitosis : a thickening of telomeres which appeared strongly coloured (heterochromatic), interchromosomal associations involving either the telomeres of two non-homologous chromosomes, or one telomere of a chromosome and the centromeric area of another chromosome and chromosomes and metaphases with various degrees of pycnosis. Because of pycnosis the chromosomes lose their outline and individuality, becoming at times an amorphous, compact, chromatic mass intensely stained.

It is worthy of note that when larger doses are administered (2 mg/ 100 g body weight), these chromosomal modifications occur with the same frequency at 24, 48 and 72 hrs from taking the substance. These modifications consist almost exclusively of generalized pycnoses. However, if a smaller dose is administered (1 mg/100 g body weight), a certain evolution of the chromosomal aberrations can be noted, both with regard to the types of chromosomal changes and with their frequency. In this respect, our study pointed out the following : at 24 hrs from taking the pesticide there occur thickened chromosomal telomeres, associations between two or more chromosomes, and relatively frequent stathmoanaphases caused by the clearing of the centromeres. At 48 hrs the changes present after 24 hrs persist, and more interchromosomal associations with a higher frequency are to be seen the short arms of the subtelocentric chromosomes are frequently heterochromatic and attached to each other and rare deletions and pycnotic metaphases also appear. 72 hours after the treatment, there

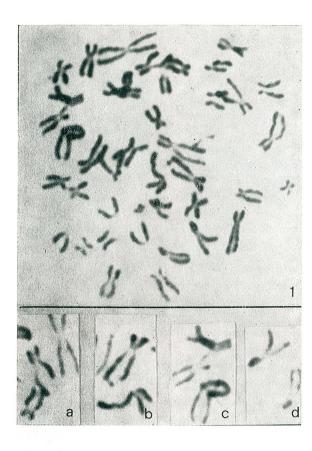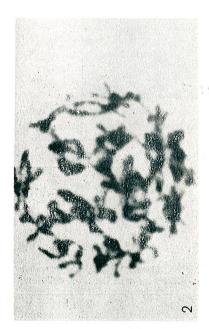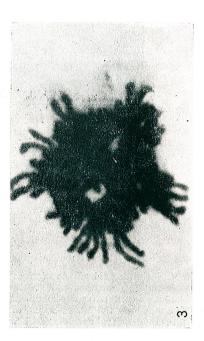*Plate 1.* — Cytological effects of zinc phosphide on mitosis in *Arvicola terrestris* and *Mesocricetus auratus*. *1*, Metaphase with different anomalies : *a*, deletion ; *b*, deletion and interchromosomal associations ; *c*, interchromosomal associations ; *d*, stickness of the short arms of chromosomes. *2*, Pycnotic metaphase. *3*, Starshaped metaphase. *4* and *5*, Various degrees of despiraled chromosomes.

is an almost exclusive presence of pycnotic metaphases, involving all the chromosomes. The star-shaped metaphases with the chromosomes gathered into an amorphous chromatic mass are particularly suggestive; also the despiraling of the chromatic material the chromosome is made of.

The frequency of chromosomal aberrations has increased from 24 hrs (28.57% of the metaphases) to 48 hrs (40% of the metaphases) and to 72 hrs (50% of the metaphase). According to the results obtained we consider that zinc phosphide causes many and varied chromosomal aberrations and that it has a lasting effect. The genetic material of the animals which have survived is rather strongly affected (Table 2).

Table 2

Evolution of the chromosomal anomalies induced by zinc phosphide in *Mesocricetus auratus*

Treatment	Recovery time (hrs)	metaphases with anomalies (%)	Predominant anomalies
Zinc phosphide 1 mg/100 g b.w.	24	28.6	Telomeric associations, telomeric thickenings, stathmoanaphases
	48	40.0	Interchromosomal associations, chromatidic pycnoses, delections, pycnotic metaphases
	72	50.0	Pycnotic metaphases, star-shaped metaphases, despiraling of the chromosome

As for Nuvacron, our investigations revealed the fact that it affects the mitotic index, causing a sharp decrease of this latter at 48 hrs from the treatment, probably inhibiting the cell division for a short time, so that the mitotic index increases at 72 hrs, significantly beyond that of the control. Thus the control mitotic index was $14.1^0/_{00}$ and that of the animals treated $13.8^0/_{00}$ at 24 hrs, $7.9^0/_{00}$ at 48 hrs and $29.7^0/_{00}$ at 72 hrs (Table 1).

Unlike zinc phosphide, Nuvacron does not affect the chromosomes morphologically, and does not produce chromosomal aberrations.

The results of our investigations on the cytogenetic effects of zinc phosphide and Nuvacron are consistent with those obtained by Kihlman [7], Sax and Sax [11], Amer Soheir and Farah [2], Yorder et al. [13], Grant [6], Kelly-Garvest and Legator [8], Georgian [5], etc. which also evidenced the mutagenic effect of some pesticides, insecticides and fungicides on the chromosomes in mitosis and meiosis.

In conclusion, our research revealed considerable differences between the cytogenetic effect of the two pesticides studied. It was found that zinc phosphide does not influence the mitotic index, but it causes numerous and severe chromosomal aberrations. This may have consequences on descendants, since the animals surviving the treatment can be the carriers of certain chromosomal aberrations and mutations. As for Nuvacron we may say that it has a more reduced influence on the genetic material and is the fore less dangerous for the descendants.

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BIOLOGICAL STUDY OF THE SPOTTED BOLLWORM, *EARIAS INSULANA* BOISD. IN THE VICINITY OF PESHAWAR, PAKISTAN

BY

KARIM ULLAH

The biological study of *Earias insulana* Boisd., a major pest, revealed that the insect is present throughout the cotton growing season and overwinters as hibernating pupae. The emergence of adults started in February. The insect lives on *Hibiscus esculentus* and on other malvaceous plants in the absence of cotton during spring and early summer.

Cotton is grown in about 60 countries of the world. The world area under cotton has exceeded 80 million acres. With regard to the area, Pakistan has occupied the fifth position with about 3.3 million acres or about 4 percent of the world area under this crop, based on the average of 4 years (1959—1963).

Numerous insect pests are causing damages to the cotton crops in cotton-growing countries. Among them, *Earias insulana* Boisd. is one of the most serious pests which is distributed in almost all the cotton-growing countries of the Old World and, particularly, in Pakistan, India, Turkey, Cyprus, as well as in Africa.

Numerous biological studies on *Earias insulana* were conducted throughout the cotton-growing countries [2 — 10].

As little work has been done on cotton pests in the Peshawar region, Pakistan, and with a view to finding out the means for its effective control, a thorough understanding of the bionomics of the pest is a must. Therefore it was attempted to study the biology of *Earias insulana* Boisd. in detail.

MATERIAL AND METHOD

The research was conducted at the Faculty of Agriculture of Peshawar, Pakistan, with *Earias insulana* as test insect, collected from cotton (*Gossypium* spp.), bhindi (*Hibiscus esculentus*) and gulkhaira (*Althea rosea*), at room temperature (Table 1), from May 1965 and continued till Novemver 1966. Bhindi fruits, a favourite host of the test insect, were fed to the larvae throughout the study.

To record the number of eggs laid and the longevity of the adults, one female with at least two males were released in single breeding chambers on a fresh leaf of gulkhaira. These adults were fed on sugar solution.

The eggs laid were counted and removed daily to record the inter-oviposition period. The eggs laid in the same days were placed in clean Petri dishes till hatching. The freshly hatched larvae were removed to other Petri dishes containing a few pieces of bhindi fruits. The food was changed every 1 to 3 days and continued till the resulting adults from these larvae died. Regular records about the larval and pupal stages and the longevity of the adults were maintained.

Studies on the seasonal history of the test insect were initiated in November 1965 and continued up to December 1966. A large number of larvae present on different hosts were collected from the field and were reared in the laboratory at room temperature in order to know the different stages and the number of generations throughout the year.

Table 1

Average fortnightly room temperature in C degrees

Period (Months)	1st Fortnight		2nd Fortnight	
	Maximum	Minimum	Maximum	Minimum
June 1966	34.6	30.3	36.6	31.4
July 1966	34.2	31.8	33.7	32.7
August 1966	33.1	31.7	33.0	31.3
September 1966	30.9	28.8	29.3	26.8
October 1966	27.7	24.7	24.7	22.7
November 1966	21.8	18.9	18.4	15.4
December 1966	14.0	11.5	14.5	12.0
January 1967	11.8	9.7	13.0	10.0

RESULTS AND DISCUSSIONS

The data obtained from the biological study of *Earias insulana* Boisd. revealed that the pest overwinters as pupa to tide over the unfavourable conditions. The adults emerge from these pupae in the forthcoming spring to repeat the seasonal life history in the next year. The emergence of the first adults was observed during February and March (Table 2). The results obtained slightly differ from the findings of Lefroy [7]. According to this author, the test insect commonly overwinters as pupae and moths, but in Peshawar, due to the severe winter season, no other forms than pupae could be found.

The adult life of the males and females lasted for 2 to 35 days with an average of 10.8 days, and 2 to 39 days with an average of 11.1 days, respectively (Table 3). Previous records [4] about the longevity of the moths showed great variations due to the difference in the environmental conditions under which the observations were conducted.

The eggs were always laid singly by the females on the leaves, flower-buds, bolls and pods, etc. The number of eggs per female showed great variations which could be assigned to factors like the size and weight of the adult females, the food available, the existing temperature and humidity and the population density during larval development. A female laid from 2 to 296 eggs with an average of 82.5 (Table 3). The differences between the data mentioned above and those of other studies [7] [10] are primarily due to the differences in the environmental factors, locality, etc.

The incubation period as determined in the present study was 2 to 16 days with an average of 5.04 days (Table 3). The findings of the previous authors [4 — 6] [10] are to a great extent in accordance with the present results.

The larval period, as determined in this study, was with an average of 10.5 days (Table 3). The present results from those of other authors [4] [5] [10]. The differences a to the effect of temperature and food.

Table 2

Duration of hibernating pupae of *Earias insulana* Boisd. in days, recorded during 1965—66

Date of		Individual pupal period
Pupation	Adult emergence	
2—12—1965	24—2—1966	84
3—12—1965	17—2—1966	76
5—12—1965	4—3—1966	89
7—12—1965	2—3—1966	85
9—12—1965	24—2—1966	77
9—12—1965	3—3—1966	84
9—12—1965	9—3—1966	90
9—12—1965	14—3—1966	95
28—12—1965	5—3—1966	67
31—12—1965	7—3—1966	66
Average period		81.3

Table 3

Duration of different stages in days an... female in the life cycle of *Earias in...* during 1965—66, at room t...

Stages in the life cycle	
Incubation period	
Larval period	
Pupal period during summer	7
Pupal period during winter	66-
Longevity of moths	
Entire life cycle during cotton season	17
Number larvae/adult	

The adult moths emerged from pupae after 7 to 13 days average of 9.7 days, and after 66 to 95 days with an average during summer and winter, respectively (Tables 2 and 3). The differences from the findings of the other authors [4] [5] [10] temperatures and months of the year in which the pupal period

Generally, it was observed that the duration of the dif of the insect is prolonged with the commencement of the co

The life cycle was completed in 17 to 51 days with a 23.1 days during the cotton-growing season (Table 3), the r in accordance with previous findings [3] [5].

Besides these, the studies carried out in Peshawar (P vealed that *Earias insulana* Boisd. has 7 to 8 generations a ye The results obtained differ from those of Planes [9] but are i with those of Pant [8].

Table 4

Generation study of *Earias insulana* Boisd., November 1965—December 1966, in days (based on ... duration)

Preoviposition period	Incubation period	Larval period	Pupal period	Duration of generation	Date of adults emergence	
..	..	..	81.3	..	14— 3—1966	
5.0	2.0	9.0	8.0	24.0	30— 5—1966	1
5.0	2.0	10.0	7.0	24.0	5— 7—1966	2
5.0	2.0	9.0	8.0	24.0	28— 7—1966	3
4.9	2.8	7.8	9.3	24.8	21— 8—1966	4
4.5	3.0	11.0	12.7	31.2	15— 9—1966	5
2.5	3.2	10.0	15.0	30.7	7—10—1966	6
2.5	3.5	10.0	10.5	26.5	25—10—1966	7
6.2	9.0	11.5	..	..	..	8

le des individus d'une même espèce, trouvés *dans une station* sorte qu'il y a presque certitude pour qu'ils soient apparentés ». le cas d'une espèce dont on sait qu'elle habite l'humus des forêts es de tout l'Ouest Paléarctique, des Pyrénées au Caucase ; si l'on part celle définition, on est en droit de se demander si une population e correspond à l'ensemble de cet aréal ? s'il y a une population pour e respectif de chaque grand massif montagneux ? si, par exemple, ue sous-division des Carpates possède sa propre population ? ou bien ue population distincte correspond à chaque forêt de hêtres, ou à ue parcelle de celle-ci ? Il est vrai que certains efforts ont été faits clure dans les définitions aussi l'aspect de la délimitation (Tudoran-[10]) : « ...une population locale est un système ouvert autoréglable nt les éléments sont les individus d'une même espèce, *localisés dans ne certaine biocénose*... ». Mayr [7] est un des rares auteurs à avoir erré de plus près le problème des graves ambiguïtés dans l'utilisation du erme : « The term "population" is used in several different ways and re-ourse to a dictionary is of little help ... a usage is spreading in biology hat restricts the term population to the *local population*, the community of potentially interbreeding individuals *at a given locality*... a group of individuals so situated that any two of them have equal probability of mating each other and producing offspring... In view of the diverse mean-ing of the word population it would be useful to have a technical term for the local population as defined above. The term *deme* was proposed by Gilmour and Gregor (1939) ; they, however, defined it vaguely, without clearly avoiding the ambiguities of the term *population*. Later, several zoologists ... gave "deme" a more specific meaning, definitely restricting the term to the local population, the interbreeding community, as defined above ».

On est surpris de constater que les problèmes de la délimitation spatiale (et temporelle) correcte des populations * sont loin d'avoir attiré l'attention dans la même mesure que ceux de la structure, du fonctionne-ment, de l'évolution de celles-ci. Afin de rendre crédible la définition de la population, il faut tenir compte non seulement de son caractère d'unité panmictique, mais aussi du fait qu'elle représente la totalité des individus d'une espèce *habitant la même biocénose et de ce fait plus ou moins nette-ment séparés des unités similaires avoisinantes* (ce qui n'exclut absolu-ment pas la possibilité des intercommunications et intergradations entre populations locales d'une même espèce). Ces discontinuités sont dues [8] à la combinaison de 3 facteurs : « (1) each species can exist only in certain ecological conditions ; (2) an area of any sizeable geographic extent con-tains ecological differences sufficient that any one species could not exist over every square foot of it ; and (3) a species is at times annihilated from local pockets within its normal range ».

Un échantillon peut être représentatif d'une population (à condition qu'il soit suffisamment important et que l'échantillonnage se fasse de manière très soignée et en essayant de tenir compte des limites spatiales de la population — le « flair » du chercheur n'est pas une qualité négli-

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* Nous nous référons constamment aux *populations naturelles,* et non à celles expéri-mentales.

geable dans cet essai !). Mais ce serait la pire des erreurs que de vouloir mettre le signe de l'égalité entre *population* et *échantillon* !

Une série d'exemples de fantaisie (mais la bibliographie fournirait d'innombrables exemples réels rentrant dans l'une ou l'autre de ces catégories) permettront de préciser nos idées sur l'utilisation correcte ou incorrecte du terme. « La chèvre noire ... est représentée dans les divers massifs des Carpates par des populations distinctes » (certainement correct). « L'espèce x de ... est représentée dans chaque lac glaciaire par une popu-lation ... » (probablement correct s'il est question d'animaux à faibles possibilités de dispersion ; probablement incorrect si c'est le contraire qui est valable). « Les populations du coléoptère troglobie ... des grottes a et b ... » (comme il s'agit de troglobies terrestres : probablement de vraies populations). « L'espèce étant strictement lithophile, dans le littoral de la Mer ... entre les localités ...et ... elle ne peut constituer une popu-lation importante que dans la station ... » (affirmation raisonnable : vraisemblablement population). « Cette espèce dont les populations sont largement répandues dans les forêts des zones montanes est représentée dans la plaine par un petit nombre de populations dans les forêts reliques de chêne » (1ère partie de la phrase : le terme utilisé est extrêmement vague et de ce fait dépourvu de valeur ; 2e partie : vraisemblablement populations réelles). « Les populations planctoniques et benthiques de la Mer ... » (terme absolument dépourvu de précision : s'agit-il d'espèces ? d'associations d'espèces ?). « Les populations de moineaux d'Europe Cen-trale ... » (même observation, bien qu'il s'agisse d'une seule espèce). « Dans la rivière..., on remarque au km 102 une population de ... qui est plus nombreuse que celle du km 98 » (fort probablement *une seule* population). « La population de X^us albus, Y^us nigrus et Z^us viridis des sols de l'étage du pin rampant se caractérise par la dominance de la 1ère espèce sur les 2 autres » (*peut-être* association ; mais il ne s'agit en aucun cas d'une population — ni de « populations »). « Les populations du néma-tode ...de l'interstitiel de la vallée ... et de son affluent... » (puisqu'il est question d'animaux interstitiels, il est pratiquement impossible qu'il y ait une limite entre « deux populations »). « Dans la forêt...3 exemplaires de ... ont été capturés ; on remarque dans cette population .... » (de toute évidence nous avons affaire à un échantillon... et fort modeste encore !) « Les différences entre les populations de *Ursus spelaeus* des grottes A et B... » (le caractère fallacieux de l'utilisation du terme saute aux yeux dans ce cas où les documents sont des ossements fossiles pouvant appartenir à des individus de nombreuses générations successives).

Les cas que je vais présenter par la suite (insectes trichoptères de la faune de Roumanie) devraient montrer que l'acception du terme « popula-tion » doit forcément être extrêmement différenciée et nuancée d'un cas à l'autre, et que parfois l'utilisation du terme n'est absolument pas jus-tifiée.

Stactobia maclachlani Kimm. Ces minuscules trichoptères habitent un biotope très particulier : les « niches madicoles », où l'eau ruisselle en couche mince, mais de façon permanente, sur des surfaces rocheuses verti-cales ; ces niches sont normalement rares, distancées par des centaines de mètres ou par des kilomètres, même dans les montagnes bien arrosées. L'es-

pèce est répandue, en Roumanie, dans les Monts du Banat, les Monts Apuseni et les Carpates Méridionales ; il est toujours possible de tracer sans difficulté la limite des diverses populations locales.

Isogamus lineatus Klap., espèce connue des Alpes Orientales, a été découverte dans les Carpates uniquement dans les Monts de Făgăraş (Carpates Méridionales), où elle habite une série de sources et de torrents presque exclusivement au-dessus de la limite supérieure de la forêt. Nous avons affaire, dans le Făgăraş, à un petit complexe de populations parfaitement isolé des populations des Alpes ; mais dans le cadre de ce complexe, quelles sont réellement les relations entre populations ?

Micrasema minimum McL. est une espèce fort abondante, partout dans les Carpates roumaines, mais surtout dans les « rivières de montagne » (zone à *Thymallus* des rivières de montagne ; « hyporhithron » dans le système de zonation longitudinale). Les cours d'eau habités occupent surtout le fond des « vallées principales » de chaque massif, et ces « vallées principales » étant d'habitude bien distancées on pourrait considérer la totalité des individus de chaque « hyporhithron » (long de plusieurs kilomètres) comme constituant une population locale ; mais parfois on trouve *M. minimum* aussi dans des grands ruisseaux (« metarhithron ») affluents des rivières respectives, ce qui complique le problème.

Un cas similaire, mais plus net, est celui d'espèces comme *Oligoplectrum maculatum* Fourcr. ou *Goera pilosa* Fbr., éléments très caractéristiques de la zone à *Barbus* et à *Chondrostoma* des rivières de basse altitude des Carpates (« epipotamon ») ; l'indépendance accentuée de cette zone permet de parler d'une population de *O. maculatum* ou *G. pilosa* pour chaque bassin hydrographique (la longueur de la zone est normalement de l'ordre des dizaines de kilomètres).

Wormaldia occipitalis Pict. est une espèce extrêmement fréquente dans les ruisseaux petits et grands, parcourant les forêts de hêtres ou d'épicéas d'absolument tous les massifs des Carpates. Ce sont des voiliers modestes, mais les distances entre divers ruisseaux d'un même bassin ou de bassins voisins sont si réduites que—le vent aidant — des individus éclos dans un ruisseau ont certainement des chances de se mélanger à ceux éclos dans un autre. Ceci pourrait faire penser à l'impossibilité de tracer des limites interpopulationnelles. Cependant, l'étude morphologique de la variabilité montre une surprenante uniformité au niveau des échantillons en provenance de chaque ruisseau, tandis que les échantillons en provenance de ruisseaux différents (même voisins) présentent des différences parfois sensibles.

Dans les basses zones des Carpates la structure populationnelle d'espèces comme *Adicella filicornis* Pict., *Lithax obscurus* Hag. ou *Notidobia ciliaris* L. est pratiquement inconnue, mais ces 3 espèces ont laissé des colonies reliques dans la Plaine Roumaine (Plaine de Danube) dans le complexe de sources de Corbii Ciungi : dans ce dernier cas, l'existence d'une population nettement individualisée pour chacune des 3 espèces est indiscutable.

Hydropsyche bulgaromanorum Mal. est une des espèces d'insectes les plus communes dans le Danube inférieur, où elle se développe en masse au moins depuis Belgrade et jusqu'aux bouches du fleuve. Il est pratiquement sûr que, le long de cet énorme secteur, il n'y a pas d'endroit qui soit vraiment impropre à son développement. Dans ces circonstances, serait-il justifié de parler de *plusieurs* populations danubiennes de *H. bulgaromanorum* ? Je pense que non, car on pourrait multiplier celles-ci à l'infini, chaque « population » correspondant en dernière instance à un échantillon, ce qui ne serait absolument pas relevant sur le plan scientifique.

Un cas à l'antipode de celui de *Stactobia* est celui d'*Oecetis furva* Ramb., espèce limnophile et phytophile banale, à large distribution, présente par exemple dans les eaux stagnantes du Delta du Danube et de ce qui reste de la zone inondable du fleuve. En dépit de l'apparente diversité des habitats, on l'y trouve partout, et il n'y a pas de variabilité. Vouloir distinguer des populations locales, par exemple dans les divers lacs et canaux du Delta, me semble insensé.

* *

Dans la recherche scientifique, les termes sont de précieux outils à condition qu'ils soient rigoureusement appliqués aux notions respectives et qu'on n'en abuse pas. Sinon, c'est le chaos et les termes se dévalorisent (ce n'est pas le cas seulement pour « population » : pensons par exemple à « biocénose » …et même à « écologie »).

Il est vrai que la difficulté, dans le cas que nous venons de discuter, est parfois aussi d'ordre linguistique : en roumain on ne dispose pas de 2 termes différents comme en français (population et peuplement) ou comme en allemand (Population et Besiedlung).

Il me semble indispensable que, si on considère que la population n'est pas un concept purement abstrait, sans dépendance des conditions naturelles, là où le terme « population » ne peut être employé en toute connaissance de cause, là où il est impossible d'argumenter son utilisation, il soit remplacé par une description claire et complète, mais néanmoins succincte, de la situation de fait.

L'étude de cas limite (espèces comprenant une seule population restreinte, populations rigoureusement isolées dans l'espace, populations périphériques) peut offrir de grandes satisfactions au systématicien, à l'écologiste, au spécialiste de la génétique des populations. Mais il faut reconnaître que ce sont là des cas relativement simples. Ce qui extrêmement difficile c'est de reconnaître et de délimiter convenablement les populations des espèces à large distribution, et ceci d'autant plus que l'aréal des populations naturelles subit des modifications avec le temps. Il est même possible que les cas les plus épineux n'aient pas, pratiquement parlant, de solution positive.

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Institut de Spéologie
« E. G. Racovitza »
78109-Bucarest, M. Moxa 9

CINQUANTE ANNÉES DE RECHERCHES MARINES EN ROUMANIE

Entre le 1er et le 3 novembre 1967 s'est déroulée à Constanța, sous l'égide de l'actuel « Institut roumain de recherches marines », une session scientifique, dédiée au cinquantenaire du commencement des recherches océanographiques roumaines.

En 1926 le professeur Ion Borcea de l'université de Jassy fondait à Agigea (à 12 km au sud de Constanța), sur un rivage qui avait en face des fonds rocheux, sablonneux et boueux, la première station de biologie marine, qui porte aujourd'hui son nom. Une année plus tard le Dr Grigore Antipa, directeur du Musée National des Sciences Naturelles de Bucarest, a commencé l'édifice d'une autre station océanographique à Mamaia, qui porte aussi aujourd'hui son nom. Pendant les cinquante années qui se sont écoulées, les deux institutions ont été le siège de très nombreuses recherches sur la Vie et sur les conditions physico-chimiques du milieu de la mer Noire. Il m'est impossible de nommer ici tous ceux qui ont travaillé dans ces deux stations pendant ces 50 années et plus spécialement ceux qui ont poursuivi des recherches à Agigea, station qui est devenue le centre principal des recherches biologiques aussi bien marines que terrestres. Le nombre de ces chercheurs dépasse de beaucoup plusieurs centaines. Parmi eux il y avait des étrangers (bulgares, polonais, hongrois, français, allemands, etc.).

Il faut mentionner qu'à Agigea on poursuivait surtout des recherches biologiques et hydrologiques, tandis qu'à Mamaia on faisait surtout des études de pêche.

Parmi ceux qui ont dirigé les destins de ces deux stations je dois citer, à Agigea : le professeur Ion Borcea (fondateur et zoologiste), Ion Botez (anatomie), Constantin Motaș (zoologie et biogéographie), Sergiu Cărăușu (amphipodes), Nicolae Gavrilescu (physiologie), Ion Borcea jr.(zoologie), Ion Andriescu (écologie) et à Mamaia : le Dr Grigore Antipa (fondateur et ichtyologiste), le professeur Teodor Bușniță (ichtyologiste), Nicolae Ionescu (ingénieur piscicole), etc.

Je considère que presque tous les biologistes de notre pays ont passé, une ou plusieurs fois par la station d'Agigea, effectuant des recherches dans le domaine de leur spécialité. Cette station était aussi le centre didactique pour la pratique océanographique des étudiants de toutes nos universités.

On a fait ici des études sur la flore et la faune, sur la physiologie, la biochimie, la parasitologie, l'écologie, etc. des organismes de la mer Noire ; on a étudié la vie sur les plages, sur les rochers, sur les sables, sur la boue ; on a poursuivi des études sur le plancton, sur le benthos, sur la plate-forme continentale des rivages roumains, etc.

Le travail à Agigea était presque continu pendant toute l'année. Périodiquement on se réunissait pour discuter les résultats obtenus, pour connaître les problèmes qui apparaissent dans le domaine de la biologie marine, pour organiser des expéditions sur la mer en vue de collecter du matériel, etc. Plusieurs rencontres internationales ont eu comme siège Agigea ou Constanța. En 1969 on a donné un cours pour 32 chercheurs méditerranéens sur la biologie des eaux saumâtres de la mer Noire (dans le cadre du MAMBO).

Je me permets de faire une sorte de parallèle avec la façon de travailler dans la station zoologique de Naples, où, sous la direction d'Anton et de Reinhard Dohrn, on se réunissait périodiquement pour discuter les résultats obtenus par différents chercheurs étrangers. Il faut mentionner que I. Borcea, ainsi que Gr. Antipa ont travaillé à cette station de prestige international.

Les résultats de toutes ces recherches ont paru dans différentes revues roumaines et étrangères ; parmi les plus importantes je cite : Annales scientifiques de l'université de Jassy, Annales et Bulletins de la section scientifique de l'Académie Roumaine, Bulletin de l'Institut Océanographique de Monaco, Revue roumaine de biologie, Studii și cercetări de biologie, etc. Beaucoup de tirés à part de ces travaux ont été réunis dans plusieurs volumes des « Travaux de la Station biologique marine d'Agigea », publication d'échange largement répandue dans les institutions marines du monde.

Vers 1948, dans le cadre de l'Académie de la République Socialiste de Roumanie, prenait naissance à Constanța une nouvelle section de recherches marines dirigée par le Dr Mihai Băcescu. Quelques années plus tard on a essayé de réunir les trois unités marines (Agigea, Mamaia et Constanța) sous une direction unique. Mais la vraie unification n'a été possible qu'en

1970, quand a été fondé sur des bases nouvelles l'actuel « Institut roumain de recherches marines », dirigé par le contre-amiral Constantin Tomescu et ayant comme adjoint scientifique le Dr Gheorghe Şerpoianu.

Mais en dehors de ces changements d'organisation, les recherches marines et océanographiques roumaines ont continué pendant tout ce temps sans interruption. De ces résultats je me permets de souligner les plus importants.

L'école de feu le professeur Ion Borcea a étudié surtout la composition et la structure des organismes de la mer Noire. Il a été secondé dans ce travaux par ses éminents élèves : Sergiu Cărăuşu (amphipodes), Mihai Băcescu (crustacés), Maria Celan (algues), Olga Necrasov (anatomie comparée), Vasile Radu (isopodes), etc. Pendant ce temps à Mamaia Grigore Antipa sortait le grand volume sur la Vie dans la mer Noire et le premier Atlas ichtyologique. Ses successions, Nicolae Ionescu, le Dr Ileana Cautiş et Nicolae Bacalbaşa mettaient au point les migrations des poissons dans la mer Noire et de nouveaux engins de pêche.

Grâce à ces travaux complexes, nous connaissons aujourd'hui assez bien la biologie et la bionomie de la mer Noire et les causes physico-chimiques et climatiques qui provoquaient des changements imprévus dans la répartition des organismes, surtout benthiques.

Les premiers disciples des fondateurs de ces deux institutions océanographiques roumaines ont eu à leur tour beaucoup d'élèves et de nouvelles disciplines se sont ajoutées à celles existantes au début : le professeur Petre Jitariu et moi-même, nous avons fondé des laboratoires de physiologie animale et de biochimie ; Madame Lucie Leon Borcea a commencé des recherches sur la parasitologie des animaux marins, études qui ont été continuées par les professeurs Radu Codreanu et Elena Chiriac ; le professeur Ion Grossu a étudié les mollusques de la mer Noire ; le Dr Gheorghe Şerpoianu a mis les bases scientifiques des études hydrologiques et chimiques des eaux et des courants de la mer Noire ; le professeur R. Zarma a commencé les premières études microbiologiques des eaux marines, etc.

Parmi les plus jeunes spécialistes d'aujourd'hui, qui travaillent dans le cadre de l'Institut roumain de recherches marines, on doit citer (tout à fait incomplètement) les noms du Dr Geza Müller (écologie), Dr Traian Gomoiu (benthos), Dr Adriana Petran (protozoaires), Dr Florica Porumb (zooplancton), Dr Pia Mihnea (phytoplancton), Dr Skolka Hilarius (macrophytes), Dr Ion Porumb (poissons), Dr Nicolae Bodeanu (diatomées), Maria Mîrza (biochimie), Octavian Şerbănescu (pollution), Dr Vasile Telembici (acclimatisation) et beaucoup d'autres.

Nous sommes fiers de ces jeunes, qui continuent les traditions cinquantenaires des recherches océanographiques roumaines, dans les nouvelles conditions mises à la disposition de la recherche par l'État roumain. Beaucoup de ces jeunes sont bien connus dans l'océanographie mondiale ; quelques-uns d'entre nous les anciens et beaucoup de jeunes ont participé comme spécialistes à différentes expéditions océanographiques internationales ; beaucoup d'entre eux ont fait des études de spécialisation dans de grands laboratoires marins du monde, sont membres des sociétés internationales de biologie marine ou participent aux réunions de spécialité qui ont lieu chaque année dans différents endroits du monde. Presque tous possèdent une ou plusieurs langues d'usage international.

L'Institut roumain de recherches marines groupe plus de cent chercheurs de différentes spécialités océanographiques : biologie, hydrologie, chimie, pêche, géologie, submersion, etc. Il possède maintenant une revue propre qui publie les résultats de ses recherches et commence à être visité et aidé par des chercheurs d'autres spécialités qui viennent des universités du pays.

Etant donné les perspectives qui s'ouvrent aux recherches de la mer dans le futur proche, nos chercheurs dans ce domaine pourront apporter leurs contributions scientifiques aux connaissances qui donneront à l'humanité de demain de nouvelles ressources alimentaires, chimiques et énergétiques. La Roumanie participe de toutes ses forces à la vie de l'humanité future.

Eugène A. Pora

C. A. PICOŞ, **Viaţa la temperaturi extreme** (Das Leben an extremen Temperaturen), Ed. ştiinţifică şi enciclopedică, Bucureşti, 1976, 312 S., 37 Abb., 22 Tafeln, 1 Anhang.

Das Buch von Dr. C. A. Picoş, „Das Leben an extremen Temperaturen" stellt ein für die rumänische Thermobiologie besonderes Ereignis dar. Diese Arbeit kommt einem Bedürfnis entgegen, sowohl für die Popularisierung in unserem Land eines Gebietes, welches sich in den letzten Jahrzehnten bedeutend entwickelt hat, als auch für die Zusammenfassung einiger interessanter von den rumänischen, in diesem Gebiet tätigen Forschern gewonnenen Ergebnisse.

Aus 9 Abschnitten zusammengesetzt, stellt das Buch in seinem ersten Teil den physischen Bestandteil der Beziehung Tierorganismus-Temperatur vor. Es werden in den nächsten Abschnitten (Kap. 3—5) die gegenwärtigen allgemeinen Kenntnisse über die tierische Wärme, physiologischen Grundlagen der Thermoregulation und thermische Toleranz der Tiere diskutiert.

Die biologischen Wirkungen der Umwelttemperatur werden in einem gesonderten Abschnitt (Kap. 6) unter die Lupe genommen. Die Abwehrmöglichkeiten gegen die Kälte (Kap. 7) und Wärme (Kap. 8) werden ausführlich im Verhältnis mit dem Niveau der phylogenetischen Entwicklung vorgestellt.

In dem letzten Teil dieses Buches (Kap. 9) betont der Verfasser die praktische Bedeutung der künstlichen- Unter- und Übertemperaturen.

Die umfassende Literatur (300 zitierte Arbeiten) und eigene Forschungsaktivität vom Verfasser haben zur Ausarbeitung einer wissenschaftlichen Arbeit von hohem Wert sowohl für die Biologen als auch für die in den angrenzenden Gebieten (Medizin und Tierzucht) tätigen Spezialisten, beigetragen .

Durch die Durchführung dieses Buches ist es dem Verfasser gelungen, seine Forschungsaktivität über zwei Jahrzehnte zusammenzufassen indem er sich als einer der bedeutendsten Fortsetzer der wertvollen rumänischen Traditionen auf dem Gebiet der Zoo-Ökophysiologie bewährt hat.

Dan Marinescu