REVUE ROUMAINE DE BIOLOGIE
SÉRIE DE ZOOLOGIE

SOMMAIRE

CUMELLA AFRICANA N. SP. AND MAKROKYLINDRUS (COALESCUMA) REYSSI N. SP. (CUMACEA, CRUSTACEA) FROM THE SAHARIAN BOTTOM OF THE ATLANTIC

BY

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The author reports 3 Cumacea from the West tropical Africa waters of which two new species, all from the sandy bottom of the Mauritanian Atlantic : *Cumella africana* n. sp., common on the bottoms of 14—96 m. and *Makrokylindrus reyssi* n.sp. (1045—1124 m), also differentiated by a dichotomic key for the 6 species of *Makrokylindrus* cited in the West Africa, between parallel 35°N and 35°S, and *M. spiniventris*.

In the present note we deal with :

1. Cumella africana n. sp.

(Fig. 1)

Description. ♂. Tegument ± glabrous. Eyelobe (Fig. 1 A and B) disc-shaped, with 2 antero-lateral setae, five ocular lenses disposed as follows : one large anterior lense, two postero-lateral smaller ones and two others antero-lateral, still smaller, all of them embedded in a black-brown pigmented mass (Fig. 1 B).

The antenna flagellum, tight in its lateral notch, reaches but the half of the free thoracomer III.

The last peraeopod, very slender, with the carpus exceeding twice the length of the propus (Fig. 1 D and E, to be compared with 1 F).

Uropods slightly longer than in ♀ (to compare figure 1 G with 1 I, according to individuals of *C. limicola* from the Black Sea).
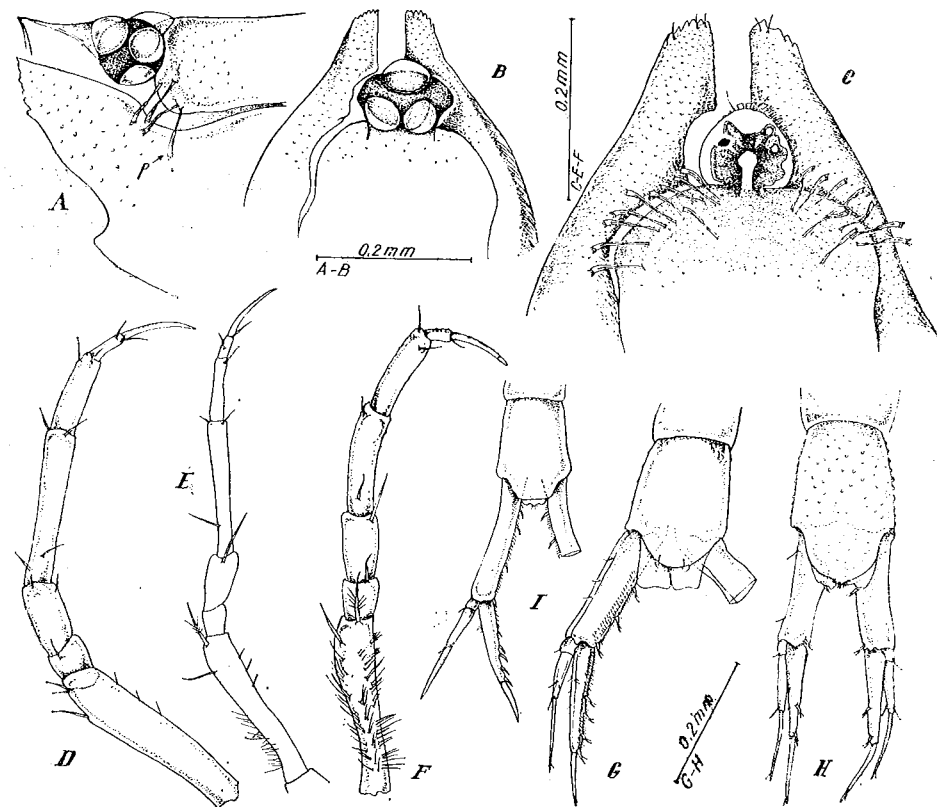

Fig. 1. — *Cumella africana* n. sp. A, B, E, G, ♂; C, D, H, ♀; F, I, *Cumella limicola* ♂; A, frontal lobe and pseudorostrum, lateral view; B, id., from above; C, id. ♀; D, prp. 5 ♀; E, id. ♂; F, id. of *C. limicola* ♂; G, pleotelson and uropods ♂; H, id. ♀; I, id. of *C. limicola* ♂ (Black Sea). I, according to Băcescu 1954; the others, orig.

♀. The whole tegument—somewhat calcified for a *Cumella* — is covered by a sort of granulations rather than by scales (Fig. 1 H) and by rare but long and visible setae. Carapace does not present true carinae; total absence of spines on the dorsal length.

Eyelobe with two very small anterior prolongations like 2 little horns, 2 lateral setae and a pigmented mass of two hemispheres, generally bound by a bridge (Fig. 1 C); four lighted points shine in this mass, the only indication — not clear — of the existence of some minute lenses. On the carapace, especially on the edge of the pseudorostral lobe, some cylindric prolongations emerge (Fig. 1 A, p, and Fig. 1 C) which look like some parasitic Protozoa.

Peraeopods I and II somewhat similar to *C. limicola*, except for the coxa which is ± trapezoid-shaped, not elongated. The last two pairs

of peraeopods are much more slender and the relation between the length of the joints is altogether different: the carpus is also in ♀ approximately twice as long as the propus (Fig. 1 D) and the basipodite is almost glabrous (Fig. 1 E).

Uropods (♂ ♀) are strikingly similar to *C. limicola*, differing only in their shorter basis and their longer rami (Fig. 1 H; fig. 1 G, to be compared with fig. 1 I, for instance).

For other features, see figures and data given in table 1.

Size : 2—2.2 mm (♂ ♀).

Material. Over 20 specimens captured exclusively by dredging, on sandy bottom, between 14 and 96 m, along the Mauritanian coast, during January-February, 1971, at the following stations of the Thalassa expedition : St. X023, 14 m., typic sand by *Branchiostoma nigeriensis* : 2 ♀♀; 2 ♂♂; 18°59′2″N; 16°22′7″W; St. X041, 52 m., sand, shells : 4 ♀♀; 5 ♂♂; 1 juv.; 20°10′5″N; 17°32′9″W; St. X068, 96 m., muddy sand : 2 ♀♀; 2 ♂♂; 1 juv.; 20°47′6″N; 17°36′6″W.

Holotype ♂ (St. X068), under No. 185 in the collection of the "Gr. Antipa" Museum; allotype ♀, id., under No. 186 and 16 paratypes, id., No. 187.

Ecology. Considering the Cumacea associations where it was taken — and where the species of *Bodotria, Iphinoe* and *Eocuma* as well as *Branchiostoma* massively predominate — *Cumella africana* should be, as opposed to the Atlantico-Mediterranean *Cumella*, psammobiontic rather than iliophyle. Since none of the specimens studied — where adult males predominated — was captured in the plankton, not even by nightlight, we assume that ♂♂ of this species do not undertake night migrations towards the surface as their parents do.

Remarks. In *Cumella africana*, features of *C. limicola* (uropods) are associated with characteristics of *C. pygmaea* (eyelobe, thinness and relation between the last peraeopod joints, the length of the flagellum of A_2). It clearly differs from *C. limicola* : the male by *the absence of the rich ocular group composed of 9 lenses* of which 2 are lodged in the pseudorostrum (character of the subgenus *Cumewingia* (Băc. 1971) and by the shortness of the flagellum of A_2; the female, by *the absence of carinae and especially of carinal spines*, and by a different relation between the last peraeopod joints (Table 1, where its pieces were compared with those of *C. limicola* from the Black Sea).

Despite the fact that 18 species of *Cumella* are known in the Atlantic, almost all quotations refer to the NE regions or to tropical America ones of this ocean. In the African waters only one single species — *Cumella limicola* — was mentioned and this only by the coast of Morocco, not far from Gibraltar [6]. Jones (1960) also reports a *Cumella* sp. at the other extremity of Africa (34°09′8″S; 18°26′1″W), equally on sandy beach, at 9 m., which he considers to be close to *C. hispida* or *C. michaelseni* Zimm.

As a matter of fact, the quotation of *C. limicola* should be confirmed in the light of the existence of the species described in the present paper, which could be easily mistaken for *C. limicola*, especially if only the ♀♀ were considered.

Table 1

The morphologic features which distinguish Cumella species previously mixed up

Some characteristics	Cumella africana		Cumella limicola	
	♂	♀	♂	♀
Carapace	glabrous, slightly tuberculated	without carina and spines; with large, rare setae	glabrous	with 2—6 spines on the carina; short setae
Eyelobe lenses	5 large lenses	4, practically non visible in the pigmented mass	9 lenses (2 in the pseudorostrum)	4 small visible lenses
Length of flagellum of A_2 ♂	it hardly reaches pereionite III	—	reaches the pleotelson	—
Length of the carpus of peraeopod V as compared with the propus	2—2.2	1.8	1.2	1
Length of the uropod basis as against the endopod	1.2—1.5	1.36	1.18	1.4
Idem, as against the breadth of the basis	4.4	4.25	5.4	4.2
Size	1.8—2.1	2.0—.22	2.5—3	2.2—31

C. africana certainly belongs to the Atlantico-Mediterranean *Cumella* group, and not to the Caribbean or the Indo-Pacific ones [2].

2. Makrokylindrus (Coalescuma) reyssi[1] n.sp.

(Fig. 2)

Description : ♀ ovigerous. Carapace large (1/3 of the total length, uropods inclusively), globulous, without crests or tubercules, soft, non-calcified. It points out some double dorso-median gibbosity following the depression of the frontal lobe (Fig. 2 A); entirely covered by minute spinules and rare, long setae. Antero-inferior side of carapace pectinated. Pseudorostral lobes ± sharp, without antennal notch and with a girdle of ventrally increasing hairs. Same rare pilosity on thoracomers and pleonites. Thoracomers 3 and 4 coalescent. Pleomers thick, the distal one, just a little shorter than the last but one, presents small spinules on the caudal edge (Fig. 2 B).

The telson, inclusively its terminal spines, does not exceed the length of the last two pleonites taken together; perfectly cylindrical and definitely shorter than the uropod basis (Fig. 2 B). Its postanal portion — terminal spines taken into account — represents but 1/6 of the telson; without spines, 1/10 (Fig. 2 C); it presents 4 pairs (3 in ♀♀ P) of lateral spinules and two strong distal spines bent towards the median line. Near the anal valves, i.e. on its inferior face, the postanal portion bears several rows of minute spinules. Rare setae on the entire telsonic cylinder.

The uropod peduncles exceed by much the telson and bear 18—19 flagellated short spines on the interior edge; on the external one, some simple setae, like those on the exopodite. The last one much longer than the exopodite (Rp. 16 :10, without taking into account the terminal phanera) (Fig. 2 D). Endopodite—3-articulated, each joint bearing 2 flagellated spines — ending with a short, strong thorn.

The small 3-joints flagellum of A_1 shorter than the proximal joint of the large flagellum. Mandible with 11 mobile laciniae between pars incisiva, sharp, and pars molaris, prominent. Maxilliped III with its basis twice broader distally; otherwise, the common type. Peraeopods are clearly seen in figure 2 A. The only one bearing spines is peraeopod II with 2 strong spines at the end of the basipodite and some smaller ones on the proximal face of the meropodite. Exopodite 4—5 articulated. Peraeopod I very bristle as it was maintained only in one *M. reyssi* of the 8 specimens examined; the basis presents 7—8 huge plumose setae around its distal end.

♂♂. The juvenile males examined have but first signs of exopodites at peraeopods III and IV and at A_2; the differentiation under way does not differ from ♀ ♀ with regard to appendage, except for A_1; this consists in thick joints (Fig. 2 E, somewhat of the *Leptostilis* type).
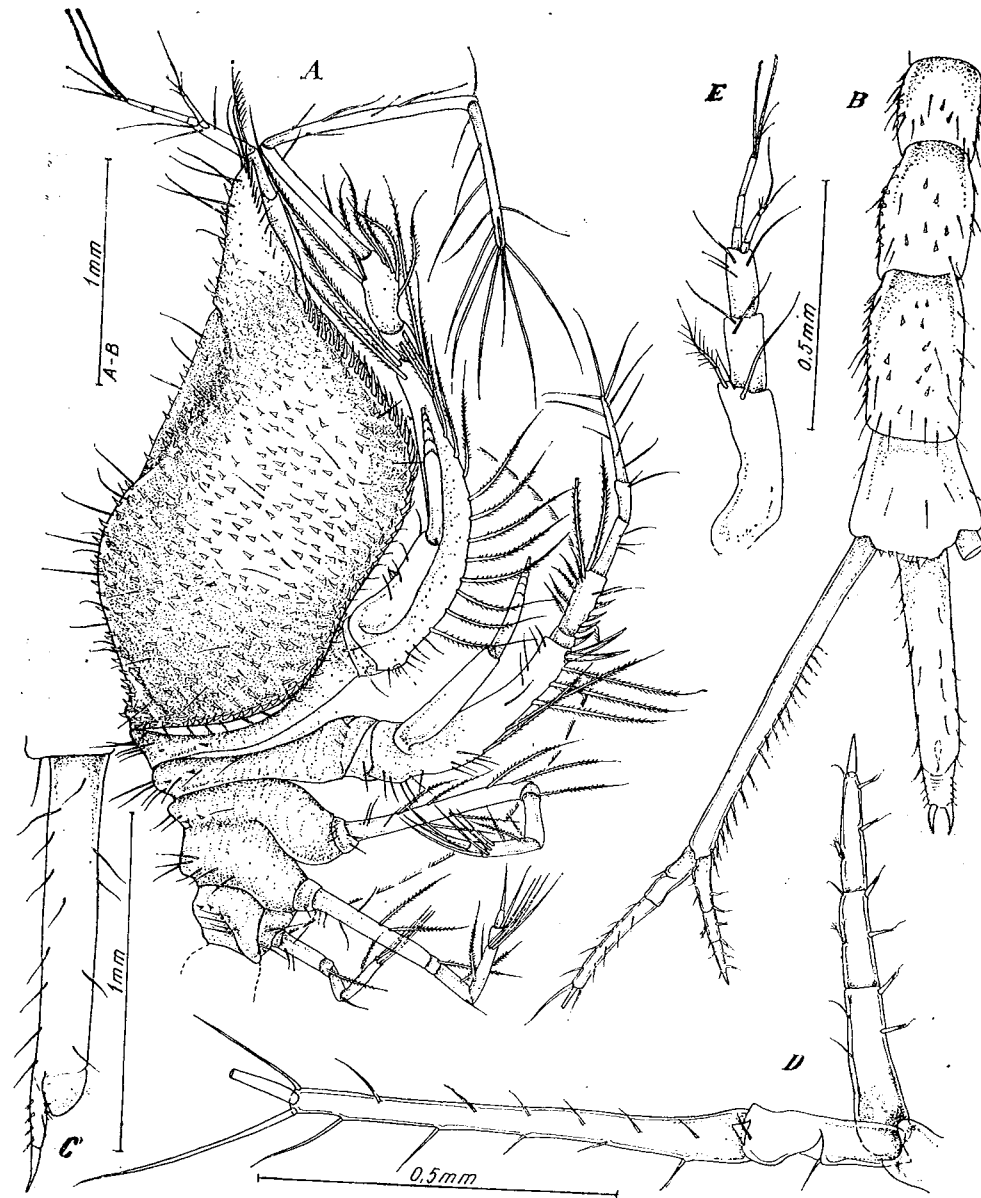
Size 9 mm.

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Fig. 2. — *Makrokylindrus reyssi* n. sp. ♀ ovigerous & ♂ P; A, ♀, lateral view; B, terminal portion; C, telson, lateral view; D, uropod rami; E, A₁ ♂ P (orig.).

Material. 1 ♀ with embryo, 5 ♀ P and 2 ♂ ♂ P. St. X046, 20° 49′9″N ; 17°58′8″W ; 1120 m., sandy mud. St. X055, 21°45′6″N ; 17°39′18″ W ; 1 ♀ with embryos and 1 juv., 1045 m., mud.

Holotype ♀ (ST. X046), No. 197, coll. "Gr. Antipa" Museum.

Remarks. M. *reyssi* is one of the few *Makrokylindrus* having not only the tegumen but also the appendage unarmed. Indeed, as against the thorny thoracopods of most species known [4], [8], [9], the characteristic of our species is precisely the absence of spines excepting the strong spines of the peraeopod II basis. All specimens have on their carapace fine sand heavily adherent, by setae secretion, of course, supplementing the carapace softness and showing a living way of the digging type in the soft substrate.

M. *reyssi* differs from all species having the telson shorter than the basis of uropods (a number of 8—9), by the particular shortness and armature of the postanal portion of the telson, hardly exceeding the anal valves. As a matter of fact, its separation from the other 6 species cited in the perimeter of the African coast of the Atlantic, encompassed between the Azores and Gibraltar parallels, North, and that of Cape-Town, South, may be done according to the following key :

1 (10) Telson longer than uropod basis **2**

2 (9) Telson exceeding the distal part of the uropod, at least by the length of its postanal portion **3**

3 (4) Postanal portion of telson long (approx. 1/3 of the total length), suddenly tapering, and glabrous. . . . .
. . . . . . **1 M. lomakinae** Băc. 1962, W. Capetown, 4885 m.

4 (3) Postanal shorter (1/4—1/5 of the total length), gradually tapered, with lateral spines or hairs; carapace glabrous **5**

5 (8) Thorns on the inferior portion of the first pleonite. **6**

6 (7) Postanal portion of the telson with lateral setae (uropods broken). Basal joint of A₁ ♀ serrated, with a spinule row; pleonites serrated with spinules . . . .
. . . . . **2 M. erinaceus** (G. O. Sars) 1887, Azores, 3700 m.

7 (6) Postanal portion of the telson without setae. Uropod rami ± equal; basal joint of A₁ ∓ smooth, but with a large apophysis spine-shaped. Pleonites ± smooth . . . .
. . . . **3 M. spiniventris** Hansen 1920, SW Faroë, 850 m. .

8 (5) Without the notified thorns, telson glabrous. Uropod endopodites hardly exceeding exopodites . . . .
. . . . . **3 M. inermis** Fage 1929, Azores, 1550 m. . . . . .

9 (2) Telson exceeding uropod basis only by the two apical spines **10**

10 (11) Granulated tegumen; telson glabrous. . . . .
. . . . **4 M. wolfi** Băc. 1962, W Capetown, 4000 m. .

11 (10) Tegumen spiny; telson with 4—6 pairs of spinules fixed on the dorsal face. . . **5 M. alleni** Reyss 1972, the Canaries, 2988 m.

12 (1) Telson shorter than uropod basis; postanal portion short (only 1/6 of the total length, spines inclusively). Uropod exopodites much longer than endopodites. . . . .
. . . . **6 M. reyssi** n. sp. W Mauritania, 1100 m. . .

For the West tropical Africa waters we mention also the presence of *Makrokylindrus spiniventris* Hansen, 1920 (St. X043, 480 m.: 30°09'18''N, 17°43'1''W, 8 specimens, and X069, 230—290 m.: 20°22'5''N, 17°40'3''W).

M. spiniventris and *M. reyssi* are the first representatives of the genus in Mauritania waters and their finding fill up an important zoogeographic gap.

CONCLUSIONS

As the abundant material of Cumacea dredged by us on the Thalassa, especially along the Mauritania coast [2], has been studied, not only Cumacea yet unknown on the tropical West African coast appeared, but also species new for science, as those just reported [3], or the two species we are dealing with now. These species, together with *Makrokylindrus spiniventris* also mentioned in the present paper, rise the number of species known from the coast of this country to 12 [3], [6], [8], [9], [10]. *Cumella africana* is kindred with the NE Atlantico-Mediterranean species *C. limicola*, from which it may be easily distinguished (see data in table 1). *Makrokylindrus reyssi* belongs to the most genuine representatives of the genus, i.e. those with a perfectly cylindric telson and the postanal portion much shorter than the rest. It seems to be of North Atlantic origin and is situated in the group of the 8—9 species with the telson obviously shorter than the uropod basis. Together with *Makrokylindrus spiniventris*, these are the first representatives of the genus. *M. reyssi* differs from the other species mentioned by the above dichotomic key.

For the Mauritanian waters we also mention as new:

Upselapsis caparti (Fage) St. X066 (3 specimens)
Heterocuma africana Zimmer
Eocuma aff. *travancoricum* Kurian St. X048 (10 spec.); St. X062 (5 spec.); St. X029 (2 spec.)
Heterocuma dimorpha (Fage) St. X060 (55 spec.)
Eocuma cadenati Fage
Eocuma ferox (P. Fischer).

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*The "Gr. Antipa" Natural History
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О ВЗАИМООТНОШЕНИЯХ МЕЖДУ РАЗНЫМИ ГЕНЕТИЧЕСКИМИ ГРУППАМИ ОТРЯДОВ AMPHIPODA И MYSIDACEA

И. И. ДЕДЮ

Взаимоотношения между различными генетическими группами высших ракообразных остаются до сих пор совершенно неизученными. Между тем раскрытие закономерностей их проявления во всем своем многообразии содействовало бы решению ряда проблем, связанных с историей происхождения той или иной генетической группы, с эволюцией *Crustacea* в целом.

В настоящей работе мы попытаемся показать, какие взаимоотношения в условиях бассейнов рек Днестра и Прута, а также в других водоемах существуют между группами амфипод и мизид разного происхождения.

В результате исследований, проведенных на протяжении ряда лет (1959—1969 гг.) нами установлено, что фауна амфипод и мизид бассейнов рек Днестра и Прута в генетическом отношении делится на следующие группы:

I. Древнепресноводные аборигены — виды и подвиды родов *Niphargus* и *Synurella* и подрода *Rivulogammarus* рода *Gammarus*.

II. Понто-Каспийские иммигранты — виды и подвиды родов *Dikerogammarus, Niphargoides, Iphigenella, Gmelina, Gmelinopsis, Corophium* и *Gammarus*.

III. Средиземноморско-атлантические колонисты—*Gammarus locusta* и *Corophium volutator*.

Все эти генетические группы не в одинаковой степени представлены в бассейнах Днестра и Прута. Так, в бассейне Днестра обнаружены не только все эти группы, но и все представители, входящие в их состав. Что же касается бассейна Прута, то здесь встречается небольшое количество представителей только первых двух групп. Третья группа пол-

ностью отсутствует. Вместе с тем в пределах бассейна Днестра не во всех типах водоемов они представлены в одинаковом объеме (см. таблицу).

Распространение генетических групп амфипод в водоемах бассейна Днестра

Генети-ческие группы	Днест-ровский лиман	Русло Днестра	Притоки Днестра	Кучур-ганский лиман	Поймен-ные водоемы	Под-земные воды	Пруды и малые водохрани-лища
I	—	—	—				
II	31*	27	3		1		
III	2	—	—	14	12	4	1

* Количество видов, входящих в соответствующую группу.

Из таблицы видно, что ни в одном из типов водоемов бассейна Днестра не представлены все генетические группы. То же относится и к количеству таксонов, входящих в ту или иную группу. Наиболее богаты видами Днестровский лиман (33) и русло Днестра (27). На втором месте стоят Кучурганский лиман (14) и пойменные водоемы (13). Беднее в этом отношении пруды и малые водохранилища, в которых обитает только один вид.

Амфиподы разного происхождения в своем распространении взаимоисключают друг друга. В этом можно убедиться на ряде конкретных примеров. Так, в Днестровском лимане средиземноморские колонисты *G. locusta* и *С. volutator* обычно не смешиваются с понто-каспийскими иммигрантами. В тех случаях, когда в составе группировок, образованных этими двумя атлантическими бокоплавами встречаются понто-каспийские виды, последние представлены единичными экземплярами. Например, ценоз *Corophium volutator + Nereidae*, как указывает Ю. М. Марковский [7], на 97,5% состоит из морских эвригалинных форм; на долю же понто-каспийских бокоплавов (*Gmelinopsis tuberculata, Niphargogammarus deminutus, Corophium nobile* и *Iphigenella andrussowi*) приходятся сотые доли процента. Случайное смешивание бокоплавов атлантического происхождения с бокоплавами — понто-каспийцами происходит на стыке ареалов солоноватоводных и пресноводных ценозов или во время сильного опреснения («в пресноводные годы», по Ю. М. Марковскому, [7]) солоноватоводной зоны.

В низовье, среднем участке и отчасти в верховье русла Днестра из амфипод обитают только понто-каспийские иммигранты, в то время как в его притоках — только представители подрода *Rivulogammarus*. Верхняя граница распространения понто-каспийских амфипод проходит в русле Днестра на 150 км ниже нижней границы распространения видов подрода *Rivulogammarus*. В русле Прута нижняя граница распространения видов подрода *Rivulogammarus* проходит в районе минимального развития понто-каспийских амфипод (г. Кагул).

В Кучурганском лимане как древнепресноводные, так и средиземноморские амфиподы полностью отсутствуют. Что же касается поименных водоемов Днестра, то здесь средиземноморские бокоплавы также отсутствуют. Вместе с тем, в некоторых пойменных озерах (Белое, Широкое,

Затланица), где обитают понто-каспийские амфиподы — иммигранты, был обнаружен древнепресноводный вид — *Niphargus* (Phaenogammarus) *valachicus*. Однако и в данном случае этот бокоплав, как правило, не встречается совместно в одном и том же ценозе с понто-каспийскими видами, т.е. здесь последние экологически изолированы от нифаргуса.

Чем же объяснить эту ярко выраженную несмешиваемость разных генетических групп амфипод? Когда и как она возникла? Какова роль этого явления в эволюции амфипод?

Дать исчерпывающие ответы на эти чрезвычайно интересные и сложные вопросы в настоящее время невозможно, так как знаем мы об образе жизни и биологии амфипод очень мало. Однако предположительно можно сказать следующее. Если отсутствие смешиваемости атлантических амфипод с амфиподами понто-каспийского происхождения в Днестровском лимане можно было бы объяснить разным отношением их к солености воды, то одной из главных причин отсутствия представителей подрода *Rivulogammarus* в русле Днестра является массовая иммиграция сюда эвригалинных понто-каспийских амфипод, вследствие чего виды подрода *Rivulogammarus* были вытеснены в притоки, где они в настоящее время развиваются в массовом количестве.

Тот факт, что в русле Прута амфиподы понто-каспийского происхождения занимают очень ограниченное пространство (только на протяжении 120 км низовья реки), объясняется, по-видимому, тем, что здесь, во-первых, они представлены только двумя видами, а во-вторых, представители подрода *Rivulogammarus* занимают господствующее положение почти во всей реке.

По-видимому, экологические особенности представителей той или иной генетической группы амфипод играют одну из главных ролей в явлении их взаимоисключения.

Наши попытки найти ответ на эти вопросы путем лабораторных опытов ничего не дали. Дело в том, что в эквариальных условиях виды подрода *Rivulogammarus* прекрасно себя чувствуют совместно с амфиподами — понто-каспийцами.

Не менее интересной оказалась экологическая изоляция между представителями родов *Niphargus* и *Gammarus*. При тщательном обследовании большого количества источников из разных географических точек бассейнов Днестра и Прута нам ни разу не удалось обнаружить совместного обитания видов и подвидов подрода *Rivulogammarus* с представителями рода *Nipargus*. В условиях исследуемых нами бассейнов те источники, в которых были найдены подвиды группы *stygius* из подрода *Niphargus* sensu stricto, в той или иной степени были связаны с артезианскими водами. Источники, в которых обитают представители подрода *Rivulogammarus*, были связаны только с грунтовыми водами.

Явление экологической изоляции между видами родов *Gammarus* и *Niphargus* замечено и другими авторами. Так, С. Muehlberger [10] при исследовании распространения пресноводных амфипод Германии ни разу не встретил совместного обитания представителей этих двух родов. На этом основании немецкий автор предлагает использовать представителей этих двух родов в качестве биоиндикаторов происхождения подземных вод. Я. А. Бирштейн [2] обнаружил то же при изучении распространения родов *Gammarus* и *Niphargus* в Крыму и Закавказье. Motaş,

Botoşăneanu, Negrea [9], исследовав большое количество источников и подземных водоемов Румынской равнины, обнаружили ясно выраженную конкуренцию между представителями рода *Gammarus*, с одностороны, и родов *Niphargus*, *Synurella* и *Orhestia* — с другой.

Ф. Д. Мордухай-Болтовской [8] установил отсутствие *Pontogammarus robustoides* в верхнем течении р. Берды, где обитает *Rivulogammarus pulex*. Н. С. Ялынская [16] отмечает отсутствие в верховье Днестра видов рода *Dikerogammarus* в районе распространения представителей подрода *Rivulogammarus*. М. М. Кожов [6] указывает на отсутствие *G. lacustris* в открытой части Байкала, т.е. там, где обычно обитают эндемичные байкальские бокоплавы.

Некоторые авторы обнаружили взаимоисключаемость пресноводных и солоноватоводных групп бокоплавов. Так, Н. Hines [12], изучив экологические особенности британских бокоплавов, установил ярко выраженный антагонизм между *Gammarus duebeni* и *G. fascitus*, с одной стороны, и *G. pulex* — с другой. Кроме того, указанный автор отмечает экологическую изоляцию между популяциями *G. pulex* и *G. lacustris*. O. Kinne [5] обнаружил вытеснение *G. duebeni* со стороны *G. salinus*.

Мы также обнаружили экологическую изоляцию между представителями одного и того же подрода. Речь идет о *R. kischineffensis* и *R. balcanicus*. Было установлено, что верхняя граница распространения *R. kischineffensis* проходит на 25 км ниже нижней границы распространения *R. balcanicus balcanicus*. В горном участке Днестра, где в массовом количестве обитает *R. balcanicus balcanicus*, *R. kischineffensis* вообще отсутствует. То же обнаружено нами и в р. Молокиш — левобережном притоке Днестра.

Основная причина несмешиваемости этих двух видов заключается, очевидно, в различном отношении их речных популяций к течению воды. Дело в том, что наибольшая плотность поселения *R. kischineffensis* обнаружена нами в среднем участке русла Прута, где скорость течения воды значительно меньше, чем в верховье реки. Наибольшая численность (1000—13000 экз/м^2) *R. balcanicus balcanicus* наблюдается в горном участке Прута. Минимум количественного развития (3—5 экз/м^2) как первого, так и второго вида обнаружен на стыке границ их распространения в русле этой реки.

Фауна мизид бассейнов Днестра и Прута в генетическом отношении также неоднородна. Она делится на следующие генетические группы: I. Понто-каспийские иммигранты — *Hemimysis anomala*, *Paramysis lacustris*, *P. intermedia*, *P. ullskyi*, *P. baeri bispinosa*, *P. kessleri sarsi*, *Katamysis warpachowskyi*, *L. benedeni*. II. Эндемики Азово-Черноморского бассейна, которые в свою очередь делятся на две группы: А. Эндемики Черноморского бассейна — *Hemimysis lamornae pontica*, *P. agigensis*. Б. Эндемики, общие для Азовского и Черноморского бассейна, — *Diamysis bachirensis mecznikowi*, *D. pengoi*,* *P. pontica*, *P. kröyeri*. III. Средиземноморско-атлантические колонисты — *Gastrosaccus sanctus* и *Mesopodopsis slabberi*.

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* Систематическая принадлежность *D. pengoi*, обнаруженой *Genovese* в оз. Скутари (бассейн Адриатического моря), и *D. bachirensis*, отмеченной Ch. Holmquist [13] в пресных водоемах Югославии, не ясна.

Эти три генетические группы обладают строго очерченной областью распространения. Так, средиземноморско-атлантические колонисты за пределы солоноватоводной зоны Днестровского лимана не заходят. Следовательно, их распространение зависит в первую очередь от степени солености воды: Азово-черноморские эндемики, за исключением *D. pengoi*, которая многими авторами (Марковский [7]; Băcescu [1]; Паули [11] вообще считается пресноводной формой), обитают как в солоноватоводной, так и в пресноводной, зоне лимана. Понто-каспийские же иммигранты встречаются в пресноводной зоне Днестровоского лимана, русле реки, Кучурганском лимане, пойменных водоемах Днестра и в низовье Прута.

Изучение взаимоотношений между этими генетическими группами мизид показало полную несмешиваемость средиземноморских колонистов с азово-черноморскими эндемиками, с одной стороны, и понто-каспийскими иммигрантами — с другой. При этом основным фактором, обусловливающим экологическую изоляцию между ними, является соленость воды. Мы не можем согласиться с Ю. М. Марковским [7], который утверждает, что степень аккумуляции органических веществ на дне и зарастание водоема являются одними и из ведущих факторов, лимитирующих распространение мизид. Известно ведь, что благодаря особенностям гидрологического режима (главным образом, течению воды) степень аккумуляции органических веществ и зарастания в пойменных водоемах Днестра и Кучурганском лимане в несколько раз превышает степень аккумуляции органических веществ и зарастание низовья Днестра. Тем не менее качественное разнообразие мизид в низовье Днестра во много раз уступает в этом отношении Кучурганскому лиману и пойменным водоемам.

Из вышеизложенного видно, что явление несмешиваемости разных генетических групп обнаруживается как среди амфипод, так и среди мизид.

Ch. Holmquist [13] на Северной Аляске также обнаружила несмешиваемость близкородственных видов мизид *Mysis relicta* и *M. litoralis*, что автор объясняет межвидовой конкуренцией.

На основе наших наблюдений, а также вышеприведенных литературных уазаний об антагонистических взаимоотношениях и экологической изоляции между разными видами и родами амфипод можно сделать вывод о том, что явление несмешиваемости различных генетических групп амфипод и мизид является для них общим биологическим правилом.

Несмешиваемость, или взаимоисключаемость, разных генетических групп амфипод и мизид можно объяснить двумя главными причинами: 1) антагонистическими биологическими взаимоотношениями (межвидовой конкуренцией), как это имеет место у амфипод между понто-каспийскими иммигрантами и древнепресноводными аборигенами, *Gammarus lacustris* и байкальскими эндемиками, представителями рода *Gammarus*, с одной стороны, и родов *Niphargus*, *Synurella* и *Orchestia* — с другой, понто-каспийскими иммигрантами и *Niphargus valachicus*, *G. pulex* и *G. duebeni* и др.; 2) экологической изоляцией, обусловленной разным отношением тех или иных видов животных к факторам абиотической среды (соленость, скорость течения, насыщенность воды кислородом, глубина водоема и др.), как это имеет место у амфипод и мизид между средиземноморскими колонистами и понто-каспийскими иммигрантами, представителями подрода *Rivulogammarus* и рода *Niphargus* и др.

И. И. ДЕДЮ

Явление взаимоисключаемости разных генетических групп по-видимому вообще широко распространено среди высших ракообразных. Например, давно известны антагонистические взаимоотношения между *Astacus astacus* и *A. leptodactylus* (Шимкевич [15]; Бирштейн и Виноградов [3]; Цукерзис [14].

Конечно, это чрезвычайно интересное явление требует еще очень углубленного и всестороннего исследования. Для его более или менее полного объяснения надо будет провести ряд полевых и лабораторных эколого-физиологических экспериментальных наблюдений.

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ADDITIONAL SPECIES OF FISHES IN THE FAUNA OF PERU TRENCH. RESULTS OF THE 11TH CRUISE OF R/V "ANTON BRUUN", 1965

BY

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In the present paper, five species of bathypelagic and abyssal fishes (*Torictus edentulus, Holtbyrnia bancholi, Lepophidium emelas, Bassogigas coheni* and *Caulophryne jordani*) are described, two of which are new ones.

The fishes forming the basis of the present paper were collected during the 11th cruise of R/V "Anton Bruun" (1965) and this represent our second contribution to this subject.

We are thanking Prof. R. Menzies, the head scientist of the expedition, and Dr. Mihai C. Bǎcescu, for their interest in collecting and preserving this material and for their generous help during the preparation of the manuscript.

Fam. ALEPOCEPHALIDAE

Torictus edentulus Alcock, 1892

Fig. 1 a and b

Material: one specimen 146.0 mm. in standard length, "Anton Bruun" 11th cruise, Peru Trench, October 1965. I.K.M.W.T., 500 m.

Gill cover flat, extended beyond the bases of pectorals, covering a large part of the fins. The insertion of first dorsal ray behind the origin of the anal fin. Maxillary with an expanded margin. The following

morphometric features are expressed in percents of the standard length: head 35.70; longitudinal diameter of eye 6.15; preorbital space 7.54; postorbital space 24.03; maxillary 11.63; greatest depth of body 21.30; predorsal space 64.25; preanal space 57.60.
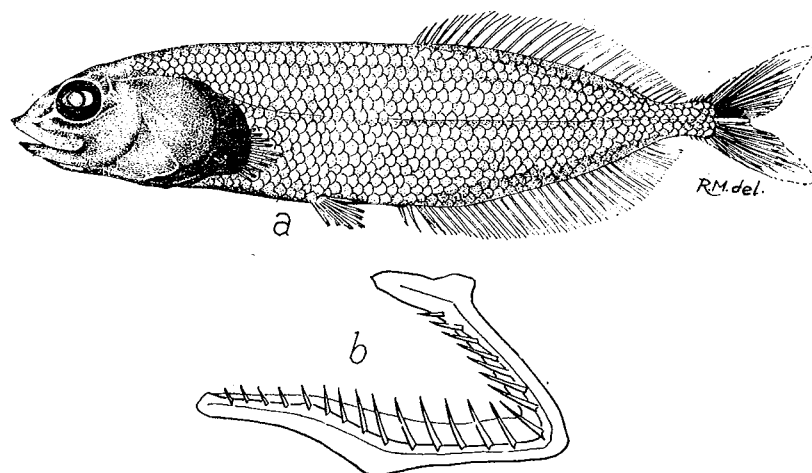

Fig. 1. — *Toriclus edentulus* Alcock: a. Lateral view of the specimen; b. First left branchial arch.

Scales moderate in size, 59—60 in lateral line.

Dentition: maxillaries, vomer and palatines, toothless. Mandibula with rare, small, pointed teeth.

Fin rays formula: D 27, A 34, V 7—7, P 10—10, C n 9+8 n.

Colour: body brown. Head deep brown, beautiful irisations. Mouth cavity and branchial chamber dark brown to iridescent black. All fins gray-brown.

Remarks: this species represents a new record for the fish fauna of the Peru-Chile Trench.

Fam. S E A R S I D A E

Holtbyrnia bauchoti sp. nov.

Fig. 2 a—c

Holotype: Type fish "Gr. Antipa" Mus. collection Cat. No. 170, one specimen 35.4 mm. in standard length. "Anton Bruun" 11th cruise, Peru Trench, October 1965.

Diagnosis: a *Holtbyrnia* with advanced pectoral fins and a small preanal space. Operculum with a striated margin.

Description: Body elongated, compressed, scaleless. Head relatively large, compressed. Eyes elliptical, well developed. Mouth cleft large,

upper jaw not exceeding the vertical from the middle of the eye. Premaxilla with two pairs of fang-like teeth. Maxillaries and dentaries with minute, pointed teeth disposed in one row. Vomerine teeth fang-like. Each palatine with one pair of pointed teeth, similar to vomerine teeth. Four to five lateral teeth on each side of the mandibula are present.
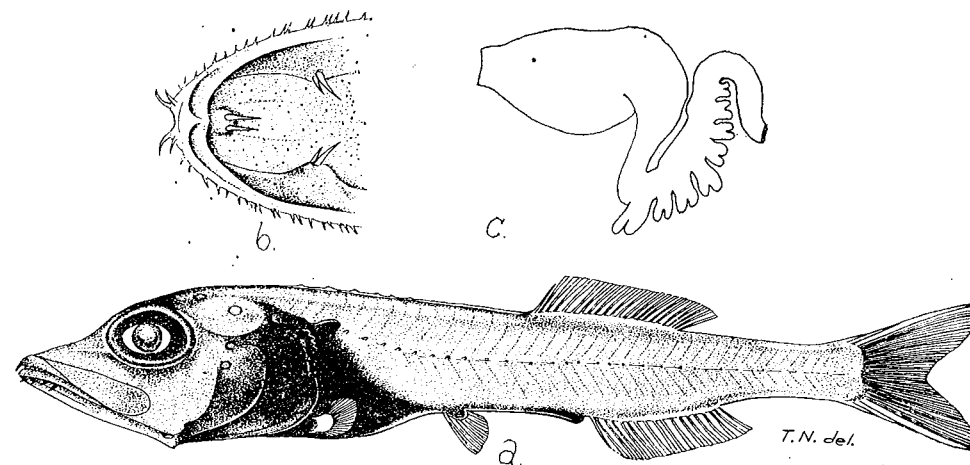

Fig. 2. — *Holtbyrnia bauchoti* sp. nov.: a. Lateral view of the holotype; b. Dentition on premaxilla, maxillaries, vomer and palatines; c. Stomach and the arrangement of the pyloric coeca.

Photophores. The terminology of luminous organs was used according to Parr [6, p. 10]. In this specimen only few photophores or groups of photophores may be discerned: Go, near the tip of mandibula, one median BRO, a transversal THO chevron-shaped, IVO chevron-shaped and two SAD. Other luminous organs are lacking.

The measurements, expressed in percentages, of *Holtbyrnia bauchoti* are given in table 1 together with the values of two specimens of *Holtbyrnia* (s. str.) *melanocephala* Vaillant captured in the same station and the values given by Parr [6, p. 78] for the holotype of *Holtbyrnia (Krefftia) macrops* Maul and *Holtbyrnia* (s. str.) *melanocephala* [7, p. 65] and by Bussing [1, p. 192] for both *macrops* and *melanocephala*. Remarkable differences between *bauchoti* and *macrops* may be observed in the greatest depth of body, preanal and predorsal spaces (compare the values).

The number of fin rays is difficult to count. The dorsal fin contains about 21 rays and the anal fin about 18.

The insertion of the ventrals is equidistant between pectorals and anal root. Anal first ray placed under the 9th dorsal ray.

Caudal well forked. Lateral line complete and distinct, underlined in its first half by small tubes. Shoulder organ well developed.

Stomach syphonal. First part of intestine with eight deeply branched coeca.

Colour: generally intense brown with beautiful iridescence, head and abdomen deep violet-brown and peritoneum jet black.

Table 1

Comparison between body proportions in some *Hollbyrnia* species

	bauchoti	macrops		melanocephala		
	holotype	Parr, 1960 holotype	Bussing, 1965	Parr, 1960	Bussing, 1965	Our specimens
h.l.	36.70	35.50	36.00	36.00	36.40—38.20	40.05—40.30
e.d.	11.32	12.00	11.80	—	10.80—12.70	12.75—13.50
pr.s.	8.46	7.90	10.90	8.80	9.10—10.40	9.60—10.62
pt.s.	16.41	—	—	—	—	16.70—19.10
h.	14.70	19.20	19.20	—	18.60—20.40	17.30—19.17
pd.s.	62.10	65.20	62.30	65.50	61.30—63.40	62.50—63.90
pa.s.	67.80	73.50	72.00	74.70	69.40—71.80	67.50—76.50
pr.s.	47.90	58.90	57.80	61.30	56.20—60.30	55.80—58.30

h.l. = head length
e.d. = eye diameter
pr.s. = preorbital space
pt.s. = postorbital space
h. = greatest depth
pd.s. = predorsal space
pa.s. = preanal space
pr.s. = preventral space

Remarks : this new species could be referred to the subgenus *Krefftia* due to its moderate head and relatively short snout.

Our new species differs essentially from *H. macrops* Maul, *H. schnakenbecki* Krefft and *H. problematica* Parr by the absence of the following groups of photophores : JO, IPO, SVO, PAO and ICO, and particularly by its advanced ventral fins.
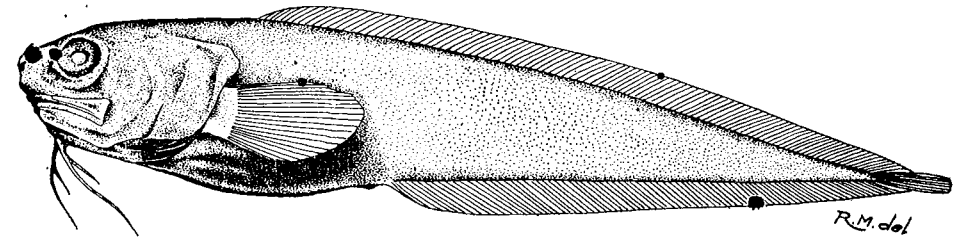
This new species is dedicated to Dr. Marie-Louise Bauchot in appreciation of her work on fishes and for her kind help given to us in different ways.

Fam. **OPHIDIIDAE**

Lepophidium emelas (Gilbert, 1890)

Fig. 3

Material : four specimens 121.0—140.0 mm. in standard length, "Anton Bruun" 11th cruise, Peru Trench, October 1965. No other data. New record for this area.

Fig. 3. — *Lepophidium emelas* Gilbert : Lateral view of the specimen.

Fam. **BROTULIDAE**
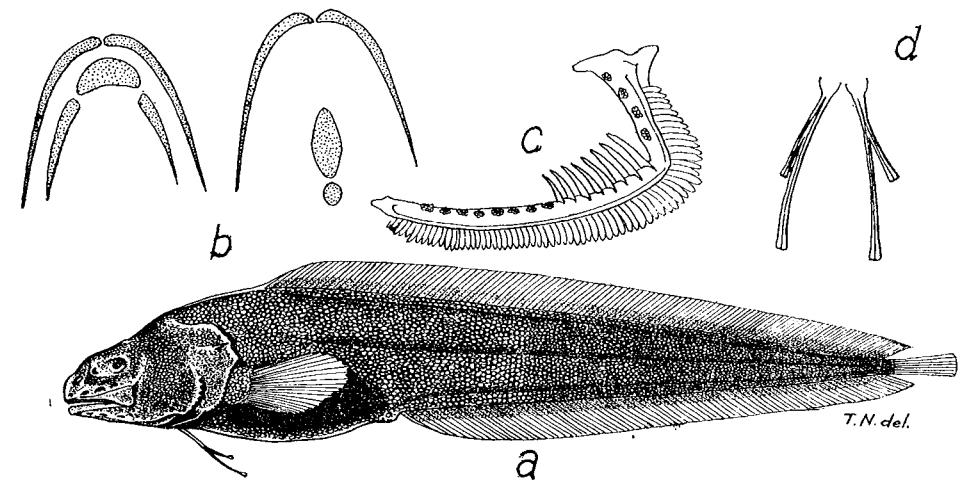
Bassogigas coheni sp. nov.

Fig. 4 a—d

Fig. 4. — *Bassogigas coheni* sp. nov.; a. Lateral view of the holotype; b. Dentition on maxillaries, vomer, palatines, dentaries and basibranchials; c. First left branchial arch; d. The ventral fin rays.

Holotype : Collection "Gr. Antipa" Mus. Cat. No. 191, one specimen 110.0 mm. standard length, "Anton Bruun" Exped. Sta. 161, Peru Trench 08°23′S — 80°25′W, depth 2945—2966 m., beam trawl, October 31, 1965. The single specimen known.

Diagnose : A *Bassogigas* with serrated preoperculum and operculum and with outer ventral ray smaller than the inner one.

The body is rather elongate and compressed, tapering gently to the caudal fin. Head robust. Eyes relatively small, covered by skin. Preoperculum and operculum slightly denticulated on their vertical edge, but not with small spines as in *Neobythites*.

Opercular spine robust. A conspicuous carina between interorbital space and the first ray of the dorsal fin. Gill rakers on the first left branchial arch : 4 small tubercles + 8 spines + 8 small tubercles (total 20).

Minute teeth on premaxillaries and palatines. Vomerine teeth arranged on a half-moon-shaped plate. Two patches of bassibranchial are present at the base of the tongue. Small papillae covers the entire mouth cavity and the tongue. Snout rounded, mouth terminal.

Posterior margin of maxillary ends slightly beyond the vertical of the posterior rim of the orbit.

Fin rays formula : D 129, A 99, V 2, P 27, C 4 + 5 (total 9).

Caudal fin possibly free, not included by dorsal and anal ones.

The following morphometric values are expressed in percentages of the standard length : head 21.30 ; eye 3.63 ; preorbital space (length of snout) 4.56 ; length of maxilla 9.10 ; postorbital space 12.70 ; interorbital space 5.46 ; greatest depth of body 16.40 ; least depth of body 1.45 ; predorsal space 24.60 ; preanal space 41.04.

Colour : generally gray-violet. Sides of head grayish-brown. A narrow postopercular blackish area. The abdomen is bluish-gray.

The mouth cavity, the gill chamber and the peritoneus are deep brown pigmented. All fins are pale.

Remarks : the present species described in a previous paper as *Bassogigas* sp. [3] appears to be distinct from all species of *Bassogigas* due to its great number of dorsal and pectoral rays, serrated preoperculars and operculars and especially to its outer ventral rays smaller than the inner ones.

However, among the other species of this genus, *B. digitatus* Garman may be considered as the closest relative to our specimen due to its general appearance and to its great number of dorsal rays. The variation of the dorsal rays, according to Garman's [2] meristic data, ranges from 105 to 121. For comparison between *Bassogigas coheni* and other species of this genus see Nybelin [5] and Nielsen [4].

This species is named *coheni* in appreciation of all the aid given to us by Dr. Daniel M. Cohen, Smithsonian Institution, Washington, D.C.

Fam. **CAULOPHRYNIDAE**

Caulophryne jordani Goode & Bean, 1896

Fig. 5 a and b

Material : one female specimen 169.0 mm. standard length, "Anton Bruun" 11th cruise, Peru Trench, October 1965.

D 1 + 15, A 15, V absent, P 18, C II 4 II. Ilicium with a bulb at its tip and numerous small and long filaments (Fig. 5 b).
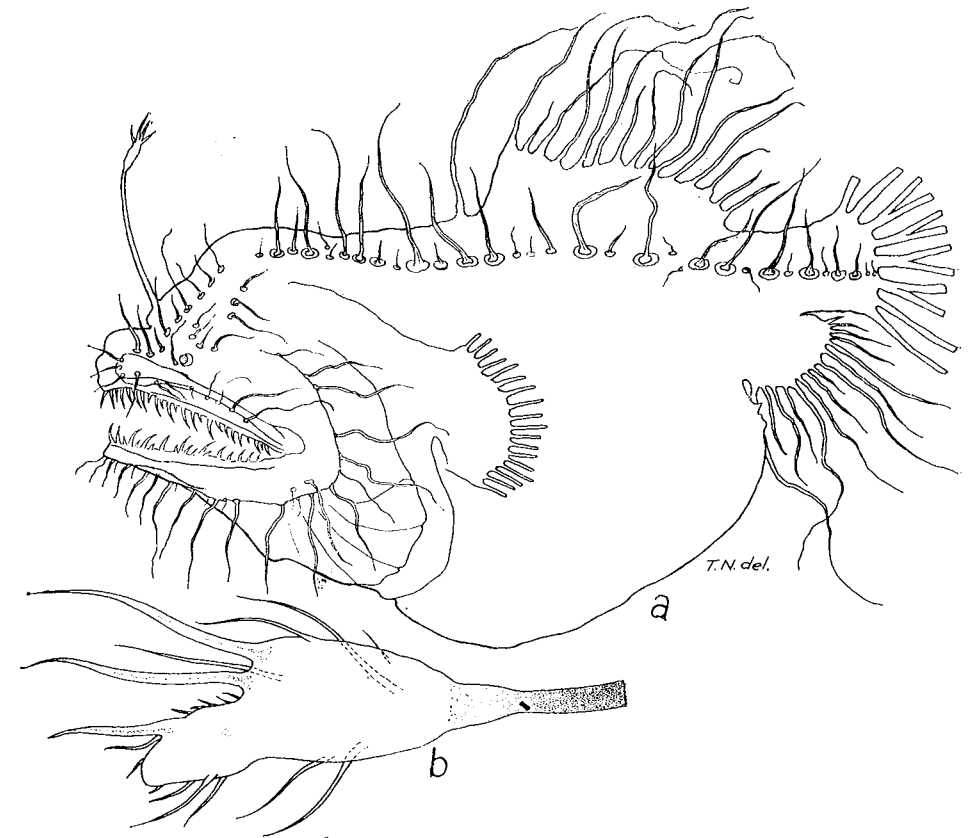
Fig. 5. — *Caulophryne jordani* Goode & Bean, 1896 ; a. Lateral view of the specimen ; b. The tip of the ilicium.

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ULTRASTRUCTURE DU SEGMENT TERMINAL DU CANAL DÉFÉRENT CHEZ *PORCELLIO SCABER* LATR.

PAR

V. GH. RADU et C. CRĂCIUN

The study of the anterior, kidneylike part of the deferent ducts in terrestrial isopods, supplied many interested data for citology, as a rule, both for the optic and the electronic microscopy. In this paper the authors investigate, also, the other parts of these ducts, firstly to make possible, the zonal comparative estimation of the morphologic ultrastructural specialization and differentiation of their glandular epithelium and secondly, to facilitate the correct interpretation of the functional aspects of the genital male ducts of the isopods.
They will further investigate the morphofunctional variation of the whole genital tract as compared with the periodicity of the sexual processes.

Chez *Porcellio scaber*, ainsi que chez les autres isopodes terrestres, en général, les deux canaux déférents se présentent comme des tubes fermés en cul-de-sac à l'extrémité antérieure et à calibre diminuant au fur et à mesure qu'on se rapproche de leurs extrémités postérieures. Par un étranglement assez accentué, ils sont incomplètement divisés en un compartiment antérieur, réniforme, de couleur blanche laiteuse (vésicule séminale selon certains auteurs), dans lequel débouchent les trois follicules testiculaires et le reste du canal, bien plus long et de couleur grise foncée (fig. 1, I). Assez dilatée au début, cette partie du canal déférent s'amincit petit à petit en un conduit assez étroit qui, au niveau du 7ème péréionite, se recourbe en angle droit et rejoint son symétrique sur la ligne médiane ventrale de l'animal. Ici, étroitement accolés, mais sans confluer, les deux canaux se dirigent vers l'arrière et s'engagent immédiatement dans l'intérieur du pénis qu'ils parcourent jusqu'à son extrémité où ils s'ouvrent séparément à l'extérieur (fig. 1, II).

À la base du pénis, les deux canaux ont une lumière très étroite ; sur le parcours du pénis, ils se dilatent sensiblement et constituent deux

petites vésicules fusiformes très allongées, pour qu'à la fin, avant l'ouverture, la lumière devienne de nouveau assez étroite. Cette disposition de la partie terminale des canaux déférents a été déjà décrite par l'un d'entre nous [7], fait resté inconnu pour Marcelle Fain-Morelle qui adopte [1] l'opinion de Gerstaecker (1900) et considère que, le long du pénis, les deux canaux confluent dans un canal unique.
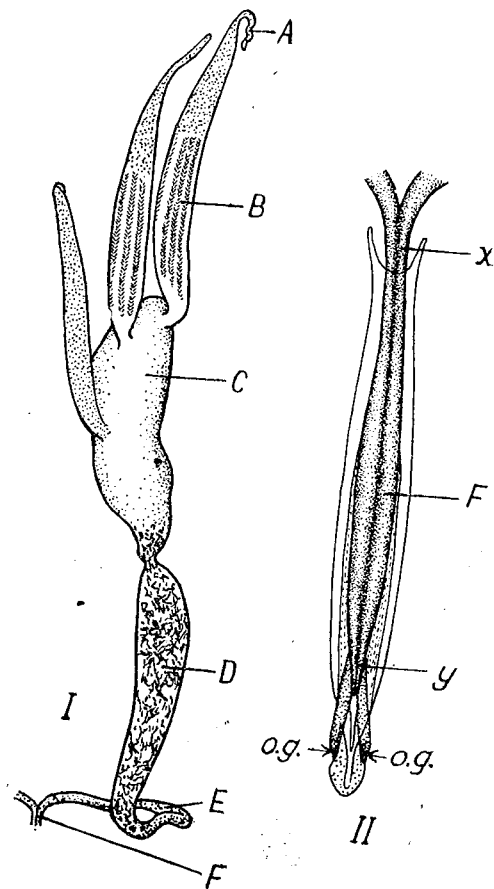

Fig. 1. — *Porcellio scaber*. I. L'appareil génital mâle du côté droit. A, glande androgène; B, follicule testiculaire; C, région antérieure, réniforme (vésicule séminale) du canal déférent; D, E, F, différentes parties de la région postérieure du canal déférent. II. Partie terminale, péniale, des deux canaux déférents, plus grossie que dans la fig. I. *x* et *y*, endroits d'emplacement des deux sphincters; *o.g.*, les deux orifices génitaux mâles.

Le segment réniforme, nommé aussi vésicule séminale par certains auteurs, a été l'objet de maintes recherches cytologiques à l'aide du microscope optique [1], [5], [6—8], [9] ou du microscope électronique [4]. Nous avons fait aussi à l'occasion de différentes réunions scientifiques quelques communications concernant l'ultrastructure des cellules glandulaires du segment réniforme, qui paraîtront prochainement.

Dans le présent travail, nous nous proposons de considérer, succinctement, quelques aspects sur l'ultrastructure de la portion mince, terminale, des canaux déférents chez *Porcellio scaber*. Pour des raisons d'orientation, nous avons sous-divisé ce canal en trois portions, *D, E, F* (fig. 1). Plus exactement, nous présenterons ici seulement les portions *E* et *F*.

RÉSULTATS

Segment E (portion transversale du segment postérieur). L'épithélium, unistratifié, repose sur une membrane basale doublée d'une couche musculaire à fibres striées, parsemée de mélanophores, qui donne au canal une couleur grise foncée (fig. 3).

Les cellules épithéliales, prismatiques, hautes de 40—50 μ, à noyau ovale, se terminent, vers la lumière du canal, par une bordure de microvilli très riche (fig. 2, 4, 5), à filaments très fins et relativement longs (3—4 μ). Les membranes intercellulaires présentent des desmosomes septés qui s'étendent sur plus d'un tiers de la hauteur des membranes.

Les structures morphologiques du cytoplasme de ces cellules présentent des aspects très particuliers. On est frappé en premier lieu par la grande richesse du réticulum endoplasmique, qui est du type granulaire, et la dominance absolue des citernes endoplasmiques dans toute la cellule, depuis la membrane basale jusqu'à la bordure apicale (fig. 2—5). De dimensions variables, ces citernes se caractérisent par leur contour irrégulier anguleux, comme des morceaux de pierre cassée. Elles sont remplies d'un contenu finement granuleux. La matrice cytoplasmique, assez réduite parmi les éléments du RE, est très riche en ribosomes (fig. 6).

Les *dictyosomes*, assez nombreux, sont constitués surtout par des microvésicules et par des macrovésicules claires (petites vacuoles) (fig. 2, 4, 7). Les saccules applatis sont rarement présents et en nombre très réduit. Au niveau des dictyosomes on observe souvent des grains fortement électrono-opaques, plus ou moins gros. Il n'est pas difficile de constater que ces grains de sécrétion dérivent des microvésicules dictyosomiques qui n'ont aucune relation visible avec les saccules applatis (fig. 7). De tels grains existent fréquemment aussi dans le cytoplasme, loin des dictyosomes.

Sous l'aspect de leur conformation, on voit que les dictyosomes de ces cellules sont très différents de ceux que nous avons décrits dans le segment antérieur, réniforme. Ils correspondent plus exactement à leur phase d'élaboration la plus poussée, où les saccules applatis sont presque ou totalement disparus.

Le chondriome est assez bien représenté, mais un peu difficilemei observable. À cause de leur crêtes faiblement esquissées, les mitochondri ont une grande ressemblance avec les citernes endoplasmiques de mêm dimensions, dont on les distingue mal à première vue (fig. 2, 4, 6, 8

Élimination du produit de sécrétion. Les grains de sécrétion électron opaques traversent la bordure des microvilli et passent dans la lumièr du canal où ils peuvent confluer et constituer des grains plus gros, quel quefois très gros (fig. 4, 5, 8). Les blocs de sécrétion plus ou moins gro constitués dans les citernes endoplasmiques, suivent la même voie, mai celle-ci n'est pas suffisante pour ce produit qui est en quantité énorme Dans un ou plusieurs points, le cytoplasme pousse la membrane apical et produit des protubérances de différentes dimensions, digitiformes o comme de petites poches, dans lesquelles il s'engage avec tous ses consti tuents et produits (fig. 4, 5). Ces protubérances claquent ou se dé tachent et déversent leur contenu dans la lumière du canal (fig. 4, 5, 8 Le matériel, ainsi éliminé, conflue en blocs irréguliers, quelquefois énor mes, mélangés avec les grains et les blocs électrono-opaques, mai sans confluer avec ceux-ci. Ensuite, tous ces blocs se désagrègent peti à petit et constituent, à la fin, dans le canal déférent, une masse d sécrétion homogène et finement granulaire, sauf les grains électrono opaques qui gardent leur contour (fig. 4, 5). Ceux-ci se désagrégeron aussi, mais un peu plus tard, surtout dans le dernier segment du cana déférent (fig. 12).

Au moment de leur ouverture dans le canal déférent, l'extrémit apicale des protubérances ou des poches d'élimination se dilate sous la form d'entonnoir et presse fortement contre les microvilli qui constituen ainsi des groupes pyramidaux, tels qu'on les voit dans les figures 4, 5 et 8

Mais, et ce fait paraît assez curieux, l'élimination de la sécrétion peut se faire aussi par les microvilli : la substance de sécrétion contenue dans les citernes endoplasmiques pénètre à l'intérieur des microvilli qu'elle gonfle inégalement, par portions, surtout à leur extrémité, qui se détache et tombe dans la lumière du canal déférent (fig. 4, 5, 8).

Segment F (partie intrapéniale du canal déférent). Les cellules épithé liales de la partie moyenne, dilatée, de ce segment sont prismatiques, de dimensions variant entre 30—35 μ de hauteur. Du point de vue cytologique, elles ont une structure tellement semblable à celle du segment transversal, que nous avons présentée plus haut, qu'il n'y a que peu de chose à ajouter (fig. 9). Nous nous limiterons à signaler la grande finesse des microvilli qui ont ici une épaisseur de 700 Å, par rapport à 900 Å dans le segment transversal, et une plus grande abon dance du chondriome, dont les éléments sont plus épais (0,4—0,6 μ) que dans le segment précédent (0,3—0,4 μ) et plus faciles à distinguer. Surtout dans la partie apicale des cellules, les mitochondries constituent à peu près la moitié du matériel qui la remplit, dispersées parmi les citernes endoplasmiques qui représentent l'autre moitié de ce matériel (fig. 9).

Le produit de sécrétion se présente pareil à celui du segment *E*. Les relations entre les grains électrono-opaques et les dictyosomes appa raissent ici d'une façon plus claire. L'élimination du produit de sécrétion suit les mêmes voies (fig. 8), mais les microvilli paraissent moins impliqués dans ce processus.

Dans la lumière de cette partie du canal, on voit des spermato hores en grand nombre, nageant dans un produit de sécrétion presque omogène. Comme particules figurées de la sécrétion, il n'en reste que le rares grains électrono-opaques, plus ou moins gros, mais toujours vec des aspects de corrosion.

Aux deux extrémités de la partie intrapéniale des deux canaux défé ents (fig. 1, II, x et y), la structure diffère radicalement de ce que nous vons montré jusqu'ici. Les cellules épithéliales sont plus basses (13—24 de hauteur) et surtout dépourvues de la bordure de microvilli (fig. 10— 11); le cytoplasme est assez remarquable par l'aspect et la disposition péciale du réticulum endoplasmique et du chondriome.

Dans la partie initiale du segment *F*, à la base du pénis (fig. 1, II, x), les cellules épithéliales, hautes d'environ 25 μ, ont un réticulum endo plasmique constitué par des tubes à parois lisses, très électrono-opaques, disposés en réseau à la base et sur les côtés des cellules, mais surtout en boucles vers la partie apicale de celles-ci (fig. 10). Leur contenu est absolument clair, sans traces de précipité. On pourrait dire que ce réti culum constitue des formations à fonction spéciale, qui ont leur origine à la base des cellules dans un feutrage très dense de réticulum endoplas mique rugueux. Dans la figure 10 on peut remarquer que les tubes de ce système sont accompagnés presque partout de brides de protoplasme très électrono-opaques, parfois de la même épaisseur que les tubes du réticulum, mais plus irrégulières. Par endroits, ces formations présentent des dilatations contenant des vésicules claires, vraisemblablement des sections transversales de tubes réticuloendoplasmiques. Dans le proto plasme fondamental de la cellule on voit aussi du réticulum endoplasmi que granulaire, mais faiblement représenté.

On observe aussi dans les cellules de ce segment des filaments (ou septes, ou membranes?) doubles qui parcourent d'un bout à l'autre la cellule (fig. 11). Ils sont rattachés à la couche intérieure de la membrane apicale et se résolvent dans le feutrage du réticulum endoplasmatique à la base de la cellule. Sur leur parcours, ils présentent des dilatations vésiculaires ou irrégulières, plus ou moins étendues, surtout vers la base de la cellule. Ils sont dépourvus de ribosomes.

Le chondriome est exceptionnellement riche, presque uniformément dispersé dans la cellule. L'épaisseur des mitochondries atteint fréquem ment 1,2 μ.

À l'extrémité terminale du segment *F* (fig. 1, II, y), le cyto plasme des cellules, hautes d'environ 13 μ, est littéralement bourré de mitochondries (fig. 12—13), parmi lesquelles l'espace est occupé par un réticulum endoplasmique pareil à celui que nous avons vu, plus haut, dans la portion x du canal (fig. 11). Le protoplasme fondamental, très réduit, est très électrono-opaque. À la base des cellules, le feutrage du réticulum endoplasmique constitue une couche très épaisse (plus de 2 μ).

Dans les deux points considérés, x et y (fig. 1, II) des canaux déférents, la couche musculaire, striée, est beaucoup mieux représentée que sur tout le reste des canaux déférents, constituant, d'après notre opinion, deux sphinctères dont la fonction serait, peut-être, de régler l'élimination du sperme pendant la copulation.

DISCUSSIONS ET CONCLUSIONS

Les images que nous avons obtenues à l'aide du microscope électronique confirment les observations de V. Radu [7], à savoir que chez les Oniscoidées, au moins chez les espèces étudiées par nous (*Armadillidium*, *Porcellio*), les deux canaux déférents restent séparés jusqu'à leur ouverture à l'extrémité du pénis. Ils ne se réunissent pas en un canal éjaculateur commun, ainsi que l'affirment J. D. Newstead et E. J. Dornfeld [4] et Marcelle A. Fain-Maurel [1], qui, assurément, ne connaissent pas notre travail de 1931. La portion dilatée, fusiforme, des deux canaux, inclus dans le pénis, constitue, d'après nous, de petits réservoires ou pompes d'éjaculation dont la fonction serait reglée par les deux sphinctères situés l'un à la base, l'autre à l'extrémité du pénis, donc aux deux extrémités des réservoires, eux-mêmes contractiles. La contraction de deux sphinctères serait antagoniste, constituant ainsi un système de pompe aspirante et foulante, assurant à la fois une certaine quantité de sperme et l'injection de celle-ci dans les voies génitales femelles.

Au niveau des réservoires, l'épithélium, constitué d'une seule sorte de cellules, manifeste une fonction glandulaire assez marquée. Le réticulum endoplasmique joue dans ces cellules le rôle essentiel, dans le sens qu'ici on voit s'accumuler la plus grande quantité de matériel qui sera éliminé dans la lumière du canal. Au niveau des dictyosomes on peut constater l'apparition de deux autres substances de sécrétion, différentes de celle du réticulum endoplasmique : une sécrétion claire, contenue dans le vacuoles issues des citernes golgiennes et des grains fortement électronoopaques, ayant comme origine les microvésicules dictyosomiques. L'hétérogénéité de la sécrétion est donc pareille à celle de la partie antérieure réniforme, du canal déférent (V. Radu et Crăciun, travaux pas encore publiés), bien que les aspects structuraux des cellules glandulaires dans les deux segments comparés soient très différents. Au contraire, dans les deux segments étudiés ici, le segment transversal (*E*) et le segment pénial (*F*, sauf la portion des deux sphinctères), ces aspects sont très semblables.

Au niveau des sphinctères, les cellules épithéliales ne manifestent pas de signes accentués de sécrétion. Les corps golgiens sont quasi absents. En échange, les mitochondries sont tellement nombreuses qu'elles impriment la particularité la plus saillante de ces cellules. On pourrait affirmer que leur présence massive est en relation directe avec la fonction musculaire des segments respectifs (on sait que dans les fibres musculaires les mitochondries sont très nombreuses, constituant 40% du volume total des fibres).

Il est très difficile de trouver une interprétation véridique pour l'abondance exceptionnelle du réticulum endoplasmique granulaire dans les cellules épithéliales au niveau des sphinctères. Nous espérons reprendre ce sujet dans d'autres travaux.
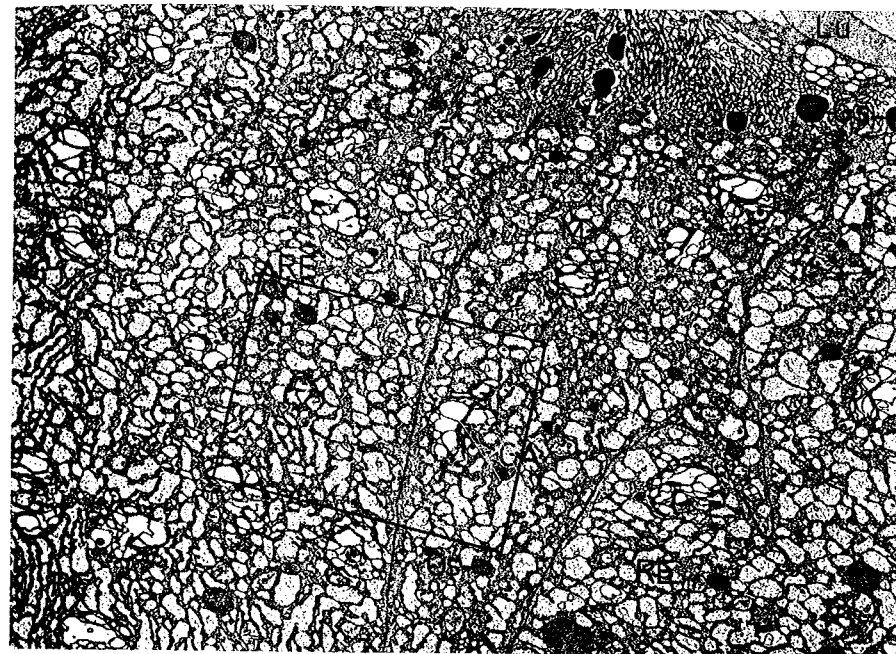

Fig. 2. — Portion *E* du canal. Aspect général, caractéristique, des cellules épithéliales, glandulaires ; il est à remarquer la grande richesse du RE, le grand nombre des dictyosomes et des mitochondries, leur répartition. × 7 800.

Explication des symboles. Nous donnons ici, une fois pour toutes les figures, l'explication des symboles employés. **C**, cordons de substance électrono-opaque qui accompagne le **REN** ; **GA**, appareil de Golgi ; **Gs**, grains de sécrétion ; **Lu**, lumière du canal déférent ; **M**, mitochondries ; **Mb**, membrane basale ; **Mi**, microvilli ; **Mm**, lames de membrane plasmatique qui traversent le cytoplasme ; **Ms**, couche musculaire striée ; **N**, noyau ; **Nu**, nucléole? **Re**, réticulum endoplasmique rugueux ; **REN**, réticulum endoplasmique lisse ; **Ri**, ribosomes ; **S**, produit de sécrétion ; **Sd**, substances de sécrétion en voie de désagrégation ; **Sp**, spermatozoïdes, en section transversale. **SPF**, spermatophores, sectionnés transversalement.
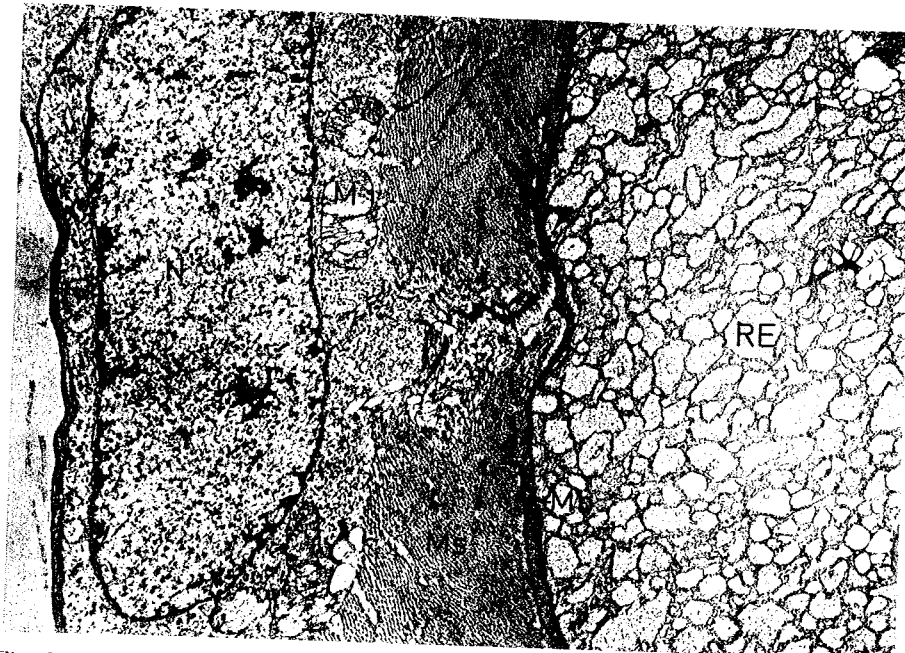La partie encadrée de cette figure constitue la figure 6, à un plus fort grossissement.

Fig. 3. — Portion *E* du canal. Partie basale de l'épithélium. Sous la membrane basale de l'épithélium, à remarquer la couche musculaire striée. × 9 200.
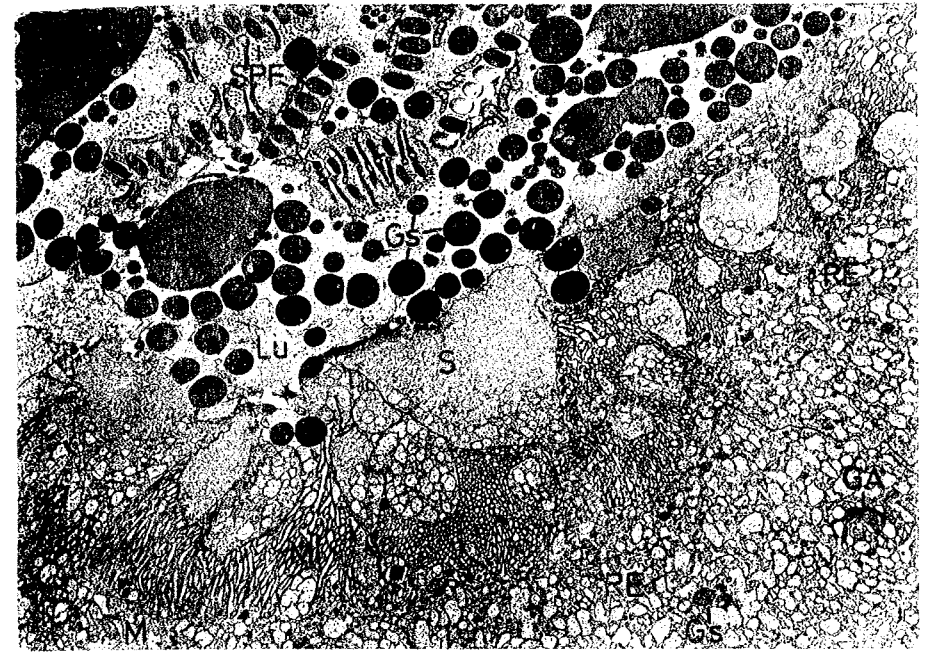
Fig. 5. — Mêmes aspects que dans la fig. 4. × 6 000.
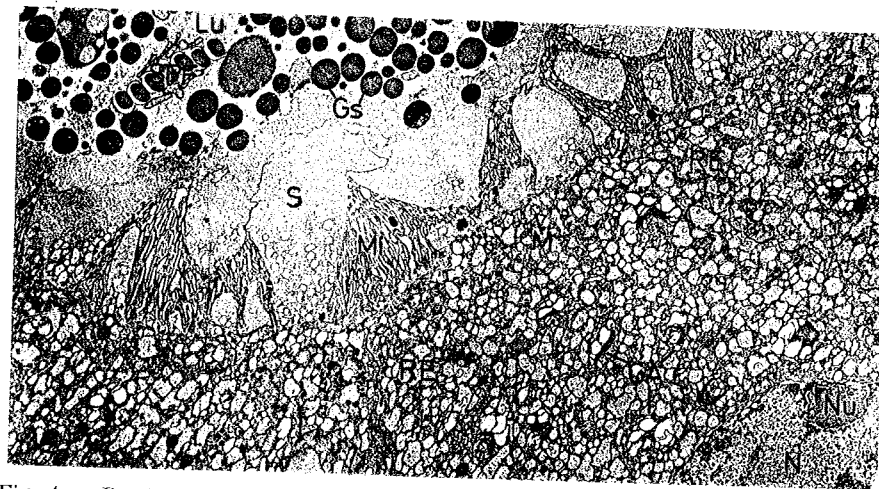
Fig. 4. — Portion *E* du canal. À remarquer la manière apo-mérocrine d'élimination du produit de sécrétion et l'hétérogénéité de ce produit, qui remplit la lumière du canal déférent, englobant les spermatophores. Section transversale. × 5 750.

Fig. 6. — Aspect plus grossi du fragment encadré de la fig. 2. On peut remarquer la constitution rugueuse du **RE**, l'abondance des ribosomes libres dans la matrice cytoplasmique, la présence de quelques citernes golgiennes dans la constitution du dictyosome. × 18 000.
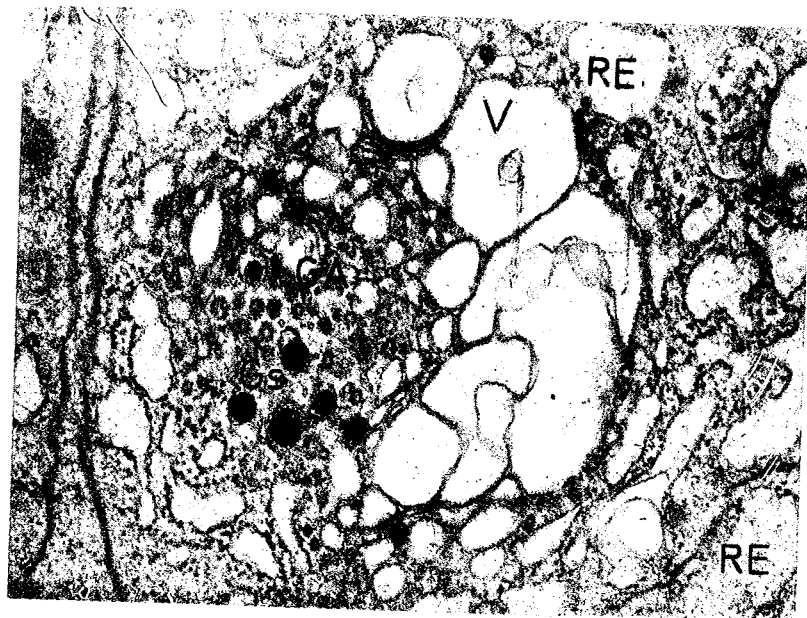
Fig. 7. — La constitution d'un dictyosome vu à un plus fort grossissement. Explications dans le texte. × 32 000.
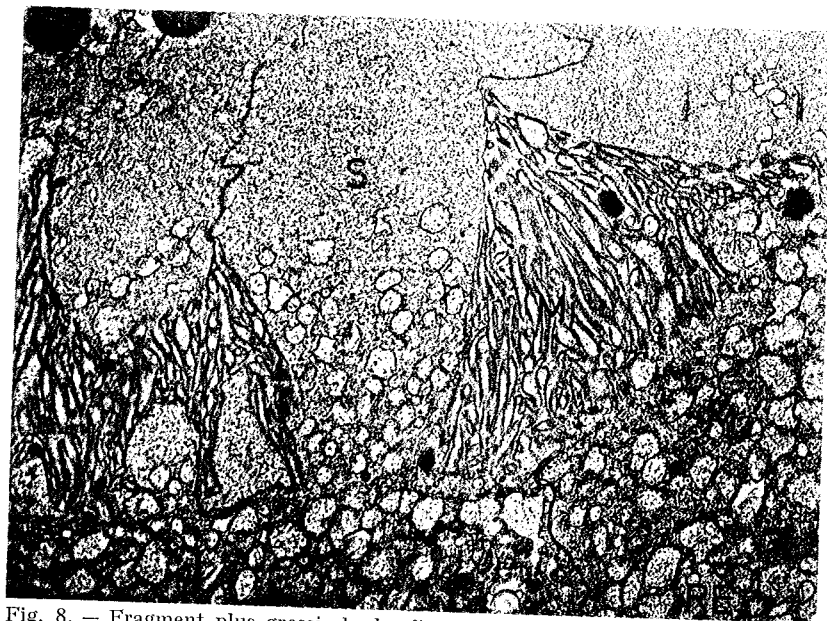
Fig. 8. — Fragment plus grossi de la fig. 4, pour pouvoir mieux observer l'élimination de la sécrétion par la voie des microvilli. × 10 000.

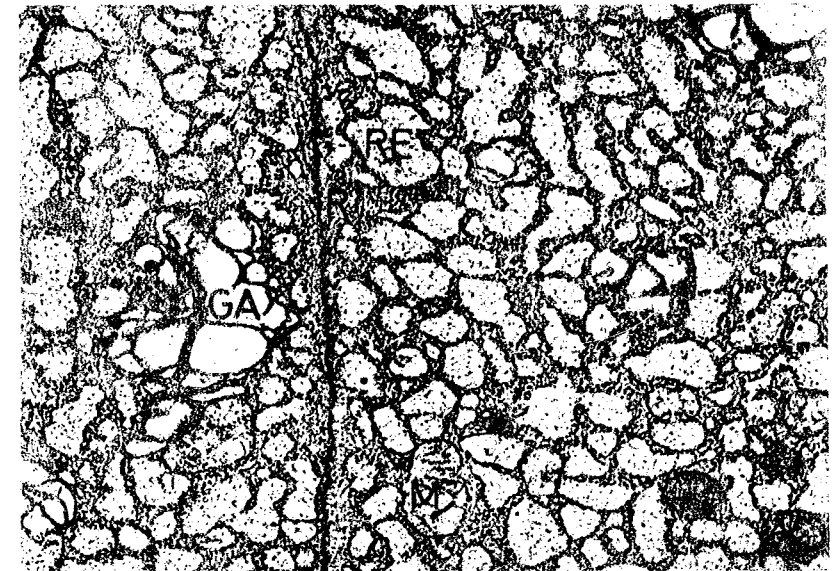Fig. 9. — Portion F du canal. Épithélium glandulaire des réservoirs d'éjaculation. À remarquer l'abondance des mitochondries et la finesse des microvilli. × 6 000.

Fig. 10. — Portion F du canal. Section de l'épithélium au niveau du premier sphincter, à la base du pénis. À remarquer l'absence des microvilli, l'abondance des mitochondries qui sont plus grosses et à crêtes très nombreuses, l'aspect particulier du RE lisse, accompagné de cordons ou brides très denses, la musculature striée en section longitudinale. Section transversale. × 5 700.
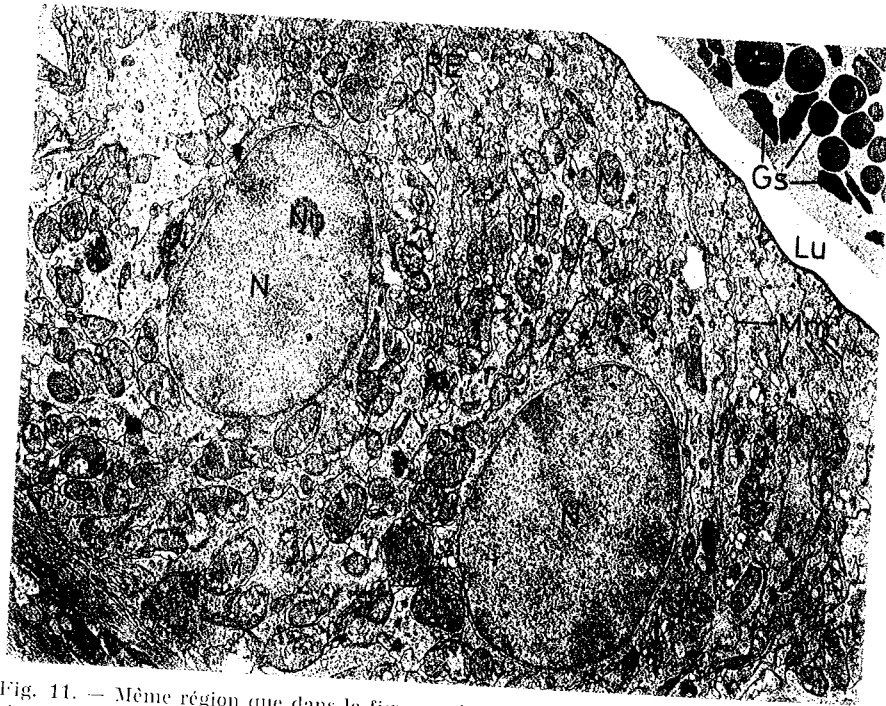
Fig. 11. — Même région que dans la figure précédente. On peut observer mieux la structure des mitochondries et remarquer la présence de lames membraneuses doubles qui parcourent le cytoplasme dans le sens de la hauteur de la cellule. × 6 300.
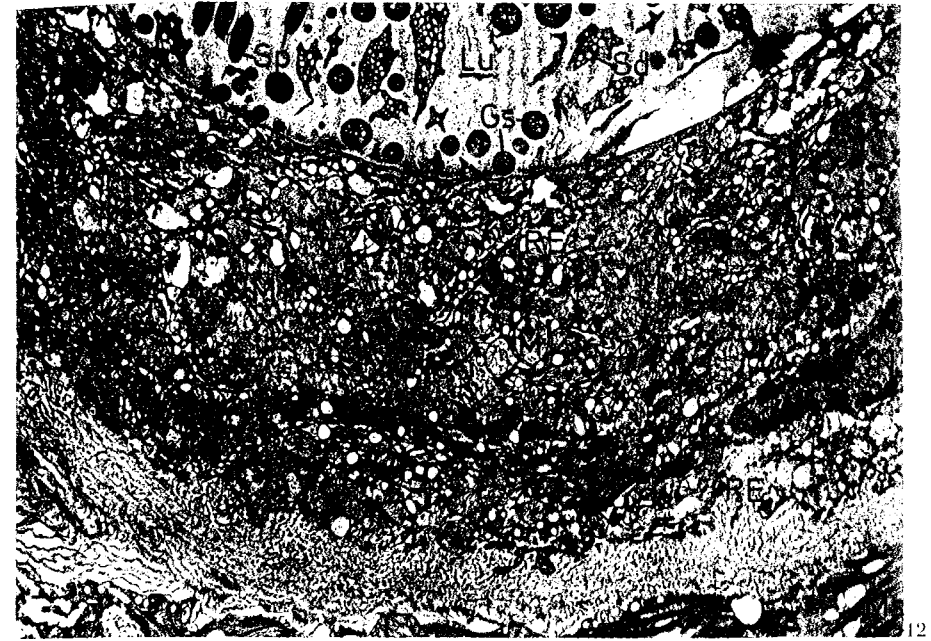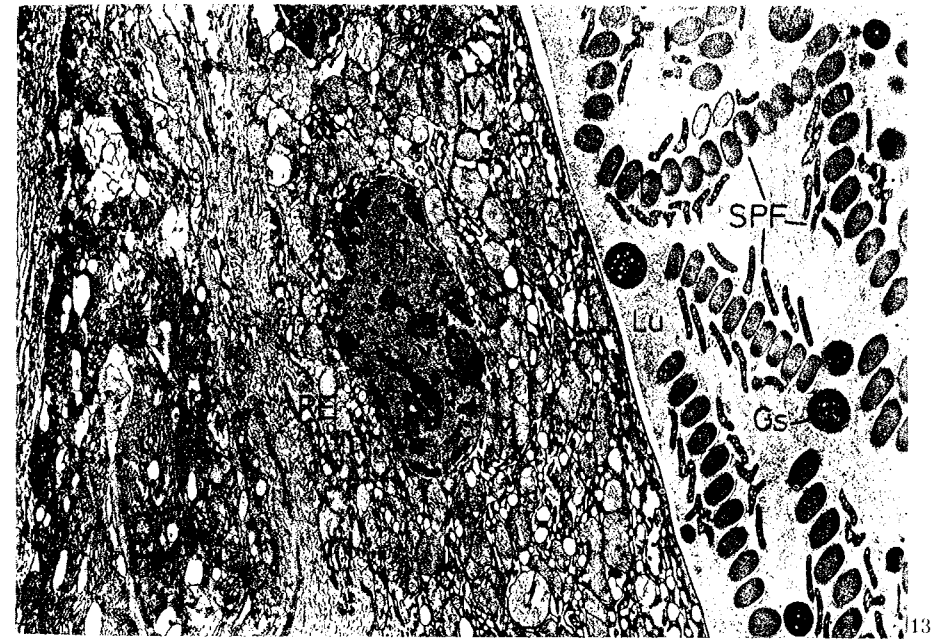
Fig. 12 et 13. — Portion F du canal. Sections au niveau du second sphinctère (y), situé près de l'extrémité du pénis. Très caractéristique l'extrême abondance du chondriome, et du RE lisse qui constitue une épaisse couche feutrée à la base de l'épithélium. En bas de la fig. 13, on voit clairement un tissu qui sépare les deux canaux déférents voisins. Fig. 12 : ×5 500 : Fig. 13 : × 6 600.

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VERGLEICHENDE TOPOCHEMIE DER BASISCHEN PROTEINE IM VERLAUF DER OOGENESE

VON

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Oocytes of animals contain in their ribosomes some basic proteins of the histones type, which are easily evidenced by means of the present histochemical techniques. These proteins appear in the oocytes nucleoli where they are produced and afterwards they are eliminated in the cytoplasm, together with the ribosomal RNA. By the oocytes development and the vitellus synthesis, the histones enrich this oocyte component.

Durch konvergente Verfahren untersuchten die Verfasser die Topochemie der Histone im Verlauf der Oogenese der Tiere, insbesondere der Fische. Während dieser Entwicklungsphase sind die Histone an die ribosomale RNS des Zytoplasmas und an die Nukleoli gebunden, deren Anzahl gleichzeitig stark zunimmt. Bei den Fischen handelt es sich um eine Folge von Histonen, und zwar bei den sehr jungen Oozyten Histone mit Lysin, bei den in langsamen Wachstum begriffenen vorherrschend Histone mit Arginin, bei den reifen Protamine und Teilungshistone mit Lysin.

Als Repressoren der Tätigkeit der Zytoplasma-Polysomen fehlen die Histone aus dem Oozytenkern während der Teilungsphasen. Sie befinden sich im Dottereiweiß, wenn dieses vorhanden ist.

EINLEITUNG

Über das Vorkommen von Histonen im Zytoplasma wurde von verschiedenen Verfassern berichtet, die sie sowohl biochemisch als auch

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histochemisch nachwiesen. Es wurde dabei festgestellt, daß sie gemeinsam mit der ribosomalen RNS vorkommen. Weil sie den Nukleohistonen ähnlich sind, nannte man sie Zytoplasmahistone. Es steht noch aus und so künftig an Versuchsmodellen geprüft werden, ob sie die gleichen Eigenschaften und Funktionen wie die Nukleohistone, oder ob sie nur ihr kurzdauernde Vorstufen haben und ihnen somit verhältnismäßig ähnlich sind. Wie dem auch sei, die Zytoplasmahistone müssen als basische Proteine betrachtet werden, die mehr oder weniger beständig an die Ribosomen gebunden sind. In verschiedenen Arbeiten [7], [8], [18] wird die Verbindung der Zytoplasmahistone mit der (wahrscheinlich mitochondrialen) DNS des Zytoplasmas der Oozyten diskutiert. In anderen Arbeiten [4], [10], [11], [12], [14], [17], [21] befassen sich die Autoren hauptsächlich mit der Verbindung solcher Oozytenhistone mit den Polysomen. Schließlich kommt es vor, daß Histone in Oozyten, Becher- und Mastzellen an saure Mucopolysaccharide gebunden sind. Es sei darauf verwiesen, daß die Basizität der Proteine allein nicht als einziges Kriterium genügt um sie als Histone zu betrachten. Bekanntlich sind Proteine mit basischem Charakter wie Globin, Globulinfraktionen, die antibakteriellen kationischen Proteine der FMN–Granula, die Zytochrome, das Lysosin die Interferrone und die Ribonuklease keine Histone.

In Anbetracht dessen, daß sich in den jungen Oozyten sowie in den Nukleolen, die aus dem Kern ausgestoßen werden, eine große Menge Riboprotein befindet und ihr Plasma arm an Organellen ist, erschien es interessant, die Histone des Zytoplasmas im Verlauf der Oogenese zu untersuchen. Besonders befaßten wir uns dabei mit Fischen, weil diese eine große Menge histochemisch nachweisbarer basischer Proteine im Zytoplasma der Oozyten enthalten.

Um allgemeine Schlüsse ziehen zu können, untersuchten wir vergleichsweise auch die Oogenesen bei verschiedenen anderen Tiergruppen.

MATERIAL UND TECHNIK

Als erstes wurden die Ovarien folgender Fischarten untersucht: *Acipenser ruthenus, Acipenser stellatus, Huso huso, Cyprinus carpio, Carassius auratus gibelio, Scomber scombri, Cobitis romanica, Mullus barbatus ponticus, Ophidion roheii, Hypophthalmyctis nobilis, Ctenopharingodon idella, Mylopharyngodon piceus*. Ferner prüften wir auch die Ovarien einiger Vertreter aus möglichst allen Tierstämmen der phylogenetischen Reihe: *Sycon raphanus, Actinia equina, Halyclyetus octoradiatus, Nereis diversicolor, Parinereis cultrifera, Syllis ramosa, Paracentrotus lividus, Anodonta piscinalis, Limax cinereus, Cardium edule, Octopus vulgaris, Eledone moschata, Astacus leptodactylus, Gammarus locusta, Tegenaria domestica, Scutigera coleoptrata, Trixa nasuta, Forficula auricularia, Rhyphus fenestralis, Lymantria dispar, Eudontomyzon dantforai, Emys orbicularis, Pelobates fuscus, Salamandra maculata, Gallus domesticus*.

Wir fixierten mit 10%iger Formaldehydlösung in neutralem Phosphatpuffer, mit 3 Glutaraldehyd in Kakodylatpuffer 0,7 M pH 7, mit Afrolein ebenfalls in Kakodylatpuffer 0,7 M pH 7, mit Methylalkohol, Carnoyscher-, Hellyscher-, Bouinscher- und Bouin-Hollandescher Lösung. Die Einbettung wurde in Paraffin vorgenommen. Gleichzeitig machten wir auch Schnitte von Karausche und Karpfen am Kryotom, die z.T. in gepufferter Formaldehydlösung nachfixiert wurden. Die Histone wurden durch konvergente Verfahren nachgewiesen: 1, nach Alfort und Geschwind [1] mit Fatsgreen FCF 0,1% in Tris-Puffer 0,1 M pH 8;

nach Bloch und Hew [6] mit Eosin Y 0,1% in der gleichen Pufferlösung; 3, nach einer Abänderung des Verfahrens mit Bromphenolblau (die Schnitte werden bei 60° 2 Std. in einer gesättigten Pikrinsäurelösung oder bei 90° 15 Min. in einer 5%igen TCS-Lösung hydrolysiert, danach 15 Min. mit 70% Alkohol gespült, 10 Min. mit 0,1%igen Bromphenolblau in Boratpuffer 0,01 M pH 8,2 gefärbt, mit Boratpuffer 0,035 M differenziert und montiert); 4, nach dem Verfahren mit Fastgreen-Eosin X (die Schnitte werden 30 Min. in einem Gemisch von 0,05%igem Fastgreen FCF und 0,05%igem Eosin Y im gleichen Puffer gefärbt); 5, mit apurinischem Schiffschem Reagens für Histone, das nach Vendrely [22] hergestellt wird; 6, nach der Millonschen Reaktion für Gesamtproteine und für nichthistonische Proteine. Die Verfahren der sauren Färbung können nach Desaminierung oder Nitrierung angewendet werden, die natürlich erst nach der üblichen Nukleinsäureextraktion möglich sind. Die Nukleinsäuren werden durch verschiedene Verfahren beseitigt: Hydrolyse bei 90°/15 Min. mit 5%igen TCS oder bei 60°/60 Min. mit einer gesättigten Pikrinsäurelösung und schließlich durch Versuche mit RNase 5 x rekristallisiert, DNase in von Love und Rabotti vorgeschriebenen Lösungen, sowie zehnmal höheren Konzentrationen. Das Lysin wurde mit FDNB nachgewiesen, die argininreichen Histone unterschied man von den lysinreichen durch das Verfahren von Black-Ansley [5] mit Silbernitrat. Die Differentialfärbung des Kernchromatins erzielten wir mit Schiff TCS, die des Nukleolus und der Zytoplasmahistone mit Fastgreen FCF. Die RNS und die Histone wurden gleichzeitig durch die zweifache Färbung mit Azur A und Eosin Y nachgewiesen. Als allgemeine Reaktionen für die basischen Proteine gebrauchte man Naphtholgelb S für die basischen Gruppen der Proteine. Die Verfahren mit Hämatoxylin Fullmer, Biebrich scarlet, Eriochromcyanin, Amidoschwarz 10 B dagegen zeitigten für die basischen Kernproteine, wie auch für die Zytoplasmaproteine gute Ergebnisse. Die SH-Gruppen der Proteine wurden durch die Reaktion von Chévrémont, die SN-Gruppen und SS-Brücken durch DDD nach Narnett-Seligman die primären NH$_2$-Gruppen nach Weiss mit Hydroxynaphtholaldehyd und durch Nachkupplung mit Tetrazolium die Phenolgruppen nachgewiesen.

Zum Studium der Feinstruktur der Fisch-Oozyten fixierte man die Ovarien von *Carassius auratus* mit der Fixierlösung von Zetterqvist bei 4°/60 Min. Nach dem üblichen Entwässern in Azeton wurden sie in Vestopal W eingeschlossen und mit Glasmessern am Ultrotom LKB geschnitten. Die Aufnahmen machte man mit einem Elektronenmikroskop OPTON.

ERGEBNISSE

Cyprinus carpio. Das Ovar der Karpfenlarven weist eine große Anzahl von Oogonien auf, die sich durch klares, nicht basophiles Zytoplasma und einen kompakten stark chromatischen Kern auszeichnen. Bei Färbung mit Feulgen oder Schiff-TCS[1] erscheint der Kern leuchtend violett, ohne klarere Zone (Abb. 1). In den gleichen Ovarien ist auch der Beginn des Wachstums der Oozyten zu erkennen. In diesem Fall ist die DNS wie eine Kugelkappe lokalisiert. Weiter sind die Chromosomen der Prophase der Meiose zu erkennen (Abb. 2). Die außerchromosomale Kalotte stellt nach Mac Grefor [20] die DNS eines außerchromosomalen Material. Während des Wachstums der Oozyte lokalisiert sich die Markierung mit Thymidin^3-H in dieser Kalotte. Nach der Fragmentierung des Nucleolus und nach der Bildung von Hunderten von Nukleoli auf

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[1] Schiff-TCS wird mit Trichloressigsäure statt mit Salzsäure hergestellt und wird nach der Hydrolyse der Präparate bei 60°/80 Min. in n HCl oder 25 Min. in n TCS angewendet.

der Innenseite des Kernmembrans, lokalisiert sich das Thymidin imm... auf Nukleolseite, die gegen das Innere des Nukleus gewandt ist. Di... Kalotte stellt eine Vervielfältigung des Nukleolusorganisators dar, a... Folge der Vervielfältigung bilden sich ebensoviele Nukleoli, die m... Thymidin markiert werden (Abb. 3). Es ist die Phase der Transkriptio... der DNS, die das Zytoplasma benötigt. In den Ovarien junger un... geschlechtsreifer Karpfen mit im Wachstum begriffenen Oozytenkerne... tritt infolge der Entspiralisierung der Chromosomen DNS and die Ober... fläche der Kernmembran. Je stärker die Oozyte wächst, desto meh... Feulgen-positive Granula erscheinen an der Peripherie des Kernes. Werde... die Schnitte auch noch mit Fastgreen 0,1 % bei pH 8,1 gefärbt, so wir... sehr gut sichtbar, wie derartige junge Oozyten im Kern 1—2 Nukleoli... in Feulgen-positives Material eingelagert enthalten. Die Färbung mi... Methylgrün OO-Pyronin Y und die vorherige Kontrolle mit Ribo... nuclease haben eindeutig erwiesen, daß die Basophilie des Zytoplasmas... und der Nukleoli auf die RNS zurückzuführen ist. Es ist bemerkenswert... daß bei den am Kryotom gemachten Schnitten sich nur das Zytoplasma... mit Pyronin rot färbt und die Nukleoli keine Farbe annehmen. Diese... färben sich erst, nachdem die Schnitte mit einer angemessenen Fixierungs... lösung nachbehandelt werden.

Nach der Hydrolyse mit RNase, sowie mit TCS, auf die die Färbung... mit Fastgreen folgt, erscheinen ganz besonders bei Paraffinschnitten... das basophile Zytoplasma und die Nukleoli stark leuchtend grünblau... gefärbt. Bei den Ovarien der geschlechtreifen Tiere sind außer Oozyten... mit Rindenvakuolen auch Oozyten vorhanden, in denen Dottereiweiß... auftritt. Die Anfärbung mit Schiff-Fastgreen (Abb. 4) nach der Hydrolyse... mit TCS der geschlechtsreifen Ovarien, die in gepufferter Formaldehyd... lösung fixiert wurden, ist in der Karyolymphe, in den Lampenbürsten... chromosomen und im Vitellus schwach. Wie im Fall der jungen Oozyten... sind hingegen die Nukleoli und besonders das Zytoplasma intensiv gefärbt,... wodurch bewiesen ist, daß die RNS an die Histone gebunden ist. Dieses... von den Oozyten während der ganzen Vitellogenese beibehaltene Kenn-... zeichen, wurde auch durch andere Verfahren nachgewiesen. Als erstes... wurde festgestellt, daß sowohl die Hydrolyse in gesättigter Pikrinsäure-... lösung, als auch diejenige mit RNase oder RNase und DNase-Mischung... erwartungsgemäß die gleichen Ergebnisse zeitigt, wie die Hydrolyse... mit TCS. Folglich färben sich ungeachtet der Hydrolyse die Nukleoli... der wachsenden Oozyten wie ihr basophiles Zytoplasma intensiv mit... Fastgreen, alkalischem Bromphenolblau (Abb. 5a und 5 b), apurinischem... Schiff bzw. mit Eosin, etc. Bei der Färbung nach Block [9] mit Fastgreen-... Eosin (Abb. 6) färben sich sowohl das Zytoplasma als auch die Nukleoli... in den mit gepuffertem Formaldehyd fixierten Schnitten rosaviolett,... während in den Kryotomschnitten ohne Nachfixierung nur das Zyto-... plasma reagiert. Nach Bloch ergeben die lysinreichen Histone derartige... blaßviolette Tönungen. Ebenfalls gestattet das Verfahren von Black... mit ammoniakalischer Silberlösung, die spektrophotometrisch getestet... wurde, die lystinreichen von den argininreichen Histonen zu unterscheiden.... Auch in diesem Fall ergibt das Zytoplasma der sehr jungen Oozyten eine... gelbbraune Tönung, die das Nebeneinanderbastehen der lysinreichen und... der argininreichen Histone bestätigt, während die schwarzen Nukleoli

sowie das Zytoplasma um den Dotter Histone mit Arginin enthalten (Abb. 7).

Die radiärgestreifte Kortikalschicht weist in diesem Verfahren auch Histone mit Lysin auf, die selbstverständlich mit den auf Schiff-Perjod-säure stark reagierenden Mucopolysachariden gemeinsam auftreten. Den Verfahren mit Fastgreen, Bromphenolblau bzw. Eosin ging nach der Nukleinsäureextraktion die Azetylierung oder Desaminierung voraus. Auf diese Weise färben sich diejenigen Histone spezifisch, die im Ver-gleich zu Lysin eine große Menge Arginin enthalten. Die Desaminierung betrifft in erster Linie die Amino-Gruppen des Lysins und läßt die Guanilgruppe des Arginins unbeeinträchtigt. Durch dieses Verfahren gewinnt man Klarheit in dem Sinne, daß die argininhaltigen Histone im Zytoplasma der jungen Oozyten ebenso wie in den Nukleoli, in denen sie sich stark färben, vorherrschen. Die Intensität der Reaktion sinkt mit dem Wachstum der Oozyte und beschränkt sich nur auf das Zytoplasma. Die radiärgestreifte Kortikalschicht weist bei allen Verfahren eine ebenso intensive Reaktion der Histone auf und scheint außer den lysinhaltigen auch argininhaltige Histone zu enthalten.

Die Verfahren zum Nachweis der kationischen Proteine waren im allgemeinen ebenso aufschlußreich für ihren Nachweis in den Nukleoli, wie auch im basophilen Zytoplasma. Mit Solochromcyanin färbt sich das Zytoplasma der nicht fixierten Schnitte intensiv ziegelrot, die radiärge-streifte Kortikalschicht blaßrosa. In den mit gepufferter Formaldehyd-lösung fixierten Präparaten scheint der Sachverhalt etwas komplizierter zu sein, denn das Zytoplasma zwischen dem Dotter ist violett und das perinukleare Zytoplasma dunkelviolett, als ob es neben der ribosomalen RNS eine Menge DNS enthalten würde (Abb. 8). Das Karyoplasma ist ziegelrot, also proteinhaltig. Mit Biebrich scarlett pH 9,6 ist das Karyo-plasma schwach positiv und die Nukleoli, das Zytoplasma und die radiär-streifige Kortikalschicht leuchtend rot (Abb. 8). Die Nukleoproteine der Oozyten, sowie die Proteine der Blastomerennukleoli zeichnen sich durch ihre Färbung mit Amidoschwarz 10 B (Abb. 10) aus. Die SH-Proteine, die durch das DDD-Verfahren bestimmt werden, erscheinen in den Nukleoli und im Zytoplasma im großen, im Lariyoplasma und in der radiärstreifen Kortikalschicht in geringeren Mengen. Die primären Amino-gruppen, die mit 2,3-Hydrosynaphthaldehyd und Kupplung mit Fastblue B in alkalischem Medium nachgewiesen wurden, sind in den Nukleoli, im Zytoplasma und in der radiärgestreiften Kortikalschicht leuchtend blau. Ähnliche Ergebnisse erzielte man mit Naphtholgelb und Hämatoxylin Fullmer, die im vorliegenden Fall ebenfalls basische Pro-teine angeben.

Carassius auratus gibelio. Die untersuchte gynogenerische Art weist in den Ovarien alle Entwicklungsstadien der Oozyten auf. Methylgrün OO-Pyronin Y zeitigt die gleichen Ergebnisse wie beim Karpfen in dem Sinn, daß das Zytoplasma der Oozyten zu Beginn des Wachstums und bis zu ihrer Geschlechtsreife voller RNS ist und die Nukleoli sich nur in fixierten Schnitten mit Pyronin färben. Die Inkubation der Ovarien in 3 H-Thymidin 2 Std. läßt auf der Autohistoradiographie eine Synthese-tätigkeit der DNS an der Innenseite der Kernmembran und um die Nucleoli in dem, dem Auftreten der Kortexvakuolen unmittelbar vorher-

gehenden Stadium, erkennen (Abb. 11). Mit Schiff TCS-Fast green FCF färben sich das Zytoplasma und die radiärgestreifte Kortikalschicht wegen ihres hohen Histongehalts dunkelgrün. Die restlichen Strukturen, einschließlich des Dotters, ergeben eine schwache Tönung. Ebenso wie die nichtfixierten, am Kryotom geschnittenen Ovarien, weisen das Zyto-plasma und die radiärgestreifte Kortikalschicht eine viel intensivere Färbung als das Dottermaterial auf, während der Rest ungefärbt bleibt. Bei Nachfixierung mit gepufferter Formaldehydlösung, sowie auf den Paraffinschnitten, erscheinen die Histone in den Nukleoli und im Zyto-plasma viel stärker gefärbt, als auf den frischen Schnitten. Mit Bromphe-nolblau sind unabhängig von der Hydrolyse das Zytoplasma, die Nukleoli und die radiärgestreifte Kortikalschicht dunkelblau (Abb. 12). Mit Fastgreen-Eosin sind Tönungen von grün (Histone mit Arginin) bis rot (Histone mit Lysin) (Abb. 13) zu unterscheiden. Karyoplasma und Dotter erscheinen blaßrosa, während Nukleoli und radiärgestreifte Kortikal-schicht violettrosa sind. Durch dieses Verfahren färbt sich das junge Zyto-plasma violettrosa, das Zytoplasma der wachsenden Oozyten grün, das des granulären Dottereiweißes violett und das der Kortikalschicht grün. Ebenso wie in der Spermatogenese enthält das Zytoplasma zu Beginn der Oogenese hauptsächlich lysinreiche Histone, später herrscht mit dem Wachstum der Oozyte Arginin in den Histonen vor. Bei der Geschlechts-reife sind Protamine vorhanden. Die Hydrolyse mit DNase, auf die die Färbung mit 0,1%igem Eosin in Tris-Puffer pH 8,1—8,3 folgt, weist auf das Vorhandensein von nukleolaren und zytoplasmatischen Protaminen in den Oozyten, in denen die Dotterbildung zum Abschluß gekommen ist, hin (Abb. 14). Die Blockierung durch Azetylierung oder Desaminie-rung beeinträchtigt nur die jungen Oozyten, doch nicht zu stark, denn sie enthalten auch eine bestimmte Menge von Histonen mit Arginin. Auf diesen Fakt weist die violette Färbung der mit Fastgreen-Eosin behan-delten Oozyten hin.

Die Reaktion mit der ammoniakalischen Silberlösung bestätigt ebenfalls die Ergebnisse, die durch die obenerwähnten Verfahren erzielt wurden (Abb. 15). Das Zytoplasma der jungen Oozyten ist gelbbraun, das der wachsenden Oozyten schwarz. Am Ende der Dottereinlagerung enthält es schwarze Zytoplasmateilchen um den Dotter, im übrigen Teil bleibt es braun. Auch durch dieses Verfahren bestätigt sich die Reihen-folge der Histone mit Lysin, dann mit Arginin und zum Schluß mit einer Mischung in der die Protamine vorherrschen.

Auch beim Karpfen färben sich die Nukleoli der Oozyten zum Unterschied von denen der somatischen Zellen mit ammoniakalischer Silberlösung und mit Amidoschwarz 10 B (Abb. 16) wegen des Gehaltes an argininreichen basischen Proteinen tiefschwarz. Die Varianten der Methode für Arginin haben bei den verschiedensten Oozyten den Reichtum der Nukleoli an Arginin in der meiotischen Prophase gezeigt. Die basischen Proteine im allgemeinen, die mit Solochromcyanin (Abb. 17) nachweisbar sind, sind im Karyoplasma, im Zytoplasma und in der radiärgestreiften Kortikalschicht vorhanden. Die SH-Proteine wachsen in Zytoplasma der Oozyten während der Vitellogenese und befinden sich auch in den Rin-denvakuolen, im Dotter und in der radiärgestreiften Kortikalschicht (Abb. 18).

Hypophthalmyctis nobilis, Ctenopharyngodon idella und *Mylopharyn-don piceus* weisen sehr spezifisch in ihren jungen Oozyten ebenso wie *cipenser ruthenus* und *stellatus* im pachytänen Stadium eine Nukleo-lenkappe mit DNS, RNS und Histonen auf. Diese ist von den Chromo-somen völlig getrennt. Derartige Strukturen sind auch bei den Acipen-seriden und bei den Amphibien beschrieben worden. In den Oogonien ist der Kern kompakt und intensiv Feulgen-positiv. Zugleich mit der Umwandlung in Oocyten ordnet sich die DNS an der Kernperipherie in eine dichte und kontinuierliche Schicht. Mit der Zeit wird diese Schicht zu einer einfachen Kappe, die völlig vom restlichen Chromatin der pachy-tänen Chromosomen getrennt ist. Beim Sterlet wird die Entwicklung dieser Kappe mit Bromphenolblau nach hydrolytischem Abbau mit DNase (Abb. 19, 20, 21) deutlich. Der Kern der jungen Oozyten ist voll Histon, das an DNS gebunden ist. Sobald aber die Kappe ausgebildet ist, ist sie viel reicher an derartigen Histonen. Wenn der Kern wächst ist nur die Kappe leuchtend blau, im restlichen Kern sind dagegen nur einige blaue Granula zu sehen. In einem späteren Stadium erscheinen in der Mitte der Kappe klarere Teile mit geringerem Histonengehalt, während im Karyoplasma jetzt 2—3 Nukleoli entstehen. Mit dem Fortschreiten des Wachstums teilt sich die Nukleolussubstanz der Kappe in einige große Blöcke, während auf der Innenseite der Kernmembran kleine kugelförmige Nukleoli erscheinen. Wenn der Kern zusammenschrumpft befinden sich alle Nukleoli, die kleinen von 0,5—1 μ sind vorherrschend, am Rand. Beim Scherg weisen die Oozyten der Larvenovarien mit Häma-toxylin Fullmer nach DNase-Behandlung außer einer Reihe kleiner aus dem Zytoplasma ausgestoßener Nukleoli auch dichte basophile Zyto-plasmateilchen um den Nukleus auf (Abb. 22). Die Färbung mit Amido-schwarz der kationischen Proteine bestätigt auch beim Scherg, den gleichen Entwicklungsverlauf wie beim Sterlet. Zu Beginn sind in der Kappe der Nukleoli im Vergleich zum Rest des Kerns die Histone sehr reich vertreten. In der Kappe treten dann sehr deutlich zwei-drei eiweißartige Einschlüsse auf. In der Vermehrungsphase der Nukleoli ordnen sich diese mit dem klareren Teil an die Innenseite der Kernmembran an, der intensiver gefärbte Teil ist gegen das Innere gewandt.

Die Färbung mit Schiff-Fastgreen FCF beweist die Anhäufung der DNS aus der Kugelkappe an dem Rand, der Histone sowie der RNS in den zentralgelegenen Granula. Die verschiedene Hydrolysedauer (3—20 Min.) mit HCl und TCS in n und 0,1 m Konzentration, auf die die einfache oder mit Fastgreen assoziierte Schiffsche Färbung folgt weist auf zwei charakteristische Eigenschaften dieser DNS aus der Nukleo-luskappe hin: große Hydrolysefestigkeit bei gleichzeitig schwacher Bindung an die Histone der kugeligen Granula aus dem Inneren der Kappe. Schon nach 3 Min. langer Hydrolyse mit n HCl oder TCS und bis zu 20 Min. mit 0,1 n HCl färben sich die Kappen durch das Schiffsche Fuchsin an. Bei der Nachfärbung mit Fastgreen FCF erscheint das Zentrum der Kappe grün, was auf die schwache Verbindung der DNS mit den Histonen hindeutet. Die violette Farbe mit Methylgrün OO-Pyronin weist auf das Vorhandensein von DNS zusammen mit RNS in der Kappe hin. Die einfache Färbung mit Fastgreen FCF nach 15 Min.

Hydrolyse bei 90° mit TCS zeigt erwartungsgemäß an, daß die beid[en] Nukleinsäuren an Histone gebunden sind (Abb. 23).

Bei *Mullus barbatus* und *Scomber scombrus* ist die Ähnlichkeit m[it] den anderen Species in bezug auf das Vorhandensein der Kappe u[nd] ihrer histochemischen Zusammensetzung erwähnenswert. Der einz[ige] Unterschied besteht im frühzeitigen Schwund der Kappe, bevor d[ie] Oozyte wächst und das spätere Vorhandensein einiger zweiteiliger Nukleo[len] deren chromatischer Teil gegen das Innere des Kerns und deren chrom[o]phober Teil gegen dessen Membran gerichtet ist.

Bei *Eudontomyzon dautfordi* zeichnen sich die Oozyten währe[nd] des Wachstums durch ein einziges Körnchen von 3 μ aus, das ebenfa[lls] zweiteilig ist und den schaumigen chromophoben Teil der Kernmembr[an] zugewandt hat (Abb. 24).

Dieses für die Fische einheitliche Bild muß noch durch eini[ge] Abweichungen ergänzt werden, die bei *Ophidion roheii* anzutreffen si[nd] wo außer dem Zytoplasma, den Nukleoli und der radiärgestreiften Kort[i]kalschicht auch die Randvakuolen bestimmbare Mengen an Histone[n] aufweisen (Abb. 25). Bei allen untersuchten Fischspezies lassen si[ch] die Histone in dem Dotter parallel mit dem Entstehen des phospho[...] proteinhaltigen Dotters, der diese telolezithalen Eier anfüllt, lokalisier[en] Bei den reifen Oozyten färben sich die Dottergranula mit allen Verfahre[n] durch die die Histone nachweisbar sind. Zudem färben sich nach de[r] Hydrolyse durch Desoxyribonuklease diese Granula mit Rosin Y, wob[ei] in Übereinstimmung mit Bloch und Hew durch dieses Verfahren auc[h] Protamin nachgewiesen wird, leuchtend rot.

Bei den Reptilien (*Emys orbicularis* (Abb. 26), *Lacerta mural[is]* (Abb. 27)), den Amphibien (*Pelobates fuscus, Salamandra maculata* (Abb. 28), *Rana ridibunda*) und den Vögeln (*Gallus domesticus*) zeichnet si[ch] das Wachstum der Oozyten durch völligen Mangel an zytochemisc[h] nachweisbaren zytoplasmatischen basischen Proteinen aus, ausgenomme[n] die Nukleolarsubstanz, die in dieser Periode besonders bei Reptilie[n] intensiv reagiert. Hier ist die Nukleolarsubstanz, die in das Zytoplasm[a] wandert, reich an RNS, Histonen und neutralen Mucopolysacchariden Der Dotter der Sauropsiden gibt eine positive Reaktion auf basisch[e] Proteine.

DISKUSSION

Um die mitgeteilten Ergebnisse deuten zu können ist es erforderlich, noch andere Beobachtungen in die Betrachtung einzubeziehen, die an Oozyten der Vertreter anderer Tierstämme gewonnen wurden. Unsere Untersuchungen können für eine einheitliche Erklärung zusammengefaßt werden und ermöglichen es festzustellen, ob das Vorhandensein der zytoplasmatischen und nukleolaren RNS im Laufe der langsamen Wachstumperiode der Oozyten an die Anwesenheit der Histone gebunden ist oder nicht. Ferner, ob der Dotter der in der Schlußperiode der Oogenese entsteht mindestens einen Teil der zytoplasmatischen Histone enthält oder nicht. Aus den Untersuchungen von Davenport [14] und den hier mitgeteilten Befunden folgt eindeutig, daß die zytoplasmatischen Histone

zumindest zum Teil auf die Dottergranula bei deren Bildung übergehen. Diese Tatsache konnte durch die verschiedenen angewandten histochemischen Reaktionen erhalten werden. Zwar reagieren die Kerne außer den Nukleoli, nicht in einigen histochemischen Verfahren, doch kann man nicht mit der Gewißheit von Horn [16] behaupten, daß im Karyoplasma und in den Chromosomen der Oozyten keine Histone vorhanden sind. Dies umsomehr, als mit Echtgrün-Eosin oder Bromphenolblau der Oozytenkern doch schwach reagiert. Es besteht kein Zweifel, daß in der radiärgestreiften Kortikalschicht lysinreiche Histone vorherrschen, die an die Mucopolysaccharide der Kortikalschicht gebunden sind.

Bei *Sycon raphanus* enthalten die nackten, follikelzellenlosen Oozyten sowohl in ihrem Kern, als auch im Kern der Dotterzelle der Spermiozyte und auch im Kern der Transportzelle basische Proteine. Im perinuklearen Zytoplasma sind einige Granula basischer Proteine der einzige Hinweis auf ihr Vorhandensein.

Bei den Coelenteraten (*Actinia equina* und *Halyclystus octoradiatus*) enthalten das Zytoplasma sowie die Oozytenkerne die darin eingewanderten Histone, jedoch nur in geringer Menge. Die Feulgensche Reaktion gestattet die Lokalisierung der DNS auch in den Nukleoli. In jedem Kern befinden sich ein bis drei derartige „Nukleoli", die eigentlich eine kugelförmige Nukleolarsubstanz darstellen von 1—2 μ im Durchmesser. Während des Wachstums der Oozyte werden sie auf zwei Arten in das Zytoplasma ausgestoßen. Entweder treten sie durch die Kernmembran oder sie werden durch Sprossen der Kernmembran zusammen mit etwas Karyolymphe aber ohne Chromatin ausgestoßen. Die Bläschen mit strukturierter Nukleolarsubstanz zersetzen sich, sobald sie ins Zytoplasma gelangen und reichern es dadurch mit RNS und Histonen ab.

Bei den Polychaeten (*Nereis diversicolor, Perinereis cultrifera, Syllis ramosus* usw.) sammelt sich das Chromatin der Oozyten in einer Feulgenpositiven Karyosphäre, die erwartungsgemäß auch Histone enthält.

Bei *Paracentrotus lividus*, sowie bei den allermeisten Echinodermen, mit Ausnahme der Crinoiden, ist die Menge an RNS im Zytoplasma der Oozyten ebenso wie die Reaktionsintensität gegen Histone gering. Davenport [14] zeigt dennoch, daß mindestens in einigen Fällen keine scharfe Parallelität zwischen der Menge an RNS und an zytoplasmatischen Histonen besteht, denn nur ein Teil der nachweisbaren basischen Proteine ist an die RNS gebunden. Derartige Fälle sind auch bei Fischen beobachtet worden, wo eine schwache Reaktion auf Histone ohne vorherige Hydrolyse eintritt. Während der ganzen Oogenese zeichnet sich der Oozytennukleolus zum Unterschied vom Zytoplasma durch eine intensive Reaktion der Histone aus.

Bei den Mollusken (*Anodonta piscinalis, Limax einereus, Cardium edule* (Abb. 29), *Octopus vulgaris, Eledone moschata* (Abb. 30)) reagieren die Histone der basischen Proteine intensiv, jedoch nur in den Frühstadien der Oogenese. Lediglich bei *Limax* besteht die Basophilie des Zytoplasmas sowie der Histonreichtum bis zu einem späteren Stadium. Auch bei dieser Abart reagiert der Nukleolus sehr stark positiv, sowohl bei den Nachweisreaktionen der RNS, als auch bei denen der Histone. Für die Nukleoli der Mollusken wurde nachgewiesen [11], daß sie auch DNS, SH-Proteine, Arginin, Tyrosin und Eisen enthalten. Durch Digestion

mit Ribonuklease konnte festgestellt werden, daß die RNS an Histo-
gebunden ist. Diese zytoplasmatischen basischen Proteine werden v-
der RNS geschützt, denn falls vor der Behandlung mit RNase eine De-
minierung vorgenommen wird, kann man nachher Histone mit eine-
großen Prozentsatz Lysin nachweisen.

Bei der Arachnide *Tagenaria domestica* weicht die Situation dahi-
gehend etwas ab, daß die jungen Oozyten in ihrem Zytoplasma d-
Balbianischen Dotterkern aufweisen. Die Histone reagieren stark ;-
diesem Kern (Abb. 31), der viel Ergastoplasma, also auch RNS enthä-
Das Zytoplasma sowie der Nukleolus reagieren weniger stark. Zuglei-
mit dem Auftreten des Dotters ergeben die Dotterkörner die Reakti-
der Histone.

Bei Chilopoden (*Scutigera coleoptrata*) sowie bei den Crustace-
Astacus ceptodactylus, Gammarus locusta) reagieren in den jungen Oozyt-
nur in der Nukleolarsubstanz die Histone stark, im Zytoplasma dageg-
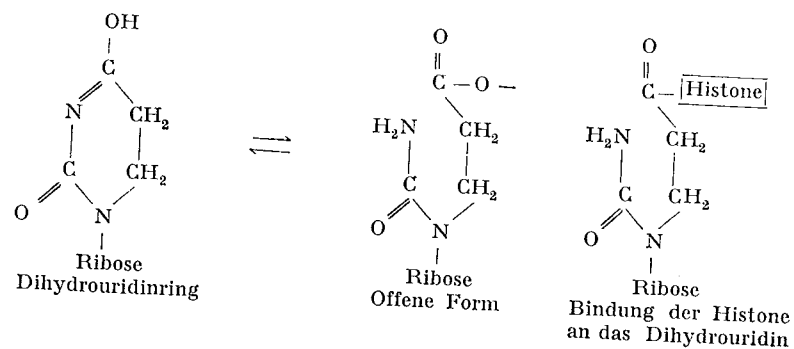sehr schwach (Abb. 32).

Mit der Entstehung des Dotters reagiert dieser in allen Verfahr-
zum Nachweis der Histone sehr stark.

Bei den Insekten ist die Rolle der Trophozyten bekannt, bei den-
die Hypertrophie des Nukleus, die Vervielfältigung der Nukleolarsubstan-
und die Anreicherung des Zytoplasmas mit Polysomen die Tätigk-
des Oozytenkerns ersetzt und ausgleicht. Die Histone und die RN-
reagieren tatsächlich sowohl im Kern als auch im Zytoplasma der Troph-
zyten stark. Bekanntlich wandern diese Stoffe in das Zytoplasma d-
Oozyten ein und reichern dieses mit Riboproteinen an.

Das Studium der Topochemie der basischen Proteine im Lauf
der Oogenese verschiedener Spezies innerhalb der phyletischen Tierska-
erleichtert die Schlußfolgerungen über das Auftreten der zytoplasmatische-
und nukleolaren Histone.

Als erstes ist die enge Beziehung zwischen der Existenz der RN-
und der der basischen Proteine vom Typ der Histone zu beobachte-
die mit Fastgreen FCF oder alkalischem Bromphenolblau immer star-
reagieren.

Die der Hydrolyse mit der RNase vorausgehende Desaminierun-
zeigt, daß ein großer Teil der Histone von der RNS geschützt wird un-
sehr reich an Arginin ist. Die Bindung der Histone an die RNS geschieh-
wahrscheinlich nach Ru-Chih und Bonner [21] durch eine Komponent-
der RNS, das Dihydrourazil, das in offener Form gebunden ist. Dies-

komplexe Verbindung Histon-RNS ergibt mit *p*-Dimethylaminoben-
zaldehyd eine gelbe Farbe, eine Reaktion, die für die Ureido-Gruppe der
Ureidopropionsäure kennzeichnend ist.

Eine derartige gelbe Farbe ergeben mit dem *p*-Dimethylamino-
benzaldehyd sowohl das Zytoplasma, als auch die Nukleoli der Oozyten.
Es gibt aber auch einige Autoren, die eine derartige Bindung zwischen der
RNS und den Histonen bestreiten und als einzige Verbindung nur die
Wasserstoffbrücken annehmen.

Die elektronenmikroskopischen Bilder der jungen Karpfen-Oozyten
zeigen die Wanderung der Nukleolarsubstanz durch die Formen der
Kernmembran in das Zytoplasma. Dieses ist mit Polysomen angefüllt,
an die die Histone gebunden sind (Abb. 33). Die Existenz der Polysomen
und die Dünne des endoplasmatischen Retikulums der jungen Oozyten
lassen die Folgerung zu, daß die Histone beim Aufbau der Struktur-
proteine, die für das Wachstum erforderlich sind, eine Rolle spielen. In
der Oogenese stammen die zytoplasmatischen Riboproteine entweder
aus den Nährzellen der Oozyte, oder aus ihrer eigenen Kerntätigkeit.
Wie dem auch sei, die Riboproteine stammen aus der Kerntätigkeit von
Keimbahnzellen, denn auch die Nährzellen haben denselben Ursprung.

Die bisherigen Forschungen über das Auftreten des Dotters und
der verschiedenen haloplasmatischen Einschlüsse haben in vielen Fällen
gezeigt, daß diese Substanzen von außerhalb stammen und in den Oozyten
in Form von Lipoidtropfen, Glykogen, Vakuolen mit Glykoproteinen
usw. vorliegen.

Verschiedene Beobachtungen an Fischen während der Entwicklung
der Oogonie zur Oozyte weisen darauf hin, daß es sich im Laufe der
Oogenese um eine Änderung der Histone handelt, die den Dotter bilden.
Wenn nun in den Oogonien der reife an Lysin reiche Typ der Histone
vorherrscht, so herrschen in den jungen Oozyten bei Beginn des Wachs-
tums die argininreichen Histone vor; nachdem der Dotter entstanden
ist, sind Protamine und lipinreiche Histone vom segmentierten Typ
vorhanden. Diese Änderungen sind nicht streng begrenzt und schwanken
auch je nach der betrachteten Art. Desgleichen sind die basischen Pro-
teine nicht nur an die RNS gebunden, denn in der radiärgestreiften
Kortikalschicht der Fisch und Reptilienoozyte sind sie an die Mucopoly-
saccharide gebunden. Ebenso sind sie in bestimmten Bereichen des Hyalo-
plasmas mit sauren Mucopolysacchariden, in den Randvakuolen der
reifen Oozyten bei *Ophidion roheii* und im Dotter der Oozyten im allge-
meinen an die sauren Gruppen der verschiedenen Mucoproteine, Phospho-
lipoide und Phosphoproteine gebunden, enthalten.

Die Rolle der zytoplasmatischen Histone könnte analog mit der
der Nukleohistone verglichen werden.

Obwohl zahlreiche Studien besonders über die Nukleohistone
unternommen wurden, ist ihre genaue Funktion in der Zelle noch unklar.
Zahlreiche Autoren, beginnend mit Stedmann haben den Histonen die
Rolle spezifische Repressoren zugewiesen, die die Wirkungsweise der
verschiedenen Gene kontrollieren.

Gegen diese Hypothese werden folgende Einwände angebracht:
die verhältnismäßig beschränkte Zahl der verschiedenen Histonmoleküle,
das Fehlen in manchen Geweben und ihre Spezifität (mit seltenen Aus-

Ribose
Dihydrouridinring

Ribose
Offene Form

Ribose
Bindung der Histone
an das Dihydrouridin

nahmen) sowie die Ähnlichkeit zwischen dem elektrophoretischen Bi bezüglich des Gehaltes an Histonen vom genetisch wirksamen und unwir samen Chromatin.

Die Histone haben tatsächlich einen weiten Hemmungsbereic Einerseits wegen ihrer Eigenschaft Komplexverbindungen mit de Enzymen und deren Substraten einzugehen und andererseits wegen di leichten Bindung zwischen der positiven und der negativen Ladur verschiedener Polymere, insbesondere der DNS [2].

Bemerkenswert ist die einhellig angenommene Tatsache, daß di argininreichen Histone, die auch in den Oozyten in beachtlichen Menge vorhanden sind, stärkere hemmend wirken, als die lysinreichen. Letzter können leichter mit Antibiotika beseitigt werden und stellen die mobile Fraktion dar, die vom physiologischen Zustand der Zelle abhängt [15 Als einfache Hypothese kann eine repressive oder derepressive Spezifitä der Histone angenommen werden. Jedoch nur in soweit, sofern sie vo einer der Struktur sekundären Heterogenität, von der Azetylierung Phosphorylierung oder anderen Reaktionen [14] gewährleistet wird. Noc wahrscheinlicher ist die funktionelle Besonderheit, die durch eine bestimmt „Spezies" der RNS [3], [21] bedingt ist. Es entsteht eine Komplexver bindung RNS-Histon, die sich zusätzlich an verschiedene DNS-Folger bindet. Dadurch ergibt sich ein Mechanismus, der den Histonen dies zusätzliche Spezifität erteilt [4]. Alle genannten Voraussetzungen betreffer die Nukleohistone.

Für die hier untersuchten zytoplasmatischen Histone kann behauptet werden, daß sie eine allgemein hemmende Wirkung besitzen, die sich in erster Linie mindestens auf die Tätigkeit eines Teiles der Polysomen des Zytoplasmas bezieht.

Diese Behauptung wird auch dadurch bewiesen, daß im Verhältnis in den metabolisch inaktiven Zellen mehr Histone im Vergleich zur DNS vorhanden sind, als in den aktiveren Zellen (Krebs- und Regenerations zellen). Das langsame Wachstum des Zytoplasmas der Oozyten ist eben durch einen Reichtum an zytoplasmatischen basischen Proteinen gekenn zeichnet. Diese Zusammensetzung des Zytoplasmas verschwindet jedoch mit dem Beginn der Dotterbildung. Bei Karpfeneiern, die drei Stunden nach der Befruchtung fixiert worden, färben sich nur die Dotterblätt chen, das Zytoplasma und die Blastomerenkerne dagegen nicht. Dies kann mit der Gesamtfähigkeit der Blastomere in diesen ersten Stadien in Verbindung stehen. Mit der Gastrulation sind die Histone durch Anfär bung erneut nachzuweisen, jedoch nur in den Kernen. Diese Tatsache läßt darauf schließen, daß die gleichen Histone auf verschiedene Teile der DNS-Ketten repressiv wirken können und zwar in Verbindung mit der biochemischen Differenzierung der Zellen.

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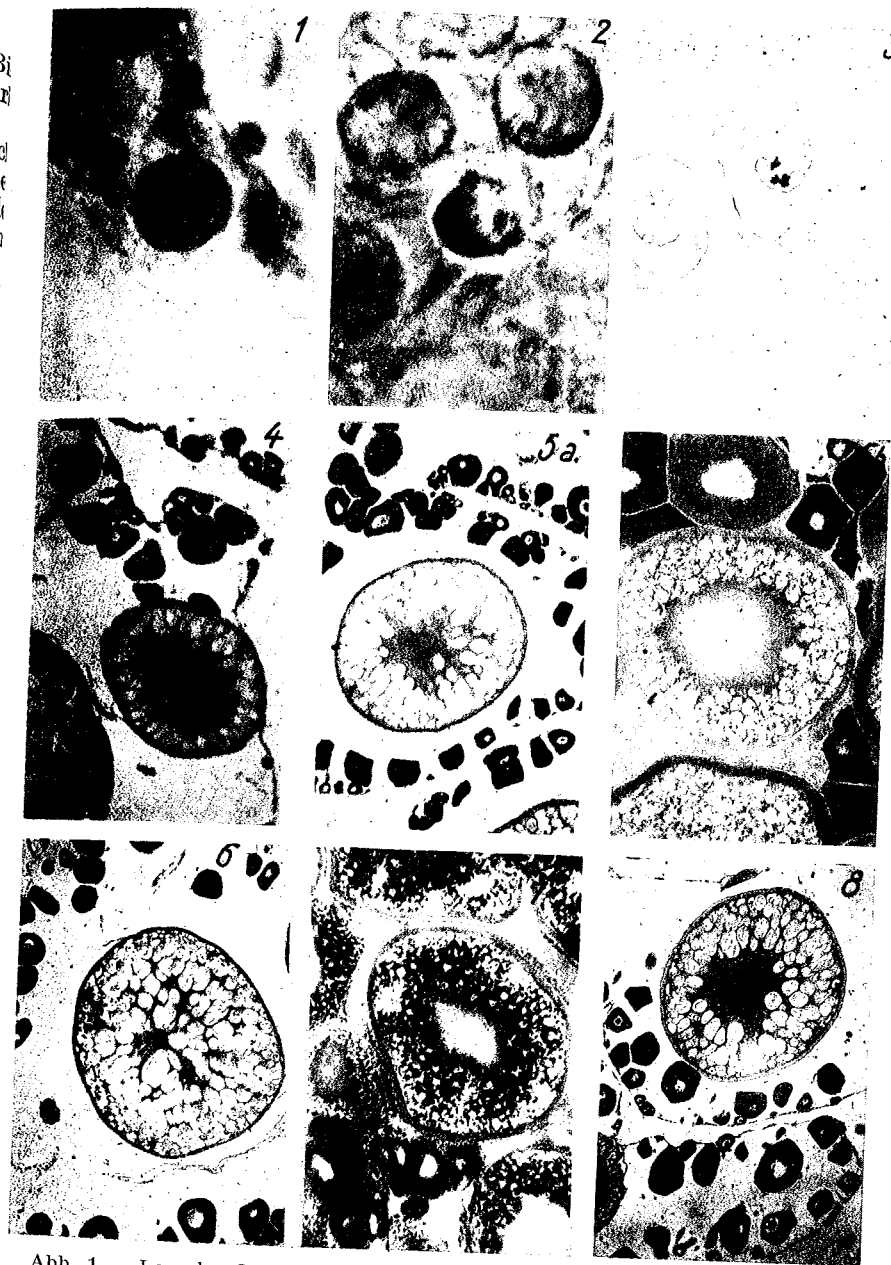
Abb. 1. — Larvales Ovar von *Cyprinus carpio* kompakter Kern einer Oogonie. Feulgen.

Abb. 2. — Junges Ovar von *Cyprinus carpio*, Kern in meiotischer Prophase mit Kappe. Feulgen.

Abb. 3. — *Cyprinus carpio*. Thymidin-³H.

Abb. 4. — *Cyprinus carpio*. Hydrolyse TCS. Fastgreen FCF.

Abb. 5. — *Cyprinus carpio*. a, Hydrolyse TCS. Bromphenolblau. b, Hydrolyse Pikrinsäure. Bromphenolblau.

Abb. 6. — *Cyprinus carpio*. Hydrolyse TCS. Fastgreen FCF-Eosin.

Abb. 7. — *Cyprinus carpio*. Ammoniakalische Silberlösung.

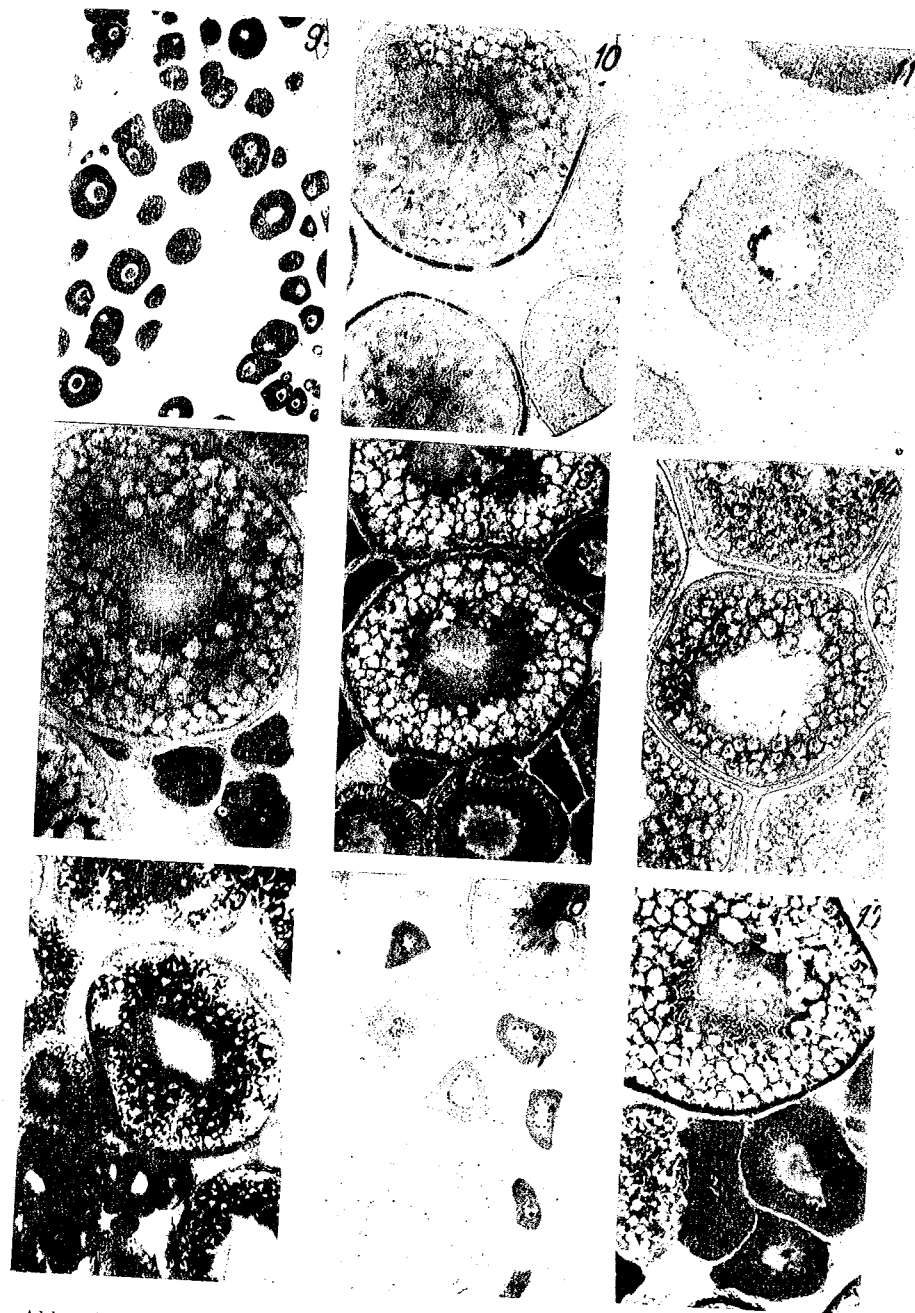Abb. 8. — *Cyprinus carpio*. Solochromcyanin.

Abb. 9. — *Cyprinus carpio*. Biebrich scarlett.
Abb. 10. — *Cyprinus carpio*. Amidoschwarz 10 B.
Abb. 11. — *Cyprinus carpio*. Thymidin — ^{3}H.
Abb. 12. — *Carassius auratus*. Hydrolyse TCS. Bromphenolblau.
Abb. 13. — *Carassius auratus*. Hydrolyse TCS. Fastgreen-Eosin.
Abb. 14. — *Carassius auratus*. Verdauung mit DN-ase. Eosin.
Abb. 15. — *Carassius auratus*. Ammoniakalische Silberlösung.
Abb. 16. — *Carassius auratus*. Amidoschwarz 10 B.
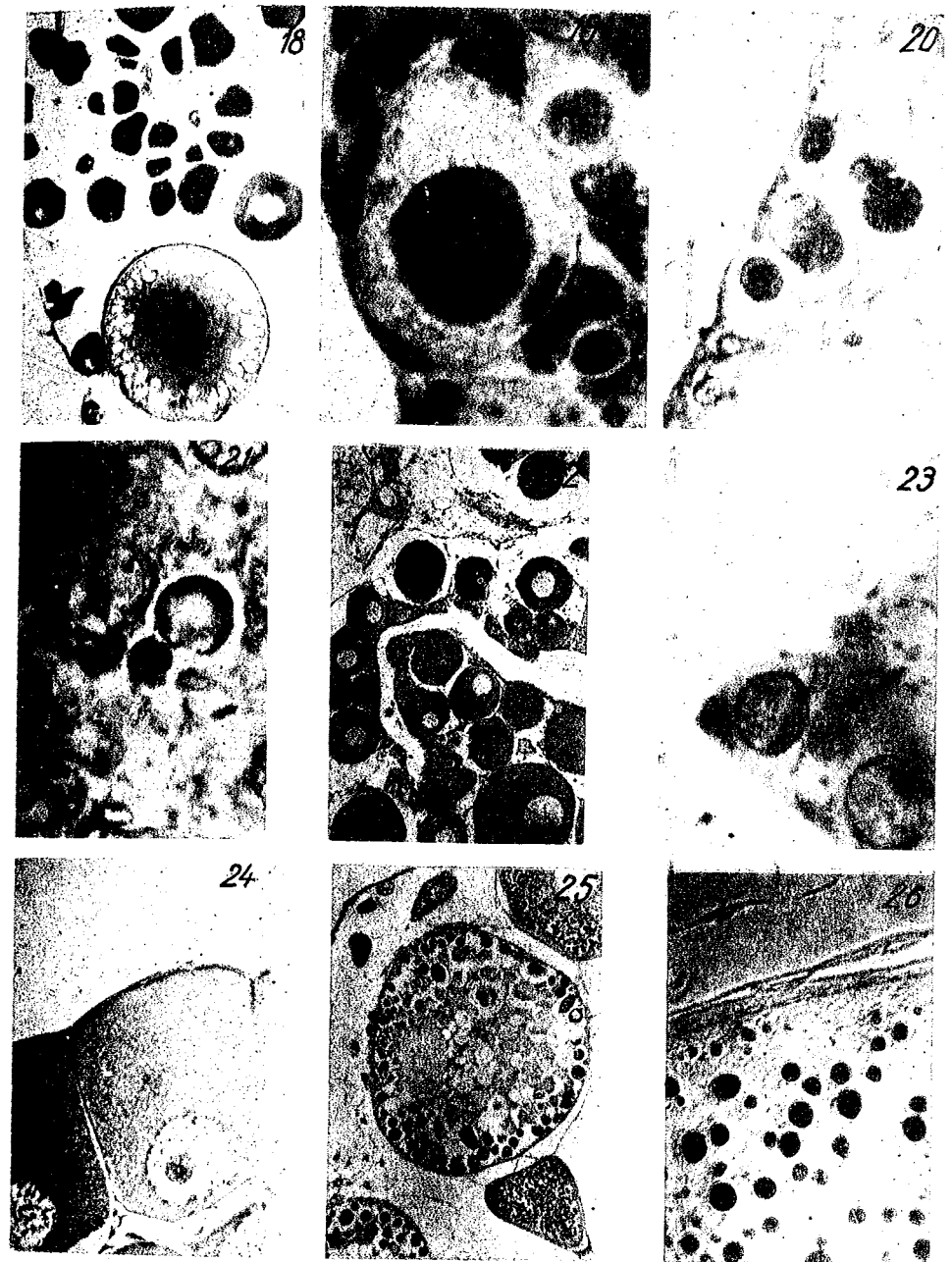Abb. 17. — *Carassius auratus*. Solochromcyanin.

Abb. 18. — *Carassius auratus*. DDD für-SH-Radikale.
Abb. 19. — *Acipenser ruthenus*. Hydrolyse TCS. Schiff-Fastgreen FCF.
Abb. 20. — *Ctenopharyngodon idella*. Schiff.
Abb. 21. — *Mylopharyngodon piceus*. Hydrolyse TCS. Schiff-Fastgreen FCF.
Abb. 22. — *Acipenser stellatus*. Verdauung mit DN-ase. Hämatoxylin Fullmer.
Abb. 23. — *Acipenser ruthenus*. Hydrolyse TCS-Schiff. Fastgreen FCF.
Abb. 24. — *Eudontomyzon danfordi*. Hydrolyse TCS. Fastgreen FCF.
Abb. 25. — *Ophidion roheii*. Hydrolyse TCS. Fastgreen FCF.
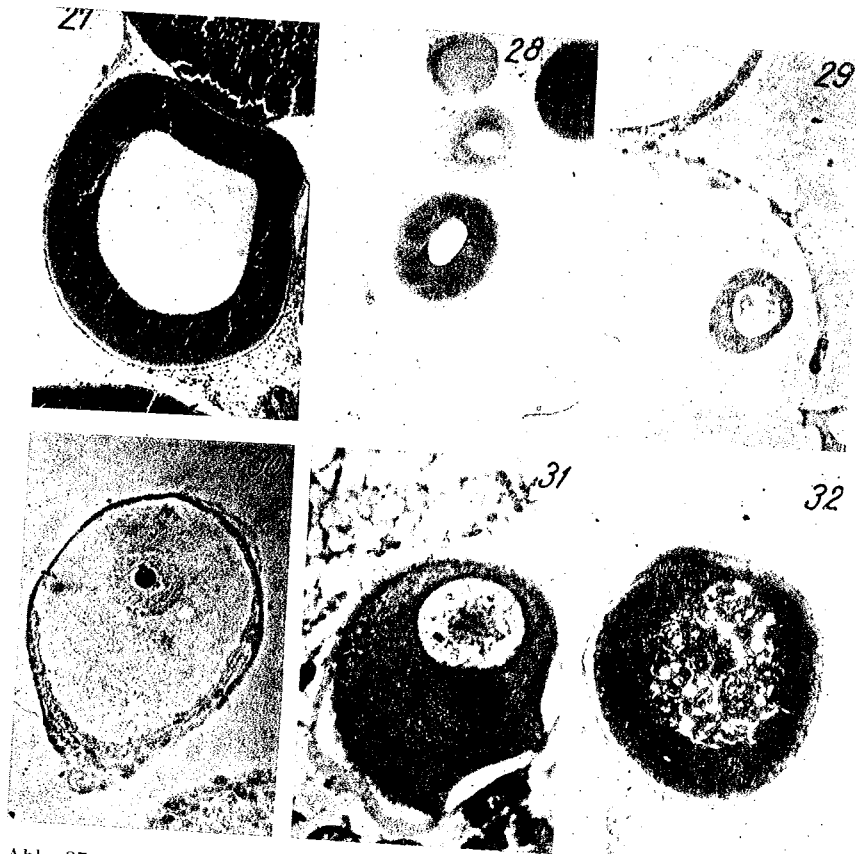Abb. 26. — *Emys orbicularis*. Hydrolyse TCS. Fastgreen FCF.

Abb. 27. — *Lacerta muralis*. Hydrolyse TCS. Bromphenolblau.
Abb. 28. — *Salamandra salamandra maculosa*. Hydrolyse TCS. Fastgreen FCF.
Abb. 29. — *Cardium edule*. Hydrolyse TCS. Bromphenolblau.
Abb. 30. — *Eledone moschata*. Hydrolyse TCS. Bromphenolblau.
Abb. 31. — *Tagenaria domestica*. Hydrolyse TCS. Bromphenolblau.
Abb. 32. — *Gammarus locusta*. Hydrolyse TCS. Bromphenolblau.

Abb. 33. — Nukleolus im Nukleus der Oozyte von *Carassius auratus*. Ammoniakalische Silberlösung × 60 000.

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CAROTENOIDS IN THE PUPA STAGE
OF *LEPTINOTARSA DECEMLINEATA* SAY

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The carotenoid pigments from the larvae of *Leptinotarsa decemlineata* Say were analysed and determined statistically at the moment of pupation and after three days of pupation.

It was ascertained that at the moment of pupation, the larvae possess a series of oxygenated carotenoid pigments such as : echinenone, hydroxy-echinenone, canthaxanthin, lutein, isolutein, and two hydroxy-β-carotene ? and also two hydroxy-keto-β-carotene forms.

After three days of pupation, besides the mentioned forms there exist yet another two new forms of hydroxy-keto-β-carotene.

The quantitative variations of some of these pigments are discussed.

The study of carotenoids in various invertebrates and particularly in the Crustacea is recorded in several interesting works. In the insects the study of these substances has, however, been more limited [3], [4], [5], [9], [15]. One of the investigated insects is the doryphorus (*Leptinotarsa decemlineata* Say). Manunta and Moritu [13] and Merlini and Cardillo [15] have analysed carotenoids in the adult insect, revealing-β-carotene, α-carotene, β-zeaxanthin, echinenone, canthaxanthin and lutein. Account being taken of the fact that neither echinenone nor canthaxanthin do occur in Solanacea [14], the authors draw the conclusion that these two pigments are intermediary metabolic products of β-carotene in insects.

As far as we know, no other studies have been carried out on carotenoid substances in the Colorado beetle, especially as concerns the investigation of the embryonic and postembryonic stages.

Our works concerning these two development stages will be gradually published. We shall first report the analysis of carotenoids existing in prepupating larvae as compared to those existing in the animal three days after pupation.

MATERIAL AND METHOD

Stage IV larvae, brought from field, were kept in terrariums till they recurved on the ventral part. Gathered immediately and kept in absolute ethanol at 4°C they formed the group of *pupae at the moment of pupation*. Part of the larvae pupating everyday were put in absolute ethanol in the third day, making up the group of *pupae after three days of pupation*. A number of pupae, removed together with a small quantity of ethanol, were filtered in a vacuum pump and then dried on a filter paper, always for the same time interval. The filtered ethanol, including a trace of dissolved pigment, was introduced into the separation funnel and the total carotenoid extract was then added. Out of dried up and weighed larvae, carotenoids were exhaustively extracted with absolute ethanol and the extract was filtered through a fast filtrating filter. Pigment traces on the filter were recovered by washing with absolute ethanol.

The carotenoid extract was saponified with 60% aqueous KOH [6, p. 498] overnight in an argon atmosphere, at room temperature and in the dark. All operations (pigment extraction, chromatographs) were carried out, as far as possible, in the dark.

The insaponifiable material transferred in light petroleum, b.p. 40—60°, after washing and drying on anhydrous Na_2SO_4 was separated into epi- and hypophase, washed again, dried and chromatographed on an Al_2O_3 Brockman u.c.b. No. 1124 column, activated for 2 hours at 110°C.

For development, solutions of light petroleum-acetone were used in various proportions and the purification of isolated pigments was obtained by their rechromatography on the same alumina.

The quantitative determination of each pigment was made according to Bonaly's method [1]. Account being taken of the volume of the total pigment extract and of the weight of pupae involved in an analysis, the amount of the total carotenoid was calculated in $\mu g/g$ fresh tissue, according to the formula: $C = \dfrac{10D}{E} \cdot V \cdot \dfrac{1000}{P}$ [1], where D is the optical density at the maximum main wavelength of each chromatographically isolated pigment; E is $E_{1cm}^{1\%}$ to which the arbitrary value of 2,500 was given [12]. All the determinations of absorption maxima, as well as the recording of the curve shape were carried out at 20°C on a Beckman D.B. spectrophotometer.

Pigment characterization was carried out by determining the partition coefficient [17] of every pigment.

The presence of ketonic groups was proved by BH_4Na treatment of the alcoholic solution [10] for 3 hours at room temperature. The reaction was stopped by the transfer of the pigment to light petroleum, washing and drying, after which it was read in the spectrophotometer. Some pigments were also recorded in I.R. at the Spectrophotometer UNICAM S.P. 200.

Part of the pigments were chromatographed in thin layer of MgO and developed with neophaline-chlorophorm-ethanol : 50 :45 :5. This solution diluted with neophaline, half or three quarters, represented two other development media.

RESULTS

The epiphasic fraction I (E_1) is eluted on Al_2O_3 column with 99/1 light petroleum-acetone. After purification, there appear 4 pigments, *the first* of which, E_1a_1, is not maintained on the column (Fig. 1). Its absorp-
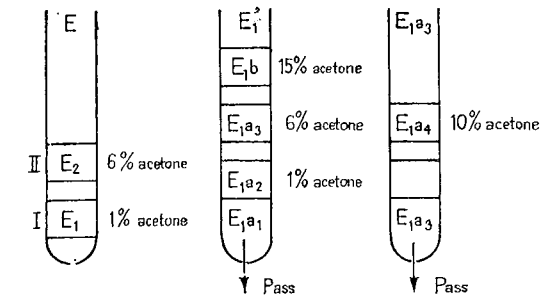
The epiphasic pigments isolation by column chromatography

Fig. 1

tion maxima are : 468(71)—443(4) mμ in light petroleum ; 488—464 mμ in benzene and 477—451 mμ in ethanol. The curve shape and the U.V. registering indicate that it is "all trans". After thin-layer chromatography it has $R_f = 0.979$. It is altogether epiphasic before and after saponification.

The second pigment (E_1a_2) (Fig. 1) is eluted from the purification column with 99/1 light petroleum-acetone. It is "all trans" and the shape of its curve (Fig. 2) is the same as that of E_1a_1. The absorption maxima are : 478—448 mμ in light petroleum, with a small shoulder at 425 mμ, 475 — 467 mμ in benzene and 481—453 mμ in ethanol.

Thin-layer chromatography yields $R_f = 0.98$.

The records of both pigments in I.R. point to an ester structure (Fig. 3) with the carbonyl stretching frequency at 1,720 cm^{-1}. Resaponified 24 hours at room temperature with methanolic KOH 15% and in a new I. R. record (Fig. 4), the pigments appear still esterified. These data make us think of a structure "ab origine" of a hydroxylated derivative of β-carotene (monoxydroxy- β-carotene ?). Further analyses, more detailed, are going to determine the structure of these two pigments.
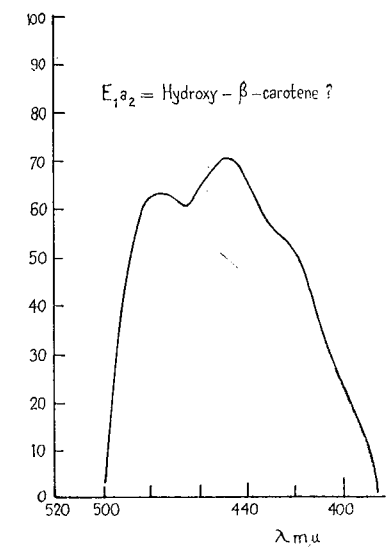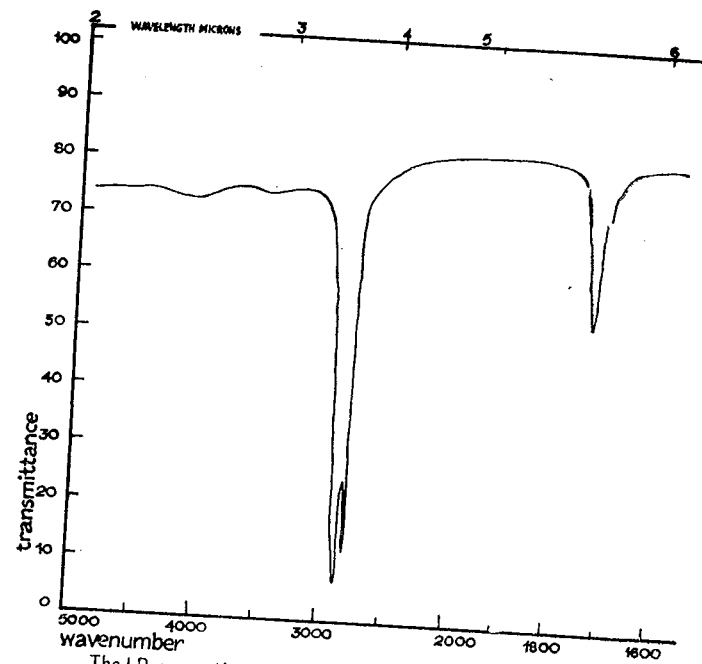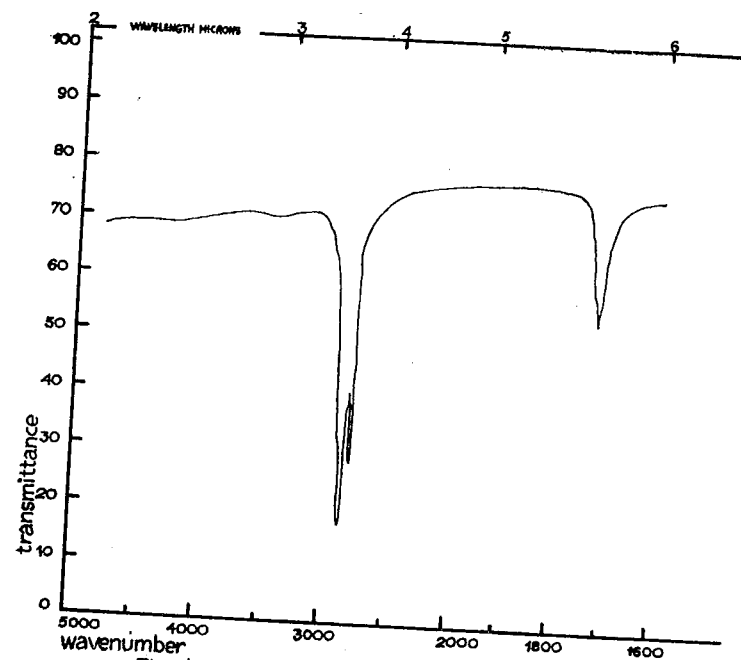

Fig. 2

The I.R. recording of the saponified pigment E_1a_2

Fig. 3

The I.R. recording of the resaponified pigment E_1a_2

Fig. 4

The third pigment, E_1a_3, is eluted with 94/6 light petroleum-acetone. After purification, there appear two pigments. The first, $E_1a_{3'}$, passes the column without being kept. The absorption maxima in light petroleum is 458 mμ [6, p. 518], in benzene = 476 mμ and in ethanol = 466 mμ. The curve shape is a little asymmetrical (Fig. 5). It is "all trans" and completely epiphasic, before and after saponification. After BH_4Na treatment, the solution turns from orange into yellow and the appeared pigment has the absorption maxima = 476—448 mμ in light petroleum, characteristic of isocryptoxanthin (Fig. 5).

Absorption maxima in different solvents, the curve shape, the behaviour on the column and its transformation, after BH_4Na treatment, into a pigment with a finer structure point to the presence of a monoketonic pigment, the *echinenone*.

The second pigment, E_1a_4, is eluted from the purification column with 90/10 light petroleum-acetone. The partition coefficient is 60/40 before and 52/48 after saponification. The absorption maxima are 466 mμ in light petroleum [6, p. 518], 482 mμ in benzene and 477 mμ in ethanol. The curve shape is a little asymmetrical (Fig. 6). The absorption maxima point to a diketo-carotenoid. Reduction with BH_4Na yields a pigment with absorption maxima at 477—451 mμ and a shoulder at 426 mμ in light petroleum (Fig. 6). Hence, the pigment is a diketo-carotenoid. Thin-layer chromatography against genuine *canthaxanthin*, yields the same R_f.
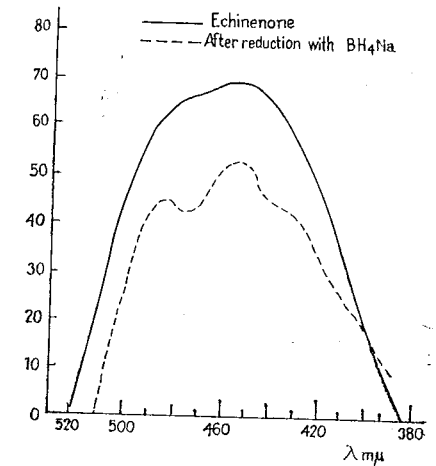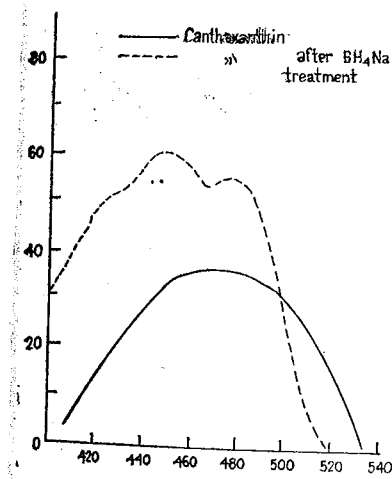
The fourth pigment, E_1b, eluted with 85/15 light petroleum-acetone, is a mono-hydroxy-monoketo-β-carotene. The absorption maxima are 455 mμ in light petroleum and 468 mμ in ethanol. The partition coefficient is 70/30 before and 71/29 after saponification. The asymmetrical shape of the curve (Fig. 7), as well as the absorption maxima suggest the existence of a ketonic group in the molecule. Treatment with BH_4Na yields a pigment with absorption maxima = 476—448 mμ and a shoulder at 427 mμ. It remains to determine whether the pigment is a 3-OH-4'-keto-β-carotene [16] or the unknown Herring keto-carotenoid [8].

Fig. 5

Fig. 6

The epiphasic fraction II is eluted with 94/6 light petroleum-acetone (Fig. 1). It is made up of a monohydroxy-keto-carotenoid, with absorption maxima = 453 mμ in light petroleum and 455 mμ in ethanol. After treatment with BH_4Na, the pigment has absorption maxima = 476—4? mμ in light petroleum. It is probably another 4-OH-4′-keto-carotenoid [11], [12]. The pigment has to be determined.

On the first alumina column there remains a trace of pink pigment which is dissolved in 5% acetic acid in acetone, but which, because of its very small amount, was not worked upon.

The hypophase also contains a series of pigments, some of which are part of the pigments discovered in epiphase: canthaxanthin, mono hydroxy-monoketo-β-carotene pigments, with absorption maxima at 455 mμ and 453 mμ, respectively.

Fraction I contains the pigment H_1, eluted with 98/2 light petroleum-acetone, but its quantity is too small to be analysed.
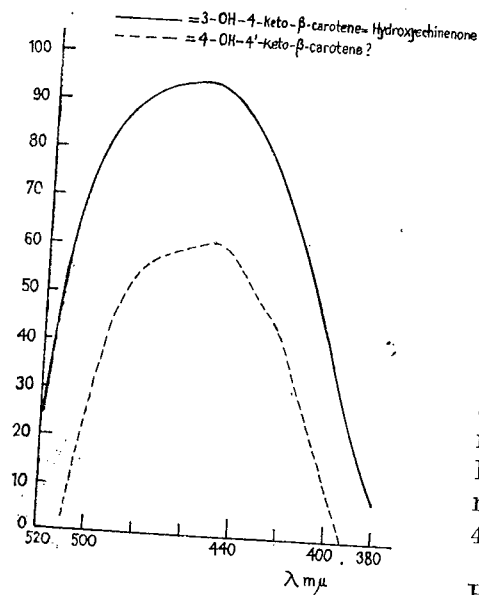

Fig. 7

Fraction II (Fig. 8) is eluted from a column with 90/10 light petroleum-acetone. At purification, it releases two pigments, *the first* of which, H_2a, is not retained by activated alumina. Its absorption maxima are 460 mμ in light petroleum, 477 mμ in benzene and 468 mμ in ethanol. The symmetrical curve shape (Fig. 7), the absorption maxima and the column behaviour point to a hydroxy-keto-carotene. The BH_4Na reduction transforms it into a pigment with absorption maxima of 474—444 mμ in light petroleum, pointing to the change of the ketonic group into a hydroxyl group. Hence, the pigment, H_2a, is a hydroxy-keto-carotene, probably 3-OH-4-keto-α-carotene, the *hydroxyechinenone*.

The second eluted pigment with 90/10 light petroleum-acetone, H_2a, is the *canthaxanthin*, whose characteristics were already stated.

Fraction III, eluted with 50/50 light petroleum-acetone, yields two pigments by purification. *The first*, H_3a, passes through activated alumina. It is 14/86 hypophasic before saponification and 62/48 after it. Absorption maxima = 473—442—422 mμ in light petroleum, 485—457 mμ in benzene and 473—445—422 mμ in ethanol. Thin-layer chromatography with genuine lutein yields the same R_f.

The second pigment of fraction III, H_3a_1, is probably the *isolutein*. It has an identical curve shape with that of pigment H_3a (Fig. 9). It is hypophasic before and after saponification, and more polar than the first, getting dissolved in 30/70 light petroleum-acetone. Absorption maxima =

470—440 mμ in light petroleum and 485—457 mμ in benzene. Thin-layer chromatography yields an R_f of 0.63 as against genuine lutein which is 0.93.
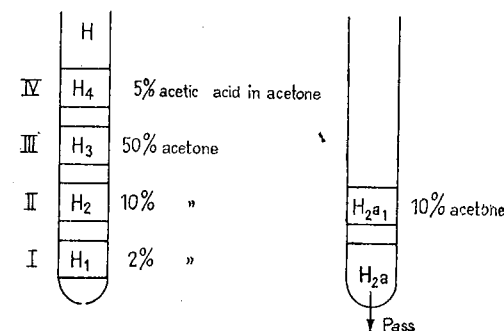
The hypophasic pigments isolation by column chromatography

Fig. 8

I. R. pigment recording points out that the pigment is esterified. The main column maintains a mixture of astacine and oxydation products. Asthaxanthin should be studied separately by an adequate method.

The totality of these pigments found in freshly pupated larvae is also met in three-day-pupated ones. Two other forms also appear which are hydroxy-keto-carotenoids. One is epiphasic, soluble in 80/20 light petroleum-acetone, with an asymmetrical shape and an absorption maximum=444 mμ, with a shoulder at 474 mμ; the other is hypophasic, with an absorption maximum at 453 mμ in light petroleum and with a shape pointing to a hydroxy-keto-carotenoid. Both are reduced by BH_4Na, yielding pigments with absorption maxima of 469—456 mμ and 477—449 mμ, respectively, in ethanol 95°.

DISCUSSIONS

We aimed at following up the evolution of carotenoid pigments in a life time altogether different from that of mature insects. We have chosen the pupation period when the animal is starving and completely immobile. Out of a series of analyses we selected only data referring to
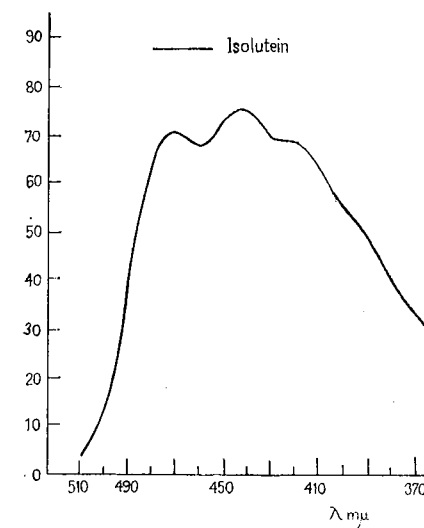

Fig. 9

precisely identified pigments. The amount of these pigments was statist[ically] ally calculated.

The per cent values of variability coefficients are certainly high[er] than expected, but this may be accounted for by the unavoidable defi- ciency in collecting the working material. Actually, larvae pupati[ng] in the evening or at night were more advanced than those pupating [in] the day time which were immediately preserved. Since the metabolis[m] of nymphosis has an amazing speed and complexity, the difference of[...] few hours, sometimes even 10, has a great importance and accounts f[or] the great variations of the obtained values.

Our data point, first of all, to the fact that the larva of this inse[ct] is supplied, at the moment of pupation, with more or less oxidate[d] carotenoid forms. Certain monoketo and diketo forms prevail quantit[a-] tively (Table 1). In the 72 hours elapsed since pupation, there occurre[d] complete destruction of some larval tissues and recovery of the tissue[s] typical of the imago — hence extremely complex and intense molecula[r] changes. During those 72 hours we also noticed that carotenoid substance[s] present a metabolism of their own, revealed by the disappearance o[f] almost half of the hydroxyechinenone. Echinenone presents no signifi[-] cant variation, the same as canthaxanthin. In turn, it is surprising tha[t] the pigments E_1a_1 and E_1a_2 significantly increase (Table 1).

Table 1

Carotenoid pigments from pupae of *Leptinotarsa decemlineata* Say (in μg/g wet tissue)

Pigment	Arithmetical mean		Difference	Significance
Hydroxy-β-carotene? 468—444 mμ	At the moment of pupation	$\bar{x} = 1.34$	— 2.39	p > 0.01 < 0.002
	After three days of pupation	$\bar{x} = 3.73$		
Hydroxy-β-carotene? 478—448 mμ	At the moment of pupation	$\bar{x} = 29.21$	— 9.89	p > 0.05 < 0.02
	After three days of pupation	$\bar{x} = 38.35$		
Echinenone 458 mμ	At the moment of pupation	$\bar{x} = 34.32$	— 3.69	p > 0.25 < 0.1
	After three days of pupation	$\bar{x} = 38.01$		
Hydroxy-echinenone 460 mμ	At the moment of pupation	$\bar{x} = 31.40$	+14.43	p < 0.001
	After three days of pupation	$\bar{x} = 16.97$		
Canthaxanthin 466 mμ	At the moment of pupation	$\bar{x} = 35.34$	+ 2.91	p > 0.05 < 0.02
	After three days of pupation	$\bar{x} = 32.43$		
Lutein 473—442—425 mμ	At the moment of pupation	$\bar{x} = 3.48$	+ 1.207	p < 0.5
	After three days of pupation	$\bar{x} = 2.27$		
Isolutein 470—440—425 mμ	At the moment of pupation	$\bar{x} = 2.52$	+ 0.912	p < 0.5
	After three days of pupation	$\bar{x} = 3.43$		

If β-carotene changes into asthaxanthin or only into canthaxanthin the pathway of a hydroxy-β-carotene [2] or a monohydroxy—monoketo-carotene [8], in the case studied by us both forms exist in the pupa of [th]at insect. These pigments could, therefore, be the synthesis source of [th]e most oxygenated form, canthaxanthin or asthaxanthin, or both.

It is interesting that lutein existing at the moment of pupation [pr]obably passes into a more oxygenated form — isolutein, since three [da]ys after pupation it decreases, while isolutein increases. Both processes [ar]e non-significant, yet they point to the direction of the metabolism of [th]is substance.

Trying to account for the significant increase of the two forms of E_1a_1 and E_1a_2, we suggest as a possible cause the long conservation period [of] the material. If three months elapsed between collecting and analysis, [th]e more labile carotenoid forms existing at pupation, as against those [ex]isting at three days of age, could be easily destroyed.

Yet, another question arises : if these carotenoid molecules make up [an] oxydo-reducing system [18], [19], [20], is it not possible that the trans- [fo]rmation reactions between carotenoids follow an opposite path, when [al]l requirements ask for it ?

Almost half of the hydroxyechinenone amount disappears, being [no]t justified by a corresponding canthaxanthin increase. On the contrary, [a]s slight, non-significant decrease may be recorded, pointing to the fact [th]at it can be used. If hydroxyechinenone molecules which disappeared [ar]e only partly the source of the maintenance of a certain canthaxanthin [am]ount, is it not possible that another amount of hydroxyechinenone [ac]count for the E_1a_1 and E_1a_2 forms? This simple supposition must be [v]erified experimentally by further studies.

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RESEARCHES CONCERNING THE ACTIVATION OF THE GLUCOSE UTILIZATION SYSTEMS IN THE CARP (*CYPRINUS CARPIO* L.)

BY

N. ŞANTA and I. MOTELICA-HEINO

It was found that during the digestive resting period — at the end of autumn and at the beginning of winter — the culture carp can digest food given by force and can absorb that nourishment, but its metabolization is very slow. Repeating the forced feeding for several days, an efficient mobilization of the specific regulatory metabolic systems can be reached, thus obtaining a real training due to their plasticity. The metabolic training has a specific character for the food used in this purpose.

In our studies published earlier [4—9] [11] about glycemia in the culture carp and in the wild one, it was found that hyperglycemia provoked by handling and capturing these animals, as well as that of nourishment is stronger in the first case than in the second one. It was considered that the observed differences could be due, at least in part, to some distinction of the digestive and metabolic training because the wild carp uses only natural food usually poor in glucides, while the culture carp, besides that, receives a supplementary food (squash maize, crushed corn and sunflower groats) rich in nourishing substances, especially glucides. We have decided to verify the above mentioned hypothesis by doing the exposed studies in the present paper.

MATERIALS AND METHODS

The experiments were carried out in culture carps of the age of two summers having the body weight of 600—800 g. The fishes were collected from the Piscicultural Research Station of Nucet and from the farm of Dridu. Those from Nucet were of the Lausitz breed,

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while those from Dridu were half-breeds with wild males from Siut-Ghiol. The fishes we captured at the beginning of October and the experiments took place in November and December. After having been brought to the laboratory, the fishes were kept in basins with running water (temperature 7—15°C) and under starvation, with the exception of the experimental periods when they were given food.

The experimental groups were of 4—6 specimens each. Two series of experiments were performed : one on the carps which were given simultaneously a mixture of hexoses (1 g mixture/kg body weight : 0.33 g of glucose, fructose and galactose) and complex food ; the other series was on the carps which received an identical mixture of hexoses but which were fed for a week afterwards with different, more or less complex, sorts of food (amidon, albumen, sunflower oil). So the carps in the first series were not trained by food, while the ones in the second series has got a moderate training during several days of forced feeding. The food was introduced directly in the gut through the mouth with a proof-stick.

The food used was Amylum soluble (Serva, Heidelberg West Germany), Albumen sanguinis (Carlo Erba, Italy) and sunflower oil from the food trade.

In the first series of the experiments it was worked on 7 groups. Each fish received a mixture of hexoses (1 g/kg body weight) and complex food (1 g/kg body weight) as follows : group I unboiled amidin, group II boiled amidin, group III unboiled albumen, group IV boiled albumen, group V sunflower oil, group VI a mixture of unboiled food and group VII a mixture of boiled food.

In the second series there were 6 groups as follows : group I boiled amidin, group II glucose, group III boiled albumen, group IV sunflower oil, group V a mixture of boiled food and group VI a mixture of hexoses (the control group).

The used hexoses were d–glucose (Fluca, A. G.), fructose (Serva, Heidelberg) and d-galactose (Merck).

The control of glycemia was made at intervals of time, respectively 2(1), 5(2), 24(3), 48(4), 72(5), 96(6), 120(7), 144(8), 168(9), 192(10), 216(11) and 240(12) hours after giving the mixture of hexoses. The numbers in parantheses represent the intervals on the graphs.

Glycemia was determined by the Hagedorn-Jensen method.

The arithmetical mean of the individual values obtained in each group was computed and reported from the mean value found before giving the mixture of hexoses, which was considered to be equal to 100.

RESULTS

In the first experimental series when the mixture of hexoses was given immediately after complex food feeding it was found primarily that the hyperglycemic effects obtained in all 7 groups are of great amplitude and of long duration and that they differ considerably from one group to another (Fig. 1).

Besides this it is quite evident that, for the same food, the hyperglycemic effect is much more intense in the case food was given boiled than unboiled (compare groups I to II, III and IV, VI to VII). The phenomenon is specially evident in the case the mixture of hexoses was given simultaneously with albumen (groups III and IV). The hyperglycemia obtained is then due not only to the intake of hexoses but also to some other factors.
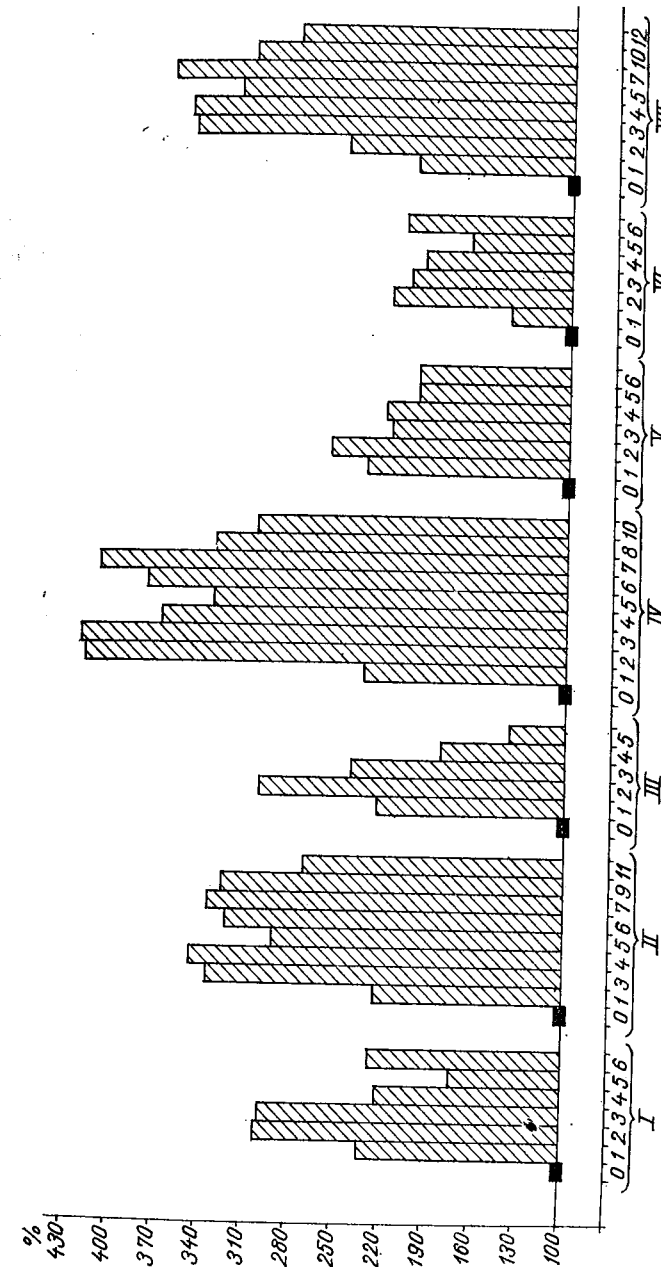

Fig. 1. — The mean glycemic values obtained in 7 groups of carps, after giving food and the mixture of hexoses. Group I, unboiled amidin ; group II, boiled amidin ; group III unboiled albumen ; group IV, boiled albumen ; group V, sunflower oil ; group VI, a mixture of unboiled food ; group VII, mixture of boiled food. Numbers 1 and 2 represent the obtained glycemic values after 2 and 5 hours and numbers 3 to 12 those after 1—10 days. The initial mean value (represented with 0) was considered equal with 100 and values obtained after are expressed as percentages of this latter.

In the second series, the results are completely different from above mentioned ones; the hyperglycemia obtained was of consider smaller amplitude, although only boiled food was used (Fig. 2).
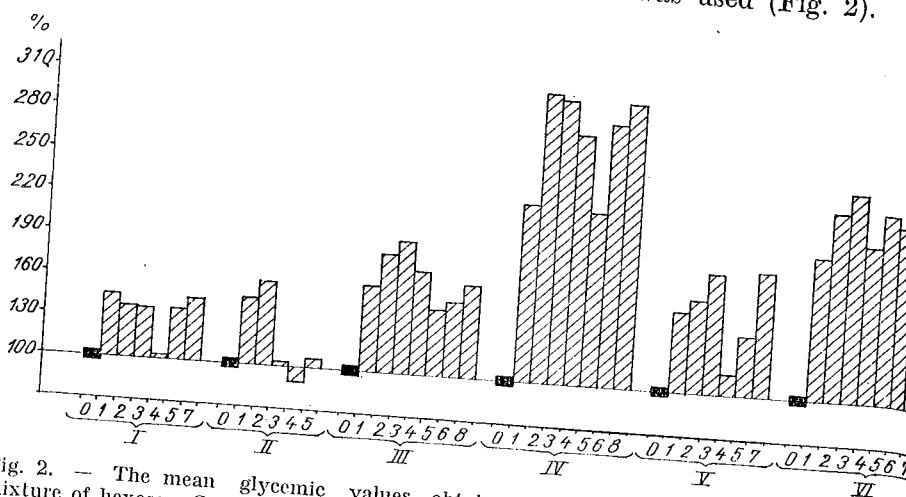

Fig. 2. — The mean glycemic values obtained in 6 groups of carps, after giving mixture of hexoses. Groups I—V were fed by force beforehand during one week with bo food, as follows: group I, boiled amidin; group II, glucose; group III, boiled albumen, gr IV, sunflower oil, group V, a mixture of boiled food, group VI, carps were kept in star tion. The other explanations as in figure 1.

The weakest effect was obtained in the carps which had receive beforehand daily some 10 doses of glucose (1 g/kg body weight/da (group II). The highest hyperglycemia, more extensive than in the cont group (group VI) was observed in group IV, that is, in the carps whi had been fed with sunflower oil.

It was also been found that glycemia of carps in group II return completely to the normal level after 24 hours and after 48 hours the appeared even a slight hypoglycemia.

DISCUSSION

From our results it can be seen that during the digestive restin period from the end of autumn and during winter, although they do n eat actively, the carps can digest a certain amount of food which is give by force to them. A sure proof of this fact is that, after forced nourishmen when giving the mixture of hexoses, very various hyperglycemic state from one group to the other are obtained, not only due to these latt but also to the nature of the given food. E.g. there is a great differenc in the effects produced by the mixture of hexoses between the specimen which were given amidin and those which were given oil. Amidin provoke greater hyperglycemia than sunflower oil.

It can also be seen that even in this time of the year the absorptio of hexoses is good and hyperglycemia has a great amplitude.

The metabolic activities bound with food transformation are uced and that is why provoked hyperglycemia is maintained at a level for a long time. Such a durable hyperglycemia state is possible ause the excretion of glucose is very small in these animals and ause their metabolism is very slow [7] [10] [12] [13].

As to the obtained results in the second series of the experiment, can be seen that a previous several-day period of feeding makes the cific metabolic activities to resume to use the respective sources. So obtained hyperglycemia in these cases is of a smaller amplitude and a shorter duration.

That is why the carps in the groups previously fed with amidin or h glucose (and especially with the latter) assimilate hexoses more ensely and quicklier than those fed with sunflower oil and even those with boiled albumen.

We think that the cause of these differences is the reappearance the internal medium of an increased quantity of glucose which provokes mobilization and the setting up of metabolic regulatory systems con cted with the processes of glucose utilisation. There is a discharge of cells from the endocrine pancreas which should be possible. Such an ect was shown in the rat [3] in which the perfusion of the isolated pan as with glucose solutions of different concentrations caused a rapid easing of insuline, proportional to the used doses. The actions of the her hexoses on the discharge of β cells seems to be much weaker (e.g. at of fructose is approximately of 1/3 from that of glucose) [1].

It is still unknown if phenomena occur in the same way in carps, t it seems that this can be possible. On the contrary, the studies done the endocrine pancreas of carps show that glucose is taken in rapidly β cells during the absence of insulin, so glucose could act on the scharge of the cells. This discharge can cause the resuming of the acti ties of biosynthesis of insulin which arrives, through endocellular self gulated mechanisms, to the intensification of insulin secretion and ally to a more efficient utilization of glucose than in the carps which in food resting conditions. There is also some morphological evidence om the Langerhans islands of the mobilizing effect of glucose on β cells. has been found that an intense and durable provoked hyperglycemia n cause, sooner or later, exhaustion of β cells and installation of dia tes [2] [14].

In normal conditions such an exhaustion is not reached; on the ntrary, as seen from our experiments, a good training in metabolic d secretory systems is obtained. As to the other functions, there is so in this case the possibility of an adequate physiological adaptation.

It is unknown if and how much supplementary food given to culture rp — during many generations — influence the metabolic process of ilizations of this food, but this possibility cannot be excluded.

CONCLUSIONS

1. During the digestive resting period from the end of autumn and eginning of winter, the culture carp can digest food given by force and an absorb that nourishment, but the metabolization is very slow.

2. Repeating the forced feeding for several days an efficient m
lization of specific metabolic regulatory systems and — due to
plasticity — a real training can be obtained.
3. The metabolic training has a specific character for the
used in this purpose.

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EFFECT OF TEMPERATURE ON THE ISOENZYMIC PATTERN OF LOACH (*MISGURNUS FOSSILIS* L.) I. GLUCOSE-6-PHOSPHATE DEHYDROGENASE, LACTATE DEHYDROGENASE, NAD- AND NADP-ISOCYTRATE DEHYDROGENASE

BY

RADU MEŞTER, DRAGOŞ SCRIPCARIU and STELIAN NICULESCU

Activity and isoenzymic pattern of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, NAD- and NADP-isocytrate dehydrogenase from muscle, liver, serum and hemolysate of loach adapted to 20°C and 0°C were determined by electrophoresis on polyacrylamide gel. In all tissues studied, from both cold- and warm-adapted fishes, the enzymes presented, generally, an enzymic activity (proportional to the staining intensity of bands) and an isoenzymic pattern unchanged, when the gels were incubated at 20°C. Incubation of the enzymes from cold- and warm-adapted fishes at 0°C showed that low temperature alters the enzymic reaction rate : the activity of some isoenzymes was completely abolished and that of the remaining ones was reduced, though they were found in tissues when determining their activity at a higher temperature. Data presented in this paper showed that low temperature induces a decrease of the enzymic activity as well as of the isoenzymic pattern of glycolytic enzymes in loach tissues, regardless of the adaptation temperature.

The action of temperature upon the different aspects of poikilotherms metabolism emphasized the importance of thermic homeostasis in adaptative mechanisms. Even though these last years many investigations were devoted to the problem of the metabolic processes regulation, the cellular mechanisms of fishes acclimation and adaptation (and of poikilotherms, in general) to low or high temperature are little known.

In fishes, metabolic compensations to environmental temperat... affect different aspects of the cellular metabolism. One of the me... nisms suggested with a view to obtain compensation during cold a... mation involves changes in the enzymic activity. Thus, in different spe... of fishes, concentration of some enzymes would appear to be increa... during cold acclimation: glucose-6-phosphate dehydrogenase from... gills of goldfish [6] and muscle of trout [12], fructose-1,6-diphosph... aldolase from gill, liver and muscle of golden orfs [14], lactate dehy... genase from liver of trout [10], [25]. Besides these, there are data sho... that glycolytic enzymes from muscle of *Idus idus* and *Rhodeus ama*... are little affected by the changes of the fishes acclimation temperat... [2], [16].

The importance of NAD-isocytrate dehydrogenase and NA... isocytrate dehydrogenase in the cellular metabolism of poikilotherm... very little known. Crabtree and Newsholme [3] demonstrated that... enzymic activity ratio of NAD- and NADP-isocytrate dehydroger... from muscle homogenates of many vertebrates is largely in favour... NADP-linked enzyme. In a recent paper, Moon and Hochachka ... studied the activity and some kinetic properties of NADP-isocytr... dehydrogenase from the liver of rainbow-trout acclimated to 2°C... 17°C. However, the relative roles of these dehydrogenases in cold accli... tion is little known.

Hochachka et al. [1], [13], [24], based on the study of Micha... constant (Km), emphasized the role of molecular mechanisms as a comp... sation factor in the adaptation of fishes to low temperatures. The auth... deem that one of the important factors in the adaptation to low tem... rature is the modification of the enzyme-substrate affinity.

Literature data concerning the changes in the enzymic activi... and the enzymic molecular mechanism under the influence of low temp... rature are scarce and controversial [13], [14], [20], [21], [24]. Th... correlation is difficult, due to the temperature limit of fish acclimatio... to the various responses depending on the species studied, to the org... specificity, and especially to the research methods.

In view to elucidate the mechanisms occurring in fishes duri... adaptation to low temperature, a study has been carried out on som... glycolytic enzymes of the liver, muscle, serum and hemolysate fro... loach, adapted to 0°C and 20°C, respectively.

MATERIAL AND METHODS

Experiments were carried out on loaches (*Misgurnus fossilis* L.), captured in Gr... pool, Olt district. In the laboratory, the fishes were divided into two groups, 7–10 spe... mens each; one group was adapted to the laboratory temperature (20—22°C), while the oth... group was acclimated to 0°C. For that purpose they had been initially adapted to an int... mediary temperature of 4°C (for two ³weeks) and then, for 30 days, to a temperature... 0°C. Neither fish group did receive any food during acclimation in the laboratory. It is... be pointed out that all fish specimens acclimated to low temperature (three groups), ha... well tolerated the influence of low temperature and no specimen died.

After the acclimation interval, both groups of fishes were killed. White muscle tissue ...s removed from the lateral parts of the body and homogenized in cold distilled water, one ...rt tissue and 3 parts water g./v. The liver was also homogenized in cold distilled water, ...e part tissue and 8 parts water g./v. For a more accurate cell destruction and enzyme extrac...on, homogenates were twice frozen and thawed. The supernatants of the two tissues, obtained ...ter centrifugation at 7000 g. for 30 min. from the two acclimated fish groups, were used for ...ectrophoresis. Blood was collected on heparin in 0.75% NaCl, from the caudal vein by tail ...tting. It was three times washed in 0.75% NaCl and, each time, centrifugated at 6000 g. ...r 20 min. The diluted serum and the hemolyzed erythrocytes subsequent to suspension in ...stilled water were used for electrophoresis.

Electrophoresis was performed in polyacrylamide gel, at a 7.5% concentration, accord...g to the method of Davis [4], disk-electrophoresis system. The tank buffer was 0.1 M tris-...ycine, pH 8.6; the gel buffer contained 0.1 M tris-HCl, pH 8.6. Electrophoresis of protein ...as run at 3 mA/tube for two hours.

In view to determine the enzymic activity of the electrophoretic fractions, the gels were ...rawn out from the tubes, washed in cold distilled water and then in 0.1 M tris-HCl buffer, ...H 7.4 for 30 min. under cold conditions. Gels were further incubated in buffers contain...g substrate adequate to the investigated enzyme. Each enzyme, from both cold- and ...arm-acclimated fish, was incubated in the specific substrate at 0°C and 20°C, respectively.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was determined according to Thul...ine et al. [26], lactate dehydrogenase (EC 1.1.1.27) according to Lush [18], while NAD- and ...NADP-isocytrate dehydrogenase (EC 1.1.1.41 and 1.1.1.42) according to Farber and Bueding ...7]. The incubation medium for the detection of NAD- and NADP-isocytrate dehydrogenase ...ontained 25 mg. % NAD, respectively NADP. Determination (staining) of enzymic activity ...as carried out on gels at 20°C and 0°C for 8—10 hours.

RESULTS

Electrophoretic study of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, NAD- and NADP-isocytrate dehydrogenase in mammals [17], [28] and fishes [10], [17], [19], [22], [28] revealed the presence of these dehydrogenases under many molecular forms. The data from literature show that the total enzymic activity and the isoenzymic pattern vary from one tissue to the other, from one species to the other, depending on their importance in the cellular metabolism.

Glucose-6-phosphate dehydrogenase. Results obtained by electrophoresis on polyacrylamide gel showed the existence of many molecular forms of the enzyme in the tissues investigated.

In muscle homogenates from both cold- and warm-acclimated fish, by incubating the gels at 20°C, four bands were revealed. At this temperature, the differences of enzymic activity and isoenzymic distribution of glucose-6-phosphate dehydrogenase in both fish groups are non significant. By incubation of the gels at 0°C, glucose-6-phosphate dehydrogenase appears with a very low enzymic activity and an altered isoenzymic pattern: two enzymic fractions with reduced enzymic activity may be distinguished in both fish groups and a very weak third one in the warm-adapted fishes. Activity differences are non significant (Fig. 1).

By incubation of the gels at 20°C, liver glucose-6-phosphate dehydrogenase appears, both in fishes adapted to low and higher temperature,

under the form of 5 bands with enzymic activity. When the enzymes are determined at 0°C, four isoenzymes with a lower staining activity are rendered evident (Fig. 1).

Important qualitative and quantitative changes in the enzyme content and in that of the isoenzymes of glucose-6-phosphate dehydrogenase are observed in the fish serum, by determining the enzyme at various temperatures. Electrophoresis of the serum of warm-adapted fishes by incubation at 20°C presents 5 isoenzymes with a medium enzymatic activity. The same homogenate, incubated at 0°C, reveals 2 bands with low enzymic activity. The electrophoretic pattern of the serum of cold-acclimated fishes undergoes the same alterations. By incubation at 0°C, an isoenzyme with reduced tinctorial activity emerges, while at 20°C, 4 fractions with a medium enzymic activity are revealed (Fig. 2).

The electrophoretic pattern of glucose-6-phosphate dehydrogenase of hemolysate in both fish groups is characterized by three enzyme fractions, irrespective of the temperature used for the enzyme determination. The staining intensity of the hemolysate isoenzymes is lowered by determination at low temperature (Fig. 2).

Lactate dehydrogenase. As it results from figures 3 and 4, there is a direct relationship between the concentration, the number of lactate dehydrogenase isoenzymes and the temperature of gels incubation. Alterations are similar to those occurred in glucose-6-phosphate dehydrogenase, but much more obvious.

By incubating the gels at 20°C, the electrophoresis of muscle homogenates reveals in both cold- and warm-adapted fishes four fractions with increased enzymic activity. By determining the enzymic activity at 0°C, only 2 isoenzymes with medium tinctorial activity appear in both fish groups (Fig. 3).

Lactate dehydrogenase from the liver of both fish groups presents 5 isoenzymes, determined at 20°C. The concentration of lactate dehydrogenase may be assessed in the two tissues acclimated to different temperatures, by the staining intensity of the enzymic fractions. Low temperature determines qualitative and quantitative lowering of lactate dehydrogenase in both liver homogenates : specific to the two electrophoretic patterns is the emergence of 3 isoenzymes with low and very low enzymic activity (Fig. 3).

The electrophoretic pattern of lactate dehydrogenase from the serum of both cold- and warm-adapted fishes is characterized by the presence of 4 enzymic fractions with similar staining activity, when the gels are incubated at 20°C. Low incubating temperature deeply changes qualitatively as well as quantitatively the isoenzymatic pattern : at 0°C, the serum of fishes acclimated at higher temperature presents 3 isoenzymes and that of cold-adapted fishes, a single isoenzyme (Fig. 4).

The hemolysate of both fish groups reveals, by electrophoresis on polyacrylamide gel, two fractions with increased activity, when the determination is made at 20°C. Incubation at low temperature shows only one enzymic fraction with a medium staining activity, regardless of the fish adaptation temperature (Fig. 4).

NAD-isocytrate dehydrogenase. Electrophoretic researches on polyacrylamide gel have revealed the presence of this enzyme in all four
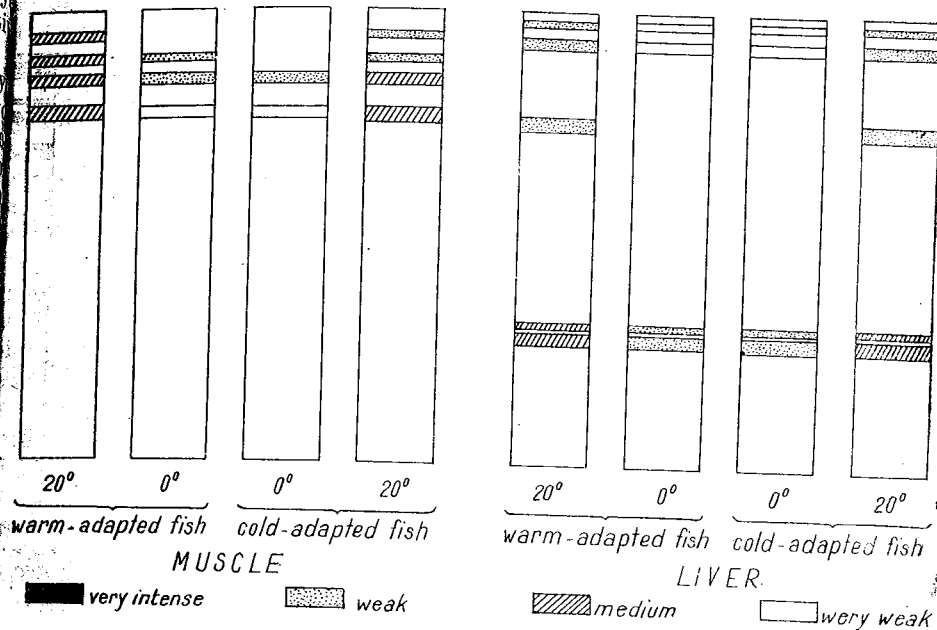

Fig. 1. — Isoenzymic pattern of glucose-6-phosphate dehydrogenase from muscle and liver, obtained by polyacrylamide gel. 20° and 0° indicate the temperature at which the gels were incubated by determining the enzymic activity.
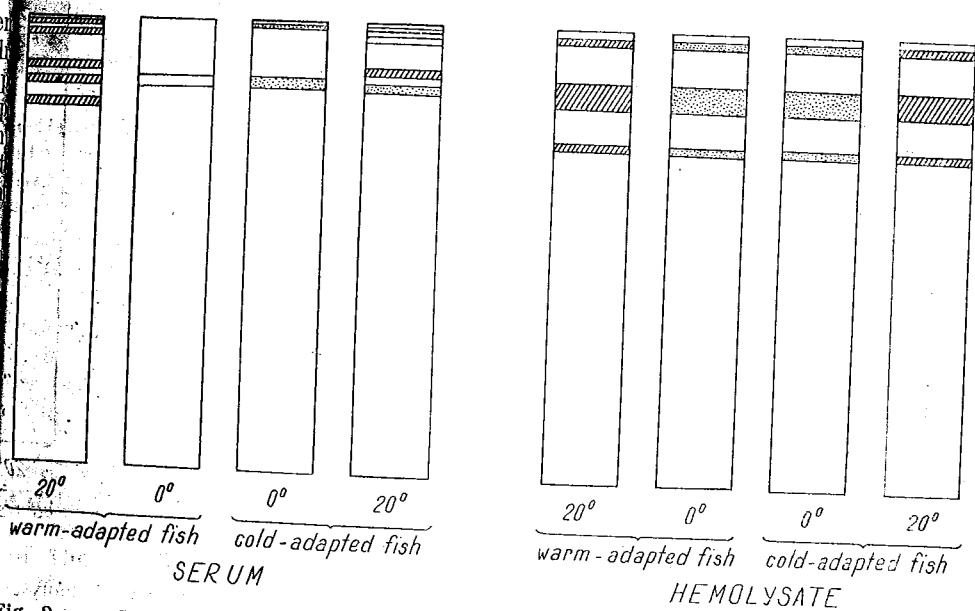

Fig. 2. — Isoenzymic pattern of glucose-6-phosphate dehydrogenase from serum and hemolysate, obtained by polyacrylamide gel. Explanation as in figure 1.
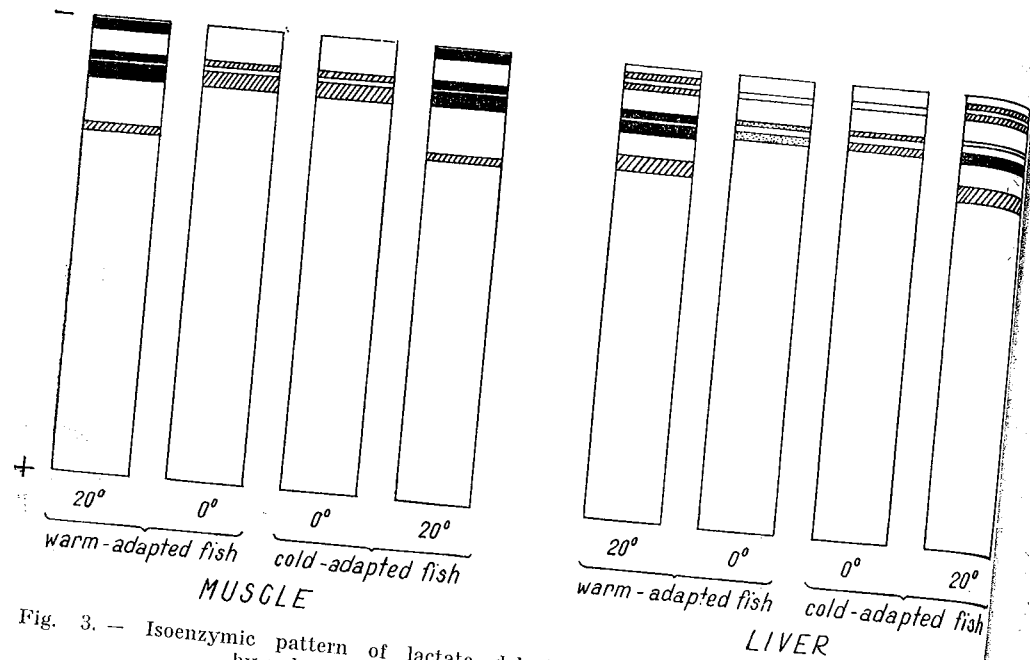
Fig. 3. — Isoenzymic pattern of lactate dehydrogenase from muscle and liver, obtained by polyacrylamide gel. Explanation as in figure 1.
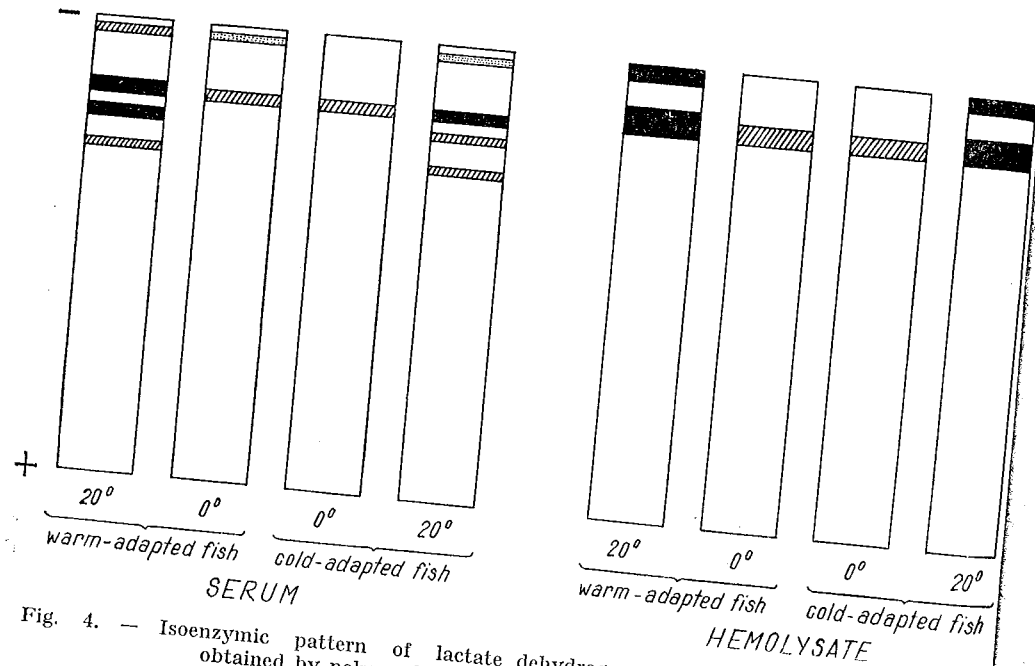

Fig. 4. — Isoenzymic pattern of lactate dehydrogenase from serum and hemolysate, obtained by polyacrylamide gel. Explanation as in figure 1.

tissues studied from loach. The electrophoregrams showed that the isoenzymes of the four tissues investigated present a low and very low enzymic activity.

In muscle homogenates from both cold- and warm-adapted fishes, by incubating the gels at 20°C, three bands with low enzymic activity were revealed. At 0°C, in both groups, two enzymic fractions with decreased staining activity were found (Fig. 5). The level of enzymic activity as well as the number of isoenzymes are not influenced by the fish acclimation temperature, but by the enzyme determination temperature.

NAD-isocytrate dehydrogenase from the liver appears in both groups under the form of 3 isoenzymes with decreased staining activity when determined at 20°C. The electrophoretic pattern of the enzyme of cold- and warm-adapted fishes reveals, by incubating with substrate at 0°C, two bands with a very low enzymic activity (Fig. 5).

The electrophoresis of serum and hemolysate present in both cold- and warm-acclimated fishes, a series of differentiations depending on the determination temperature. As shown in figure 6, when the gels are incubated at 20°C the enzyme from serum and hemolysate contains two distinct forms, regardless of the fish acclimation temperature. Determination of NAD-isocytrate dehydrogenase at 0°C did not reveal any enzymic activity band in the two tissues of both groups. Enzyme determination at this low temperature induced its inactivation in both serum and hemolysate, though the enzyme is present when staining the gel at a higher temperature (Fig. 6).

NADP-isocytrate dehydrogenase. Electrophoresis on polyacrylamide gel of loach tissue homogenates generally revealed several molecular forms of NADP-isocytrate dehydrogenase with an increased enzymic activity (proportional to the staining intensity of the bands) as compared with NAD-linked enzyme. Thus, the more active metabolic role of NADP-linked enzyme was noticed during metabolic processes in loach.

When incubating the gels at 20°C, the electrophoregrams of muscle extracts from both groups contained four enzymic fractions with decreased staining activity. When determined at 0°C, the muscle NADP-isocytrate dehydrogenase underwent a considerable decrease of the activity and an alteration of the enzymic pattern: the band with a higher electrophoretic mobility disappeared and the other three were hardly visible (Fig. 7).

NADP-isocytrate dehydrogenase from loach liver contains three isoenzymes when its activity is determined at 20°C, irrespective of the fish acclimation temperature. Enzyme determination at 0°C revealed in both groups a single isoenzyme having a very low staining activity (Fig. 7).

The electrophoretic pattern of enzyme of the serum from cold- and warm-acclimated fishes presents important qualitative and quantitative alterations. As it results from figure 8, the electrophoresis of serum from warm-adapted fishes reveals four enzymic fractions, regardless of the gel incubation temperature; yet, a decrease of the isoenzyme activity was noticed when their determination was made at a low temperature. When incubated at 0°C and 20°C, the serum of cold-adapted
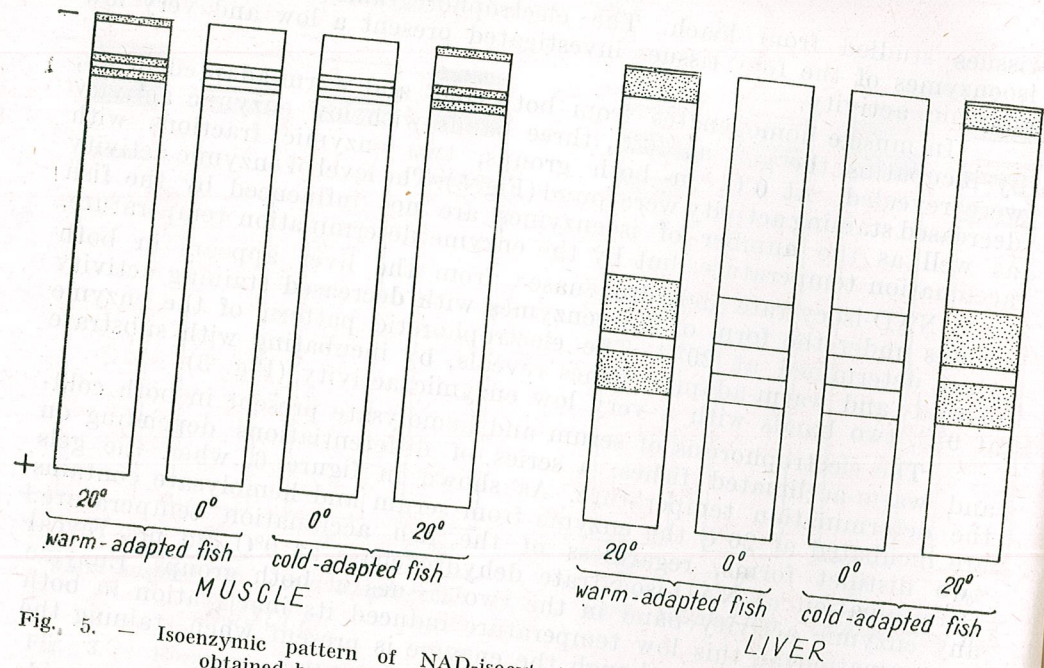
Fig. 5. — Isoenzymic pattern of NAD-isocytrate dehydrogenase from muscle and liver, obtained by polyacrylamide gel. Explanation as in figure 1.
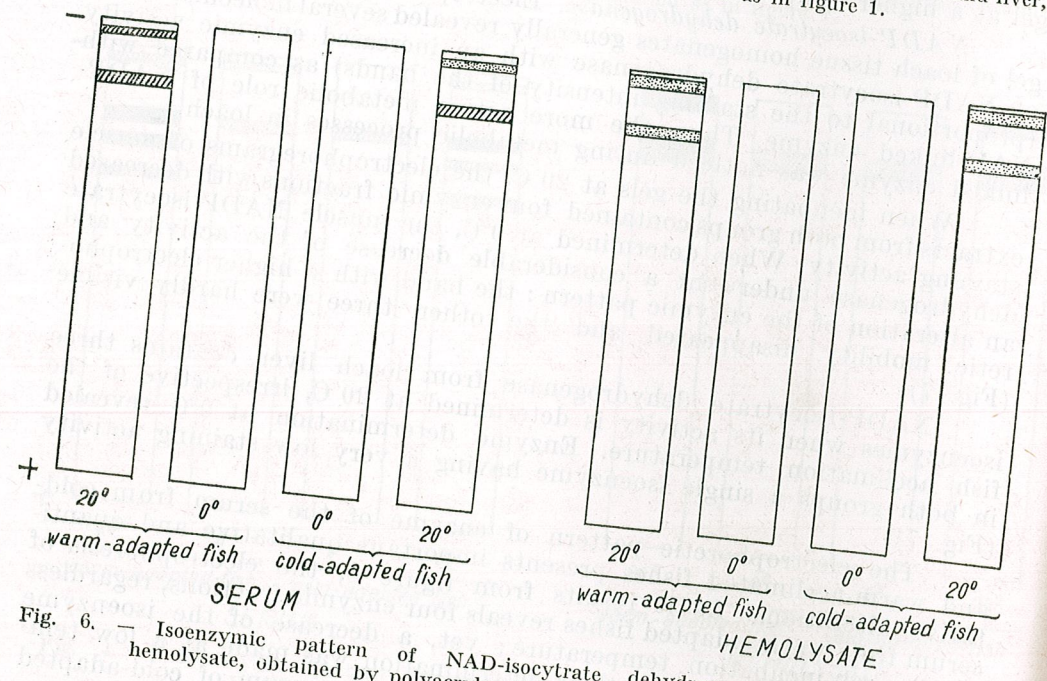
MUSCLE   LIVER

warm-adapted fish   cold-adapted fish   warm-adapted fish   cold-adapted fish

Fig. 7. — Isoenzymic pattern of NADP-isocytrate dehydrogenase from muscle and liver, obtained by polyacrylamide gel. Explanation as in figure 1.

MUSCLE   LIVER

warm-adapted fish   cold-adapted fish   warm-adapted fish   cold-adapted fish

Fig. 6. — Isoenzymic pattern of NAD-isocytrate dehydrogenase from serum and hemolysate, obtained by polyacrylamide gel. Explanation as in figure 1.
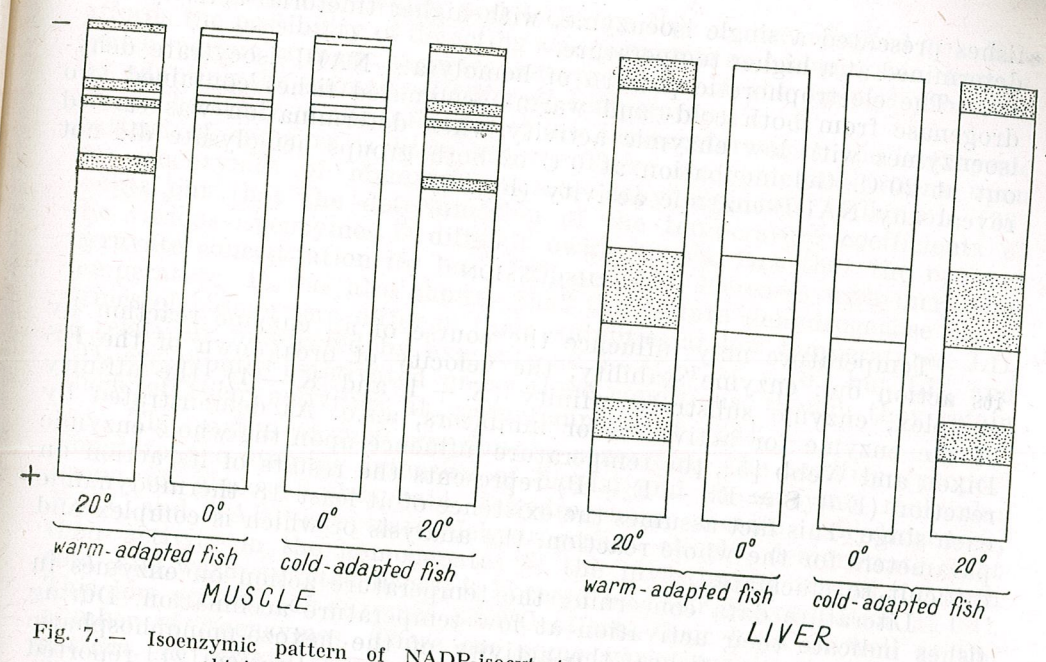
SERUM   HEMOLYSATE

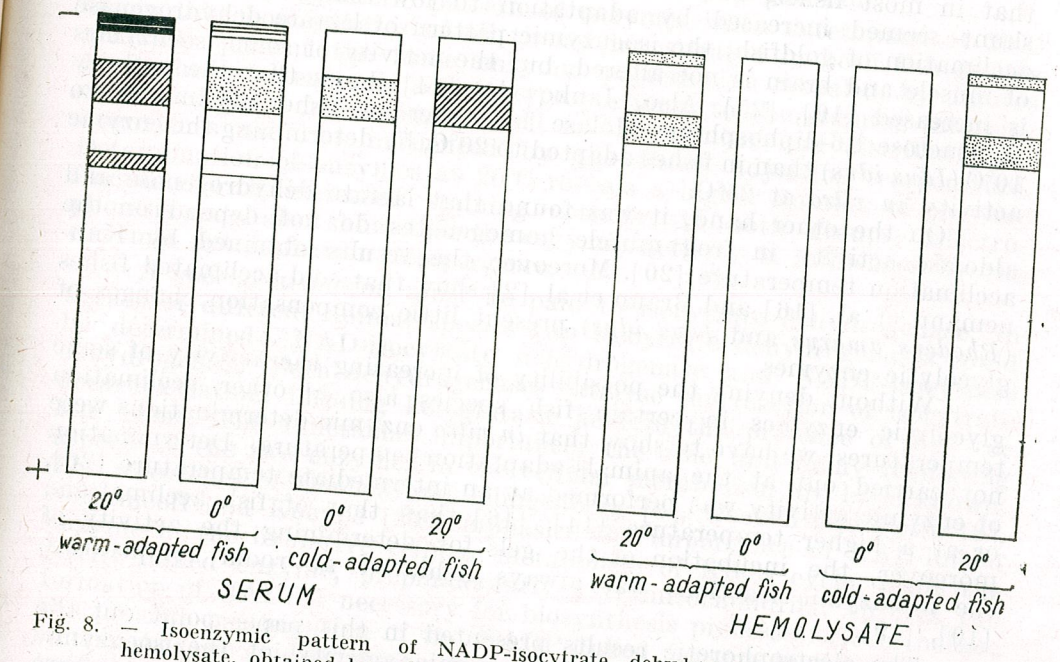warm-adapted fish   cold-adapted fish   warm-adapted fish   cold-adapted fish

Fig. 8. — Isoenzymic pattern of NADP-isocytrate dehydrogenase from serum and hemolysate, obtained by polyacrylamide gel. Explanation as in figure 1.

SERUM   HEMOLYSATE

warm-adapted fish   cold-adapted fish   warm-adapted fish   cold-adapted fish

fishes presented a single isoenzyme, with higher tinctorial activity when determined at a higher temperature.

The electrophoretic pattern of hemolysate NADP-isocytrate dehydrogenase from both cold- and warm- acclimated fishes contained two isoenzymes with low enzymic activity when determination was carried out at 20°C. Gel incubation at 0°C of both groups hemolysate did not reveal any NADP-enzymic activity (Fig. 8).

DISCUSSION

Temperature may influence the course of an enzyme reaction by its action on : enzyme stability, the velocity of breakdown of the ES complex, enzyme substrate affinity ($K + 1$ and $K - 1$), the affinity of the enzyme for activators or inhibitors, a.s.o. As demonstrated by Dixon and Webb [5], the temperature influence upon the whole enzymic reaction ($E + S \rightleftarrows ES \rightarrow E + P$) represents the results of its action on each stage. This fact assumes the existence of at least 18 thermodynamic parameters for the whole reaction, the analysis of which is complex and difficult to elucidate.

Literature data concerning the temperature action on enzymes in fishes indicate their activation at low temperature acclimation. During cold acclimation of fishes, the activity of the hexozo-monophosphatic shunt is increased [6], [12]. Hochachka [11] and Prosser [21] reported that in most fishes, both anaerobic glycolysis and pentozo-phosphatic shunt seemed increased by adaptation to low temperature. By cold acclimation of goldfish, the isoenzymic pattern of lactate dehydrogenase of muscle and brain is not altered, but the activity of some isoenzymes is increased [10], [25]. Also, Jankowsky [14] shows that the activity of fructose-1,6-diphosphate-aldolase is higher in fishes acclimated to 10°C (*Idus idus*) than in fishes adapted to 20°C, by determining the enzyme activity *in vitro* at 25°C.

On the other hand, it was found that lactate dehydrogenase and aldolase activity in frog muscle homogenates do not depend on the acclimation temperature [20]. Moreover, the results obtained by Kunnemann et al. [16] and Braun et al. [2] show that cold-acclimated fishes (*Rhodeus amarus* and *Idus idus*) present little compensation changes of glycolytic enzymes.

Without denying the possibility of increasing the activity of some glycolytic enzymes in certain fish species also at other acclimation temperatures, we have to show that *in vitro* enzymic determinations were not carried out at the animals adaptation temperature. Determination of enzymic activity was performed at an intermediate temperature [20], or at a higher temperature [14], [16] than that of fish acclimation; moreover, the incubation of the gels for determining the activity of the isoenzymic fractions was always achieved at room temperature [10], [19].

The electrophoretic results presented in this paper point out the importance of the study of the enzymic activity, of the isoenzymic pattern and of the enzymic kinetics at the acclimation temperature

(the temperature at which poikilotherms live or are adapted to). This affords the possibility of detecting some enzymic and isoenzymic changes, which permit a more accurate interpretation of the *in vivo* processes, by adapting the animals to different temperatures.

The study of the influence of low temperature upon the isoenzymic pattern of enzymes is little known. There are some indications upon the isoenzymes of mammals lactate dehydrogenase. Wilkinson [28] points out that the determination of the temperature coefficients of the various isoenzymes is difficult owing to the fact that the optimal pyruvate concentration for both LD_1 and LD_5 increases with increasing temperature. It was also shown that the lactate dehydrogenase isoenzymes of human sera differ in their stabilities at low temperatures. LD_1 is relatively unaffected by exposure at $0-4°$ or at $-10°$, but LD_4 and LD_5 are rapidly inactivated under these conditions, though they retain much of their activities at room temperature [28].

The results of our experiments concerning the activity (proportional to the staining intensity of bands) and the enzymic molecular forms of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, NAD- and NADP-isocytrate dehydrogenase clearly demonstrate that their activity in the homogenates of the investigated tissues, in both cold- and warm-adapted fishes, is lowered by their determination at 0°C. This low activity of enzymes at 0°C affects their level as well as the number of isoenzymes. There are some variations in the isoenzymic pattern, in certain tissues, more evident in serum, by adapting the fishes to different temperatures, which are non significant.

The electrophoretic pattern of enzymes when determined at 20°C shows that homogenates of all tissues investigated in both fish groups generally present the same enzymic pattern. As revealed by figures 1—8, the total enzymic activity (proportional to the staining intensity of the enzymic fractions), as well as the number and distribution of isoenzymes are not influenced by the fishes adjusting temperature. Determination of enzymes at 20°C reveals a higher number of enzymic fractions with increased staining activity, in both groups. Electrophoresis data also show that the enzymic activity is influenced by the enzymes determination temperature.

A special attention must be paid to isocytrate dehydrogenase. In tissues of different animal species two isocytrate dehydrogenases could be determined : NAD-isocytrate dehydrogenase and NADP-isocytrate dehydrogenase. Both dehydrogenases catalyse the reaction of isocytrate decarboxylation, forming ketoglutaric acid in the presence of Mg^{++} or Mn^{++} ions. The functional importance, the role and many regulating aspects of the two enzymes in the cellular metabolism are little known. The researches of Goebell and Klingenberg [8], Goebell and Pette [9], Solganicoff and Koeppe [23] emphasize the importance of NAD-linked enzyme in the oxidative cellular metabolism of Krebs cycle, while the NADP-linked enzyme, preponderantly extramitochondrial, ensures the formation of NADPH necessary for biosynthesis processes. Nevertheless, the possible participation of the NADP-linked enzyme in the oxidation of tricarboxylic acid substrates from the muscle tissue of numerous vertebrates was also made evident [27].

Literature data concerning the influence of low adjusting temperatures on the enzymic activity of NADP-isocytrate dehydrogenase in fishes do not show any particular differences related to acclimation temperature [2], [16].

Kanungo and Prosser [15], by studying the P/O ratio in the liver of goldfish acclimated to cold, found that the phosphorylating system in the mitochondria of the cold-acclimated fish has a decreased efficiency. The authors assume that metabolic adaptation to temperature occurs in the goldfish at the cellular level by quantitative changes in the activity of several enzyme systems. If we take into account the NAD- and NADP-isocytrate dehydrogenase enzymic activity, qualitatively and quantitatively decreased in all loach tissues investigated, as well as that of other respiratory enzymes (unpublished data), it obviously appears that the dehydrogenases activity related to the Krebs cycle of mitochondria is decreased under the influence of low temperature.

Our results complete the data obtained by Moon and Hochachka [19] concerning the NADP-isocytrate dehydrogenase of liver from rainbow-trout adapted to 2°C and 17°C. On the basis of enzymic kinetics results, the authors showed that the total activity of NADP-isocytrate dehydrogenase of the liver from both cold- and warm-adapted fishes seems to be the same when the enzyme is determined at the same temperature. Unable to detect particular alterations of isoenzymic pattern, due to the fact that the gels were incubated at room temperature, the authors assume that : "If there is any acclimatization effect, it is to slightly lower the activity of the enzyme during cold acclimatization".

Comparing the enzymic activity and isoenzymic distribution of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, NAD- and NADP-isocytrate dehydrogenase, by determining each enzyme at 20°C and 0°C (corresponding to the adaptation temperature of the two fish groups) it was found that loach adaptation to low temperature does not lead to proteosynthesis *de novo*. Low temperature alters the enzymic reaction rate, its functionality (catalysis), reveals that or those isoenzymes which are able to work at low temperatures. In some cases, low temperature leads to inactivation of the enzyme, though the enzyme is found in tissues by determining its activity at a higher temperature.

On the basis of the results obtained, one may assume that low temperature determines a slowing down of glycolysis in *Misgurnus fossilis*, regardless of the fact whether or not the fishes are acclimated.

Concerning the mechanism of the low temperature action upon the enzymic catalysis, this remains a problem to be further discussed. It was suggested that low temperature alters the enzyme-substrate affinity [13], [19], [24]. However, this cannot be the only explanation for the mechanism of enzyme action at low temperature. For the time being, the factors able to alter the enzyme catalytic activity at low temperature are difficult to assume.

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INVESTIGATIONS BEARING ON SPATIAL DISTRIBUTION IN SPIDERS

BY

FLORIANA BURLACU

This work is meant to study the distribution of Aranea on the soil of mixed-foliage forests (Brănești).

By using the Fischer-Débauche dispersion coefficient — which in the cases investigated was found to be superunitary — and by comparing the distribution of the frequencies observed to the theoretical ones checked through the χ^2 test, the author found out that the spiders are distributed according to the theoretical pattern of the binomially negative distribution, fact that points out an aggregation state.

In certain critical moments of the development, the serious competition determines a more uniform distribution, reflected by the Poisson distribution type. The aggregation manifested by the spiders is the results of some causes pertaining to the biotic and abiotic medium.

In animals, distribution is the result of a normal life [3]. It is determined by numerous factors, such as : the possibilities of satisfying food requirements, phenology and gradation of the organisms.

The knowledge of the distribution mode of animals has a peculiar ecological significance. The distribution reaches not only the collecting mode [4] and the method of data analysis, but may as well give indications upon population dimensions ; the distribution may also by itself describe the population structure, the fact being known that the changes of the distribution pattern may represent the result of the quantitative changes of a population.

The study of organisms surface dispersion pointed out three main distribution types [1] :

— the random distribution, according to which, for each individual, an equal probability exists to occupy any point in the space ;

the presence of each one is not influenced by the distribution of the other one. Such a distribution type requires the existance of a uniform medium ;

— the regular distribution, in which the position of each individual is conditioned by the presence of the other ones and is the result of a severe competition ;

— the grouped (aggregated) distribution, in which the individuals tend to form varying-size groups, as a consequence of the nonuniformity of the abiotical and biotical conditions.

The mathematical expression by means of certain theoretical distribution patterns gave an image of the organisms surface spreading. The coincidence between the theoretical pattern and the data observed allows to characterize a population under the aspect of its individuals distribution.

As far as spiders distribution in the space is concerned, very few works are dealing with this problem and spiders preference to a certain biotope type is emphasized.

The present work investigated the distribution of Aranea on the soil of a mixed-foliage-forest type of plain (Brănești). The frequency data in several spider species collected over a three-year interval (1966—1968) and in different time periods (monthly, seasonally, yearly) were considered.

In order to estimate the distribution mode of the spiders, we used the Fischer-Débauche dispersion coefficient expressed by the variance (S^2) and the individual mean ($\bar{x}$) ratio in the samples.

As seen in table 1 the value of the dispersion coefficient is superunitary, fact that corresponds to a mathematic model expressing a super-dispersion state.

Starting from this finding, we concluded that the mathematical pattern of the theoretical distributions which suits the best to the frequencies distribution observed is the binomially negative distribution. Consequently, we used the k coefficient which in the binomially negative distribution allows the obtention of frequencies calculated according to the following relation [6] :

$$k = \frac{\bar{x}^2}{S^2 - \bar{x}^2}$$

The checking of the null hypothesis was performed by comparing the distribution observed to that calculated, on the basis of the test χ^2. Working with a degree of freedom and using the distribution table of the χ^2 parameter distribution, we could determine precisely the extent to which the estimators deviations are significant as compared to the corresponding parameters.

In our researches bearing on spiders distribution on the forest soil, nine species and seven families were taken into consideration, among that ones which by their great number of individuals are proving to be the main components of the arachnid cenose present here.

As pointed out from table 1, the following species were investigated : *Harpactes rubicundus, Amaurobius pallidus, Zelotes villicus, Zelotes*

Table 1

Spatial distribution in spiders checked by means of the χ^2 test

Species	S^2/φ	Distribution type	$P\chi^2$	$P\chi^2$		
Harpactes rubicundus	1	Poisson	2.7267	30%	> $P\chi^2$ >	10%
	1.39	Bin. neg.	3.296	30%	> $P\chi^2$ >	10%
	3.13	Bin. neg.	0.2525	97.5%	> $P\chi^2$ >	95%
Amaurobius pallidus	2.45	Bin. neg.	8.0769	30%	> $P\chi^2$ >	10%
	1.31	Poisson	2.0489	50%	> $P\chi^2$ >	30%
	1.31	Bin. neg.	2.3268	50%	> $P\chi^2$ >	30%
	2.11	Bin. neg.	6.4615	30%	> $P\chi^2$ >	10%
	1.89	Bin. neg.	5.2287	30%	> $P\chi^2$ >	10%
	1.65	Bin. neg.	2.7665	30%	> $P\chi^2$ >	10%
Zelotes villicus	2.13	Bin. neg.	4.4385	50%	> $P\chi^2$ >	30%
	2.38	Bin. neg.	0.5351	90%	> $P\chi^2$ >	70%
	1.89	Bin. neg.	8.6077	10%	> $P\chi^2$ >	5%
	1.71	Poisson	0.5655	50%	> $P\chi^2$ >	30%
	1.71	Bin. neg.	1.6079	30%	> $P\chi^2$ >	10%
	2.26	Bin. neg.	3.6847	70%	> $P\chi^2$ >	50%
	1.96	Bin. neg.	7.153	10%	> $P\chi^2$ >	5%
Zelotes apricorum	2.43	Bin. neg.	2.3936	50%	> $P\chi^2$ >	30%
Xysticus cambridgi	1.40	Bin. neg.	0.2772	97.5%	> $P\chi^2$ >	95%
	1.15	Poisson	0.0813	90%	> $P\chi^2$ >	70%
	1.15	Bin. neg.	0.6172	50%	> $P\chi^2$ >	30%
	2.00	Bin. neg.	8.2793	10%	> $P\chi^2$ >	5%
Pardosa hortensis	3.53	Bin. neg.	0.6915	90%	> $P\chi^2$ >	70%
	1.11	Poisson	0.0404	90%	> $P\chi^2$ >	70%
	1.11	Bin. neg.	1.039	70%	> $P\chi^2$ >	50%
	1.96	Bin. neg.	2.6329	30%	> $P\chi^2$ >	10%
	2.55	Bin. neg.	0.1291	95%	> $P\chi^2$ >	90%
Pardosa lugubris	4.18	Bin. neg.	0.3481	95%	> $P\chi^2$ >	90%
	2.74	Bin. neg.	0.6228	90%	> $P\chi^2$ >	70%
	1.22	Poisson	0.4475	90%	> $P\chi^2$ >	70%
	1.22	Bin. neg.	0.5820	90%	> $P\chi^2$ >	70%
	4.46	Bin. neg.	2.0157	90%	> $P\chi^2$ >	70%
	3.12	Bin. neg.	2.734	50%	> $P\chi^2$ >	30%
	1.69	Bin. neg.	0.3282	97%	> $P\chi^2$ >	95%
	3.89	Bin. neg.	1.946	50%	> $P\chi^2$ >	30%
	1.75	Bin. neg.	0.1297	95%	> $P\chi^2$ >	90%
	4.66	Bin. neg.	1.1694	90%	> Px^2 >	70%
Coelotes falciger	1.72	Bin. neg.	4.1007	50%	> $P\chi^2$ >	30%
	2.00	Bin. neg.	1.2723	70%	> $P\chi^2$ >	50%
	2.92	Bin. neg.	1.6301	90%	> $P\chi^2$ >	70%
	1.08	Poisson	0.0634	90%	> $P\chi^2$ >	70%
	1.08	Bin. neg.	0.1197	90%	> $P\chi^2$ >	70%
Lepthyphantes zimmermanni	1.45	Bin. neg.	5.8257	30%	> $P\chi^2$ >	10%
	1.00	Poisson	1.4766	50%	> $P\chi^2$ >	30%

apricorum, Pardosa hortensis, Pardosa lugubris, Coelotes falciger and
Lepthyphantes zimmermanni. From all the cases studied, 35 favorably
plead for the hypothesis according to which the peculiarities of the spatial
distribution of the spider populations corresponds to the theoretical
pattern of the binomially negative distribution. The comparison of the
frequencies distribution observed to the theoretical ones showed non
significant deviations (as against the null hypothesis) with a χ^2 proba-
bility ranging between 5 % and 97.5 %. Only in 11 cases studied, the
deviations are significant, getting values of α transgression probability
under the 5 % accepted limit.

In two cases, i.e. in species *Harpactes rubicundus* and *Lepthyphantes
zimmermanni*, the value of the $S^2/\overline{x}$ ratio let us come to the idea of the
appurtenance of the observed patterns to the Poisson-type theoretical
pattern. The phenomenon takes place when the population level is
decreasing, registering small values of the mean of the individuals tested.

Such situations are met with in each spider population in certain
moments owing to the environmental less favorable conditions, or to
the phenology which causes the diminution of the number of individuals,
and thus, the value of the $S^2\overline{x}$ ratio will be equal or near the unit.

The researches bearing upon the spatial distribution of species
belonging to some more spider families : *Agelenidae, Amaurobidae,
Dysderidae, Gnaphosidae, Linyphiidae, Lycosidae* and *Thomisidae* led
to the finding that the phenomenon described in species is also available
for families. From 117 tests performed, in 109 cases the estimators
deviations as against the corresponding parameters are not significant;
this fact demonstrates that the spiders occurring on the forest soil are
distributed according to the binomially negative distribution pattern
(86 cases) and, in certain periods, following Poisson's distribution (23
cases). The above results show that, upon the periods of optimal biolo-
gical activity, the Aranea of the forest soil agglomerate, in certain habitat
portions, in aggregates, a fact exhibited by the binomially negative
distribution pattern.

The aggregation phenomenon, discovered by means of the theore-
tical pattern of the binomially negative distribution, may have two
causes : on the one hand, it may be due to an active behavioral, instinc-
tive aggregation or, on the other hand, to a certain biotope heterogeneity
(microclimate, plants, soil, natural ennemies).

Blackith [2] shows that the causes of organisms aggregation can
be put into evidence by calculating the mean number of organisms which
enter an aggregation. When the mean number of aggregated individuals
is smaller than 2, the grouping phenomenon is the results of the environ-
mental factors action. The aggregation of two or some more individuals
is induced by other factors. The mean number of aggregated organisms
may be calculated according to Arbous & Kerrich's formula (1951)
$\left(\lambda = \dfrac{\overline{x}}{2k} \cdot v \right)$.

By transposing our data on a graph [5] in which $\lambda = 2$ is calcu-
lated for various values of the mean and of k in the binomially negative
distribution, one may found out (Fig. 1) that all the values obtained in
the species we have studied are to be registered below the curve calcu-
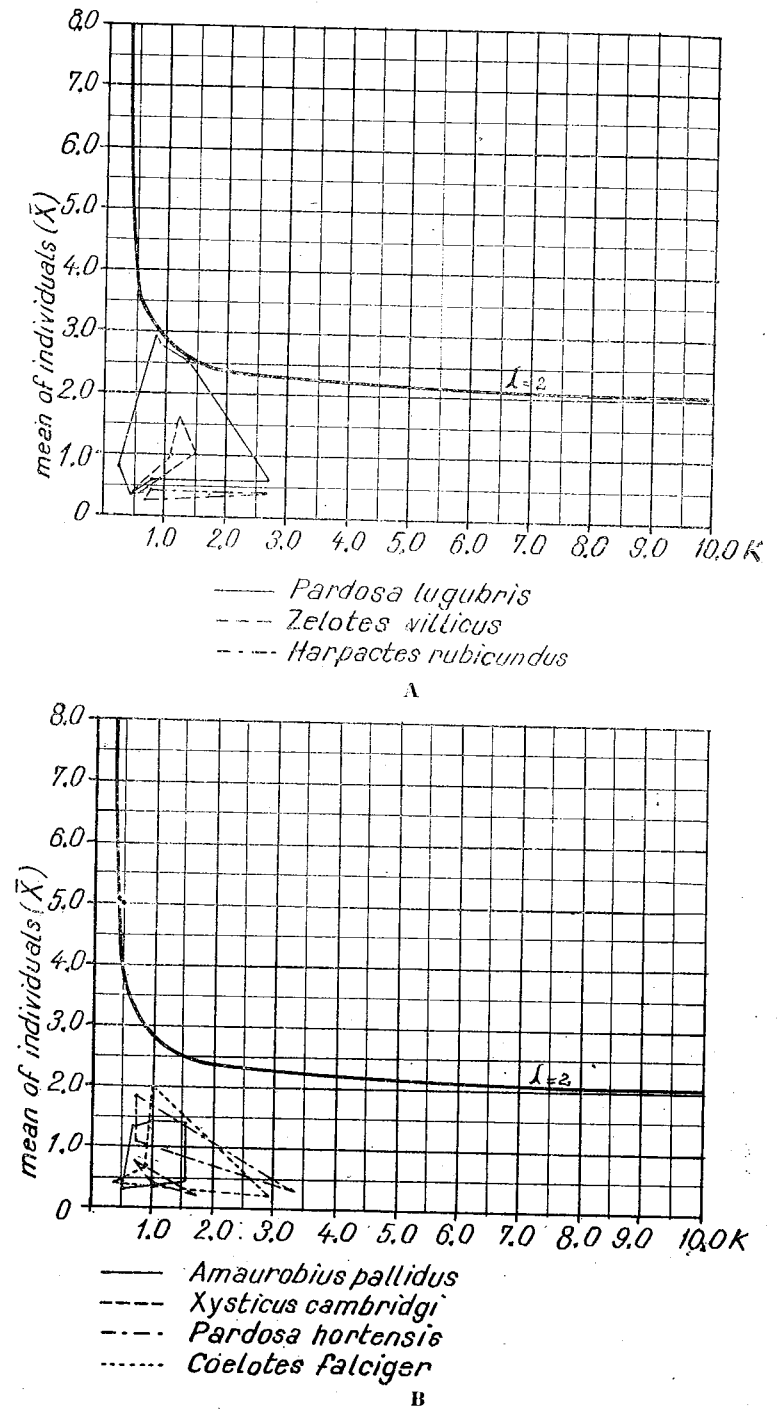

———— *Pardosa lugubris*
– – – – *Zelotes villicus*
– · – · *Harpactes rubicundus*

A

———— *Amaurobius pallidus*
– – – – *Xysticus cambridgi*
– · – · *Pardosa hortensis*
······· *Coelotes falciger*

B

Fig. 1.—Aggregation of Aranea depending on the existent environmental
conditions A, In species *Pardosa lugubris, Zelotes villicus, Harpactes
rubicundus*, B, In species *Amaurobius pallidus, Xysticus cambridgi, Parodosa
hortensis, Coelotes falcigar*.

lated for $\lambda = 2$; this fact shows that the aggregation exhibited by spiders is the result of environmental causes.

This phenomenon is clear-cut so much to more it is found out that the environmental factors changes (temperature, humidity, food quantity) lead to the modification of spatial distribution; the appearance of some critical periods in the development of a population increases the competition, diminishes the number and, herefrom, the tendency appears towards a more uniform distribution reflected in the Poisson's pattern of distribution.

CONCLUSIONS

— The Aranea occurring on the soil of a forest are distributed on the surface according to the theoretical pattern of the binomially negative distribution, fact which points out an aggregation state.

— In the critical moments of the development, the serious competition determines a change of distribution tending to a more uniform distribution reflected by the Poisson distribution pattern.

— The aggregation phenomenon found in spiders in [normal period of development is owed to the changes of the environmental conditions.

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