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DEUX ESPÈCES AFROTROPICALES NOUVELLES DU GENRE *BLAESOXIPHA* LOEW *(DIPTERA, SARCOPHAGIDAE)*

A. Z. LEHRER

Two new afrotropical species of the genus *Blaesoxipha* Loew are described: *Bl. montalciniae* sp. n. from South Africa and *Bl. gordimerae* sp. n. from Kenya.

Pour la faune africaine, Zumpt [1] a mentionné seulement cinq espèces de *Blaesoxipha, qu'ils sont difficiles d'être identifiées* d'après ses clés, descriptions et surtout ses dessins des genitalia mâles. Mais, dans les riches collections du Natal Museum (Pietermaritzburg), qui nous ont été offertes pour étude avec une particulière amabilité par MM Dr. B. R. Stuckenberg et Dr. D. A. Barraclough, nous avons découvert encore deux espèces nouvelles, qui sont décrites plus bas.

DIAGNOSES

1. Blaesoxipha montalciniae sp. n.

MÂLE

Tête : Noire et couverte d'un tomentum argenté. Le front, vu du dessus et au lieu le plus étroit, mesure 1/2 de la largeur d'un œil. La bande frontale est 2,3 fois plus large qu'une parafrontalie. Les antennes noires à teinte brune sur les articles basaux ont le troisième article 1,5 fois plus long que le deuxième. Arista noire brunâtre est pourvue de poils longs sur les deux parties. Les palpes et la trompe sont noirs. Le péristome mesure 1/3 du grand diamètre oculaire.

Chétotaxie de la tête : Les macrochètes verticaux internes sont longs, forts et rétroclines; les macrochètes verticaux externes plus ou moins distincts; les ocellaires proclines sont plus fins que les préverticaux rétroclines; les macrochètes frontaux sont au nombre de 9—10 paires; au long de la marge antéro-inférieure de l'œil on voit les macrochètes parafaciaux fins et courts; les petites vibrisses montent un peu sur les bordures faciales; il y a 1 postocellaire et 1 postvertical sur chaque côté de l'occiput; les microchètes occipitaux sont disposés sur 2 rangs. Le péristome est couvert de poils noirs; la partie postérieure de la tête est couverte de poils blancs jaunâtre courts.

Thorax : Noir, avec tomentum argenté, trois bandes médio-longitudinales noires et larges et deux bandes latérales courtes. Les propleures et le prosternum sont glabres. Les stigmates antérieurs sont noirâtres; les stigmates postérieurs sont bruns. Les pattes noires ont les fémurs médians pourvus d'un cténidium typique.

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Chétotaxie du thorax : ac = 2 + 1, dc = 2 + 3, ia = 0 + 2, prs = = 1, sa = 3, h = 3, ph = 2, n= 4, pa = 2, sc = 3 + 1, pp = 1, pst = = 1, st = 1 : 1 : 1.

Ailes : Transparentes. Epaulette noire ; basicosta et costagium sont jaunes. La nervure r_1 est glabre ; la nervure r_{4+5} est ciliée sur 2/3 de la distance entre sont origine et r—m. Cubitulus courbé en angle droit est prolongé d'un pli. L'épine costale est distincte. Les écailles sont blanches jaunâtre et les balanciers bruns.

Chétotaxie des tibias : Les tibias antérieurs ont 2 ad proximaux et 1 pv ; les tibias médians sont pourvus de 1 ad, 1 av, 1 pd, et 1 pv ; les tibias postérieurs ont 2 ad, 2 av, 2 pd et sont dépourvus de la longue pilosité

Abdomen : Noir à tomentum argenté ; les dessins en damier ont les taches noires allongées sur les tergites III et IV. La formule chétotaxique : 0 + 2 (couchés) + série + série. Les tergites génital et anal sont noirs ; le premier a un peu de tomentum et une paire de macrochètes médio-marginaux très forts et longs et une paire latérale plus courte et faible.

Armature génitale : fig. 1. Le sternite V (A) a une base très courte et les lames latérales longues, larges et arrondies aux bouts. Les cerques (B) sont très minces et se ploient en angle obtus ; leur partie distale se

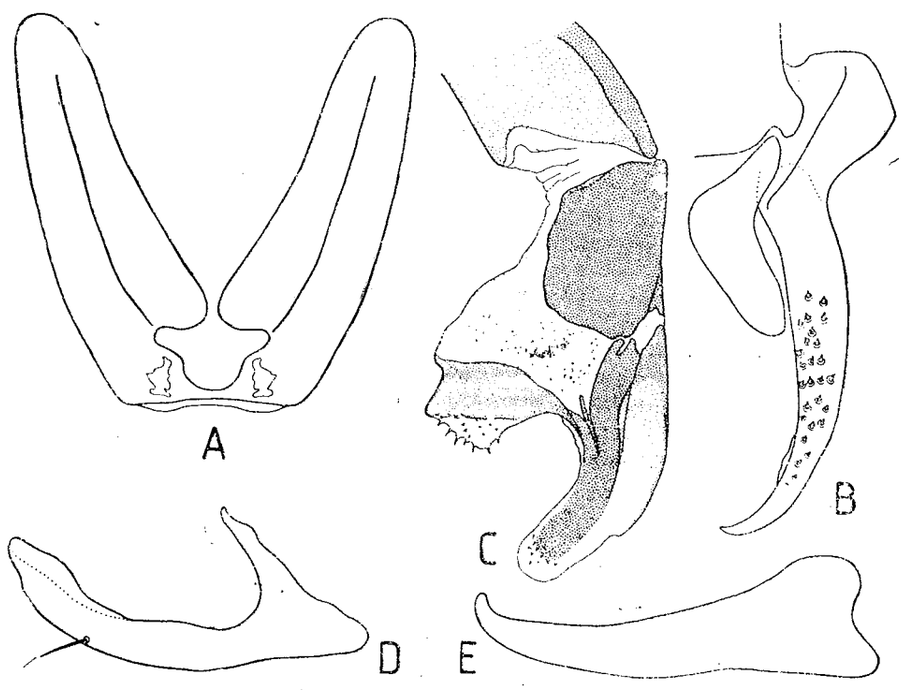

Fig. 1 — Armature génitale mâle de *Blaesoxipha montalciniae* sp. n. — A : sternite V ; B : cerques et paralobes ; C : distiphallus ; D : prégonite ; E : postgonite.

courbe sous la forme d'une faux, ayant un sommet long, aigu et sa surface antéro-médiane pourvue d'épines ; les paralobes ont la forme d'un boumerang, avec la partie distale plus longue et large. Le distiphallus (C) est très petit. La partie basale du paraphallus est courte. La partie apicale du paraphallus est relativement étroite, longue, plus ou moins transparente et avec quelques épines microscopiques sur l'apex arrondi. Le hypophallus est pigmenté, long et mince. Membrana est très développée et les lobes membranaux sont pigmentés et spinulés. Les prégonites (D) sont plus courts que les postgonites (E) ; les premiers sont légèrement courbés et ont un macrochète subterminal ; les seconds ont plus ou moins la forme d'un crochet.

Longueur du corps : 7 mm.

FEMELLE : inconnue.

Matériel-type : Holotype ♂, avec l'étiquette : « S. AFRICA : E. Cape, 5 km ENE of Rhodes, 30°47' S : 28°00 E' 2066 m, Date ; 5.II.1992, Natal Museum Expedition Wet, stream gully ». Déposé dans les collections du Natal Museum (Pietermaritzburg), avec les préparations microscopiques de l'armature génitale.

2. **Blaesoxipha gordimerae sp. n.**

MÂLE

Tête : Noire à tomentum argenté ; le vibrissarium avec ses branches péristomale, suboculaire et parafaciale sont d'un brun rougeâtre et, par ailleurs, un peu plus foncé. Le front, vu du dessus et au lieu le plus étroit, mesure un peu moins que 1/2 de la largeur d'un oeil. La bande frontale est brune sur la moitié antérieure et deux fois plus large qu'une parafrontalie. Les antennes brunes noirâtre sur toute leur longueur ont le troisième article presque deux fois plus long que le deuxième. Arista noire brunâtre a de poils moyens sur les deux parties. Les palpes et la trompe noirs ont une teinte brunâtre. Le péristome mesure 1/3 du grand diamètre oculaire.

Chétotaxie de la tête : Les macrochètes verticaux internes sont longs, forts et rétroclines ; les macrochètes verticaux externes indistincts ; les ocellaires proclines sont plus courts et fins que les préverticaux rétroclines ; les macrochètes frontaux sont au nombre de 11 paires ; les macrochètes parafaciaux sont assez développés à la marge antéro-inférieure de l'œil ; les petites vibrisses montent un peu sur les bordures faciales ; il y a 1 postocellaire et 1 postvertical sur chaque côté de l'occiput ; les microchètes occipitaux sont disposés sur 3 rangs irréguliers. Le péristome est couvert de poils noirs ; la partie postérieure de la tête est couverte de poils blancs assez courts et rares.

Thorax : Noir, avec tomentum argenté moins dense, trois bandes médio-dorsales longitudinales noires et larges et deux bandes latérales plus étroites. Les propleures et le prosternum sont glabres. Les stigmates antérieurs et postérieurs sont bruns noirâtre. Les pattes noires ont les tibias bruns noirâtre et les fémurs médians pourvus d'un ctenidium typique et court.

Chétotaxie du thorax : ac = 2 —3 + 1, dc = 3 — 4 + 3, ia = = 1 + 3, prs = 1, h = 3, ph = 2, n = 4, sa = 3, pa = 2, sc = 3 + 1, pp = 1, pst = 1, st = 1 : 1 : 1.

Ailes : Transparentes. Epaulette brune ; basicosta et costagium jaunes. La nervure r_1 est glabre ; la nervure r_{4+5} est ciliée rarement jusqu'à la proximité de r—m. Cubitulus courbé en angle droit est prolongé d'un pli. L'épine costale manque. Les écailles sont blanches jaunâtre et les balanciers jaunes brunâtre.

Chétotaxie des tibias : Les tibias antérieurs ont 2 ad et 1 pv ; les tibias médians sont pourvue de 1 ad, 1 av, 2 pd et 1 pv ; les tibias postérieurs ont 2—3 ad grands, 1 av, 2 pd et sont dépourvus de longue pilosité.

Abdomen : Noir, avec tomentum argenté et dessins en damier atténués ; les taches noires sont plus allongées et peu délimitées. La formule chétotaxique : 0 + 2 + série + série. Le tergite genital est court, noir et possède seulement une paire médiane de poils macrochètiformes courts. Le tergite anal est orange.

Armature génitale : fig. 2. Le sternite V (A) est relativement grand, ayant une base très courte, sous la forme d'une bande étroite et les lames

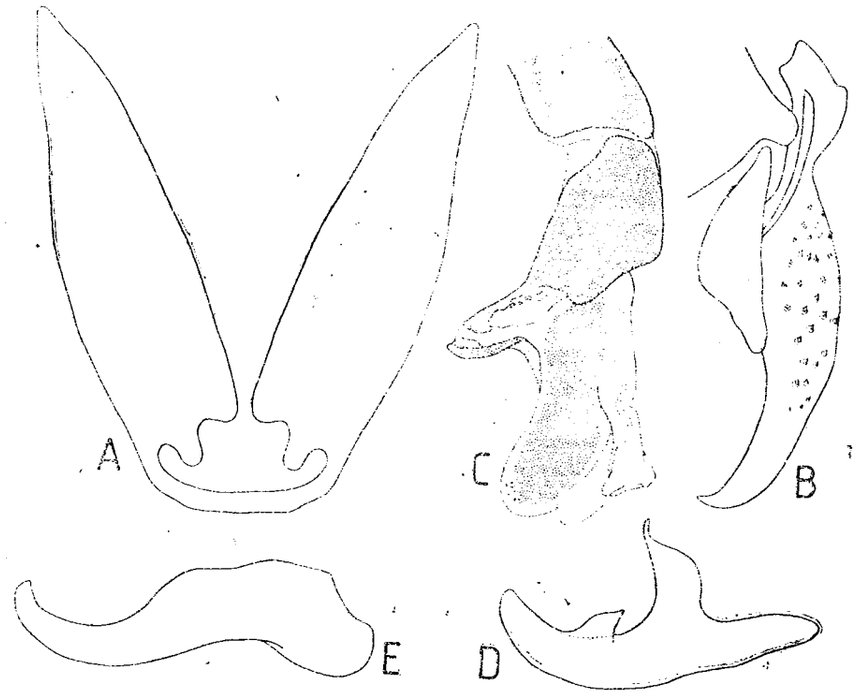

Fig. 2 — Armature génitale mâle de *Blaesoxipha gordimerae* sp. n. —A : sternite V ; B : cerques et paralobes ; C : distiphallus ; D : prégonite ; — E : postgonite.

latérales longues, larges et plus ou moins aiguës aux bouts. Les cerques (B) sont assez larges et ployés dans le quart basal ; leur partie distale se courbe légèrement jusqu'au sommet, qui se ploie brusquement ; les paralobes sont plus ou moins triangulaires. Le distiphallus est très petit (C).

La partie basale du paraphallus est courte et se prolonge d'une baguette antéro-inférieure fortement sclérifiée et pigmentée. La partie apicale du paraphallus est 1, 5 fois plus longue que celle basale et transparente ; le hypophallus est long, large, arrondi au bout et avec 5—6 épines microscopiques apicales. Les sclérites qui miment les lobes membranaux sont étroits et courbés en angle droit. Les prégonites (D) sont un peu plus courts que les postgonites (E) ; les premiers sont courbés et ont une dent médiane sur la marge supérieure ; les seconds sont légèrement ondulés.

Longueur du corps : 7 mm.

FEMELLE : inconnue.

Matériel-type : Holotype ♂, KENIA : Sigor, 31.I.1973, leg. I. Bampton. Déposé dans les collections du Natal Museum (Pietermaritzburg), avec les préparations microscopiques de l'armature génitale.

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SOME PROBLEMS IN THE SYSTEMATICS OF THE GENUS *COBITIS* AND ITS RELATIVES (PISCES, OSTARIOPHYSI, COBITIDAE)

T. T. NALBANT

New taxa of Cobitinae were described : *Iksookimia* new genus, *I. hugowolfeldi* new species, *Cobitis megaspila* new species and *Cobitis taenia danubialis* Bacescu, new subspecies.

The systematics of loaches (Cobitidae) formed the subject of many papers of different ichthyologists along the years from the capital work of Linnaeus, 1758, till now. This was due, that Cobitidae (in my sense with four subfamilies : Botiinae, Vaillantellinae, Noemacheilinae and Cobitinae, and not of Sawada, (8), is the richest Euro-Asian family except Cyprinidae.

Among the Cobitinae, the genus *Cobitis* must be revised with care because it is one of the richest and some of its species being very difficult to be recognised. On the other hand, a number of species referred to this genus belong now to other genera such as *Sabanejewia*, *Niwaëlla* and others. The members of the genus *Cobitis* are characterised by a special osseous plate *(lamina circularis* or *Canestrini's scale)* at the base of the second, often also at the first, pectoral ray in males. This bony plate is lacking in the males of the members of the subgenus *Acanestrinia*, *elongata* and *calderoni*. Another generic characteristic of *Cobitis* concerns the colour pattern, the spots along of the body ranging, according to Gambetta (3), in four pigmentary zones. This feature is lacking in other genera of Cobitinae.

1. THE GENERIC POSITION OF SOME SPECIES FROM THE FAR EAST

On the basis of the two characters mentionedabove which can define the genus *Cobitis*, I am able to separate into a new generic assemblage a number of five species from the rivers of South Korea and Amur basin.

Iksookimia gen. nov.

Type species : *Cobitis koreensis* Kim, 1976

Etymology : this genus is named in honour of the south Korean ichthyologist Ik Soo Kim, most of the species placed now in this genus being described by him. The name must be regarded as a noun, gender feminine.

Diagnosis : body elongate (Figs 1, 2, 3) but more stout than in *Cobitis* species, except *Cobitis bilseli* Battalgazi, 1942. Barbels generally longer than in *Cobitis*. Mental lobes (Figs 4, 5, 6) well developed, sometimes longer than lower lip and pointed. Suborbital spine (Figs 7, 9, 11) gene-

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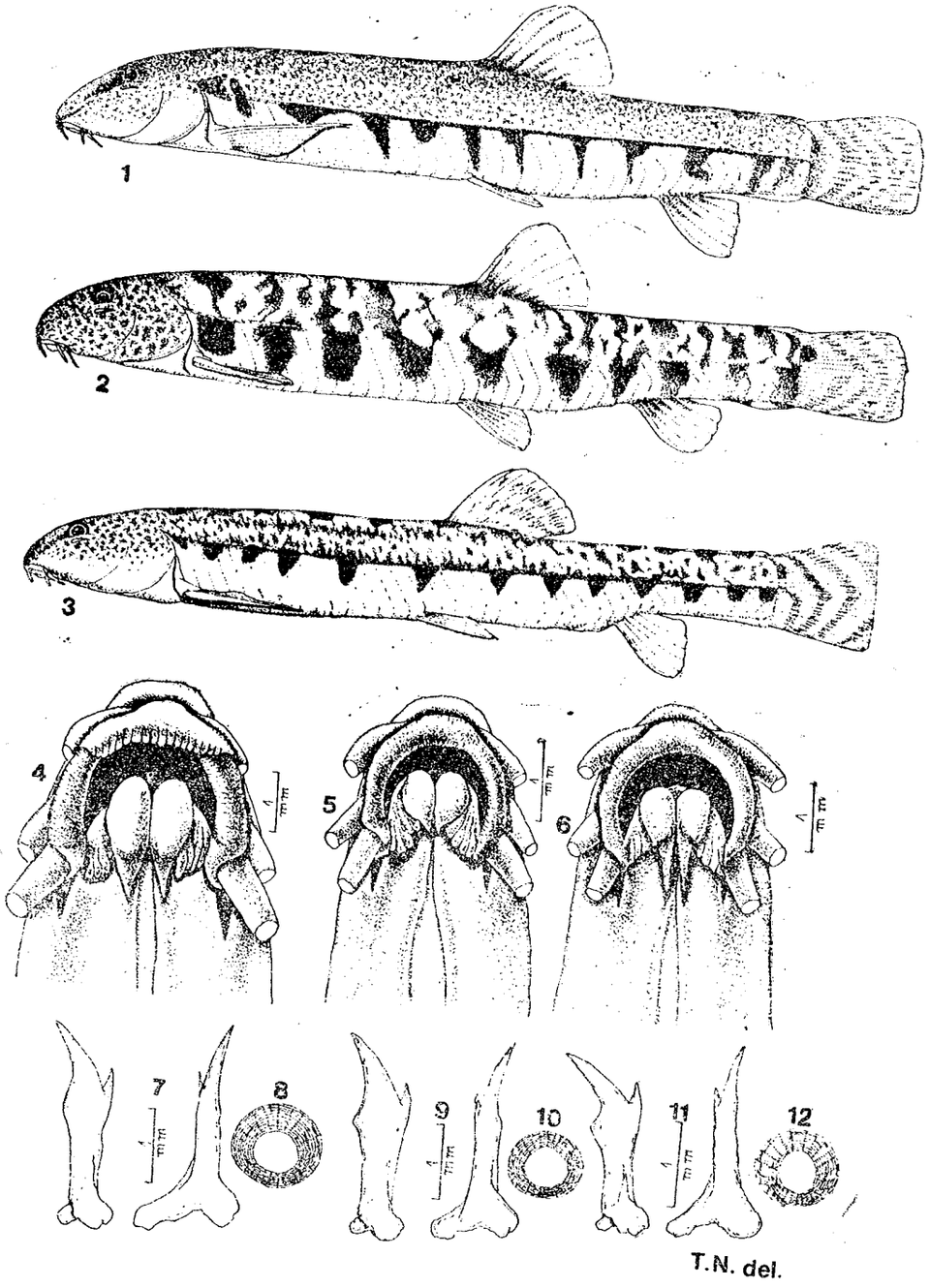

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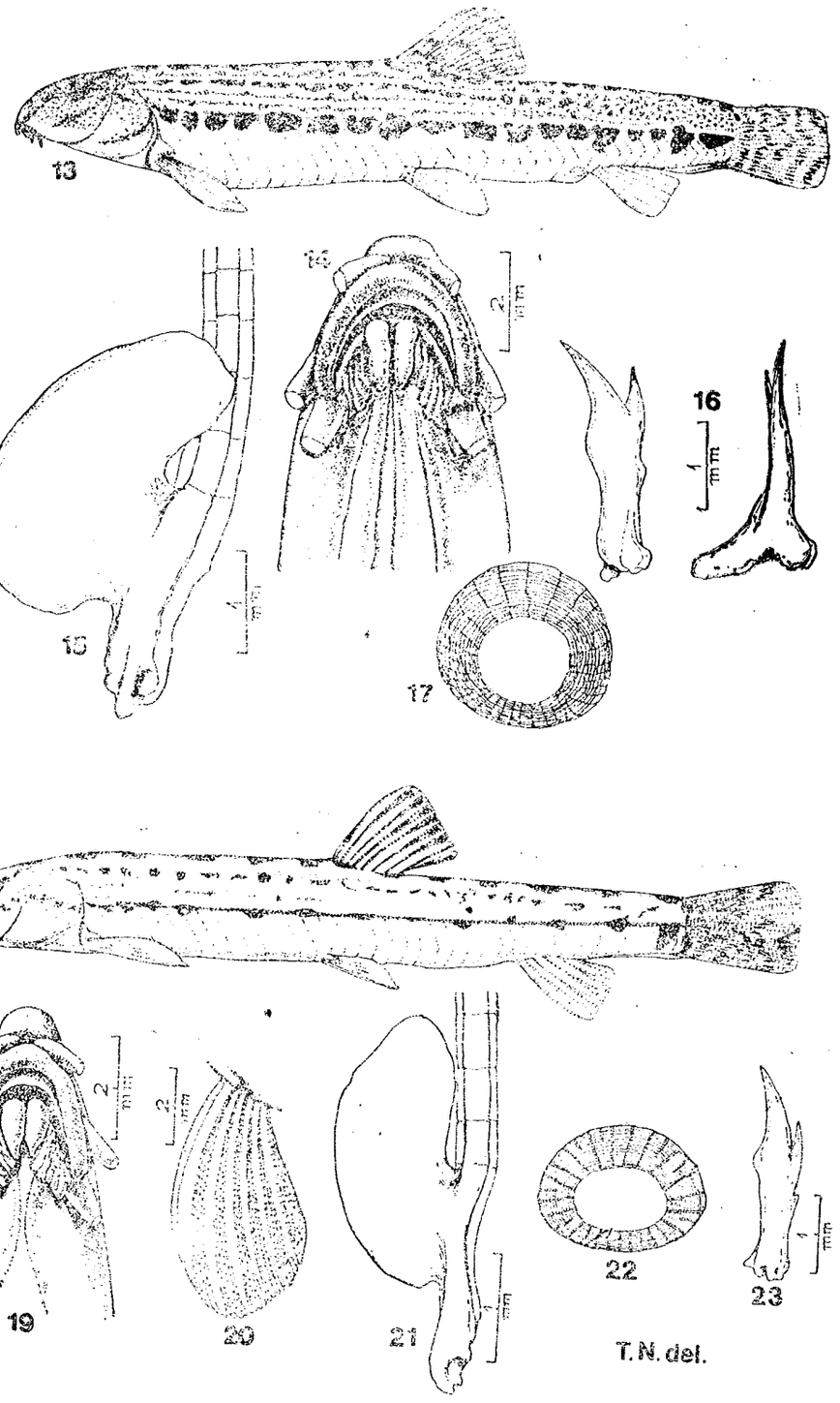

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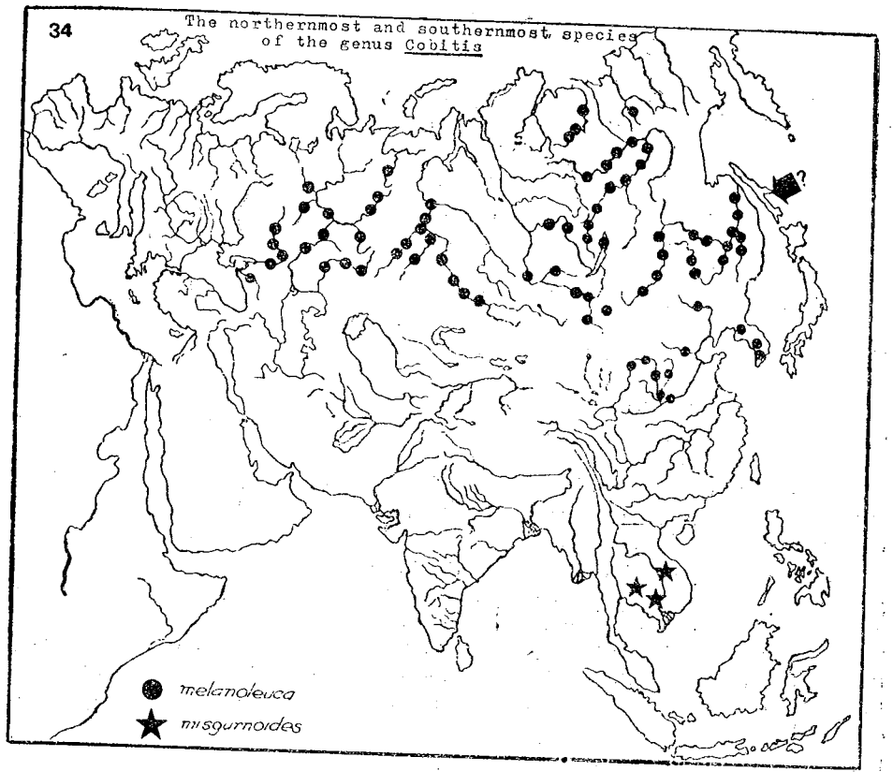
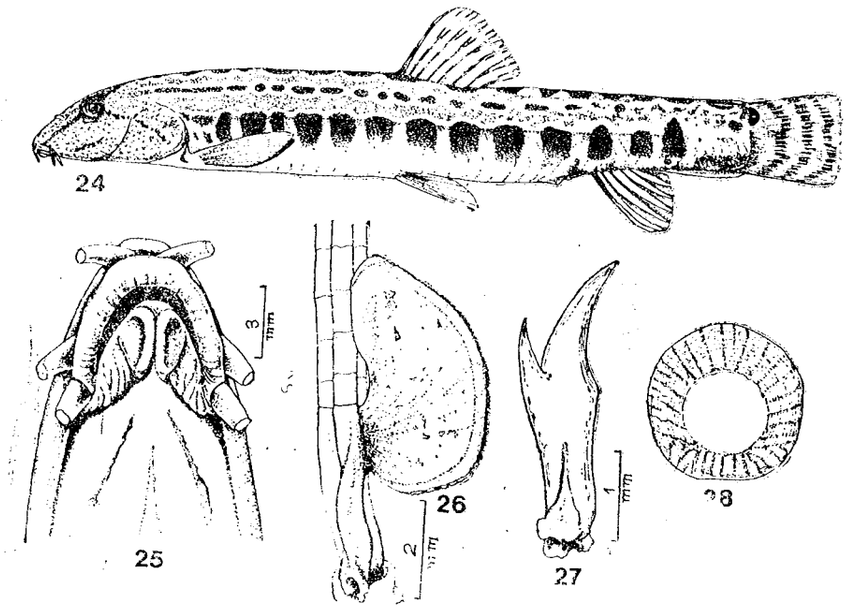


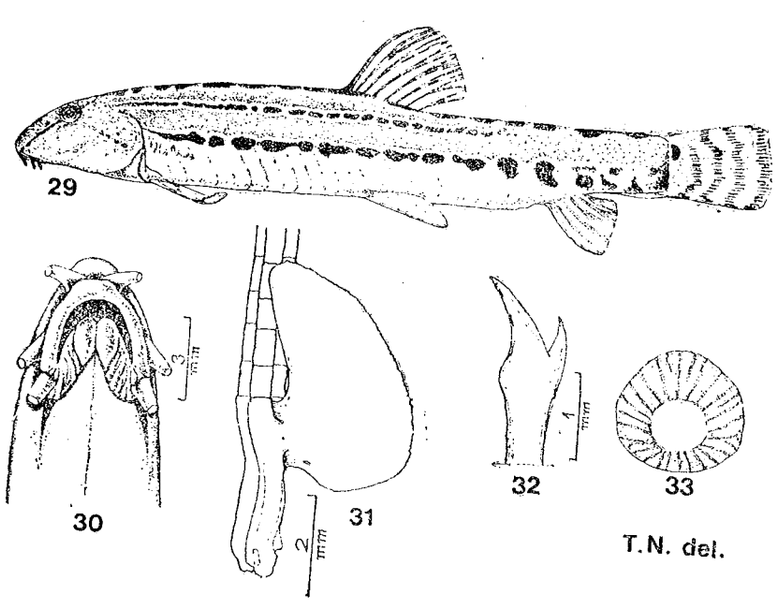
Fig. 34. Range of *Cobitis melanoleuca* and *C. misgurnoides* (northernmost and southernmost species of the genus).

rally reduced with the smallest thorn less than a half the longest. Sexual dimorphism : males have a well developed *lamina circularis*, sometimes elongated and with outer edge serrate. In addition the first pectoral ray is elongate, nearly filamentous (Figs 1, 3), as in males of *Misgurnus anguillicaudatus* (Cantor, 1842), a character not met in *Cobitis* species. Scales (Figs 8, 10, 12) with large focal zone.

Colour pattern is based on a row of large dark brown dorsal spots and a lateral row of spots more or less large of the same colour. Between them a wide band of brown dots or speckles. No disposition in four zones of Gambetta as in *Cobitis*. A jet black spot at the base of caudal fin is present. Dorsal and caudal fins with transversal rows of small dots (see Figs 1, 2, 3).

Species : *Iksookimia koreensis* (Kim, 1976) Figs 3, 5, 9, 10, *I. longicorpus* (Kim, Choi and Nalbant, 1976) Figs 2, 4, 7, 8, *I. choii* (Kim and Son, 1984), *I. pumila* (Kim and Lee, 1987) and *I. hugowolfeldi* new species described below. *Cobitis lebedeci* Vasilieva and Vasiliev, 1985, belongs to this genus and it is a junior synonym of *I. choii*. In its features, pigmentation and sexual dimorphism, *Iksookimia* appear more plesiomorphic than *Cobitis*.





Iksookimia hugowolfeldi sp. nov.

Figs 1, 6, 11 and 12

Material : ISBB 4495, adult male 64.5 mm SL, South Korea : Yung San river, no locality, May 31, 1973, Ik Soo Kim coll., as *holotype* (Fig. 1). ISBB 4496, two adult females 75.0 and 93.6 mm SL, bearing same location as the holotype, considered as *paratypes*.

Etymology : this species was named in the memory of Hugo Wolfeld, Bucharest, Romania, one of the most able aquarium fish breder and amateur ichthyologist.

Diagnosis : D II—III 7, A II 5, V I 6 — I 6, P I 8 — I 8, Cn 7 + 7 n. Body relatively stout, head large with the eye equidistant from the tip of snout to hind margin of operculum. Suborbital spine relatively small and slender (Fig. 11). The three pairs of barbels long both lips being furrowed (Fig. 6). Mental lobes well developed, longer than the lower (posterior) lip, with pointed tips. Predorsal space much longer than the postdorsal one. Insertion of ventral fins on the same line of the insertion of dorsal. Sexual dimorphism present, the males having the second pectoral ray thickened and well longer than the others. Lamina circularis as a wide blade-like process at the base of the second pectoral ray.

Colour pattern based on the alcohol preserved specimens : generally ground colour creamy-yellow ; on the dorsum 11—13 dark gray-brown spots, the dorsal fin being placed generally in the middle two blotches ; along the sides of body there are 9—11 very thin dark-brown cross bands ; dorso-lateral pigmentation based on a wide row of brown dots and speckles, the same pigmentation on the head and its sides : a dark gray-brown stripe from tip of snout to eye and then to nape ; at the upper part of the base of caudal fin a relatively small jet black dot ; dorsal and caudal fins with 3—4 rows of brown dots.

Range : *Iksookimia hugowolfeldi* apparently is distributed (till now) only in the rivers southern of the mountain chains Noryeong and Sobaik, i.e. in the basin of Yung San ; the species is also present in a small river of an island in the yellow Sea, before the entrance of the Seom Jin river in this sea and also in a short river, tributary of Japan Sea, locality Ulju, in southern-east South Korea. Other locations of this species than Yung San basin are from Kim (4).

2. THE GENUS *COBITIS* LINNAEUS, 1758

As I mentioned above this genus is characterized by the presence of a marked sexual dimorphism (except the species of the subgenus *Acanestrinia*) in the presence of one or two lamina circularis at the base of first pectoral rays and four zones of pigmentary spots or dots and no scales on head or sides of head as in *Lepidocephalus*, *Lepidocephalichthys* or *Neoeucirrichthys*. On the basis of these features, although the description of *Lepidocephalichthys pristes* Roberts, 1989, from Kapuas river, Borneo, is quite incomplete, I suspect that this species belongs to *Cobitis*, being the southernmost species of this genus. Examination of the type material of *pristes* can confirm or not this supposition. Till now *Cobitis misgurnoides* Rendahl, 1944, is the southernmost species of the genus (Fig. 34).

The type of genus is *Cobitis taenia* Linnaeus, 1758, established by subsequent designation of Jordan, 1917 and later by the Opinion 1500, BZN 45 (2) 1988, at a proposal of Kottelat (5). The linnean type locality was "*in Europae aquis dulcibus*" but the presumed type specimen, 76 mm SL, Alströmer-Linnaeus donation, 1749 (see Wheeler (9), pag. 163—164) came from Sweden. Therefore, any comparison of a species of *Cobitis* with *taenia* must be referred to the Swedish population and not from other part of Europe. Having the possibility to examine a number of lots of *taenia* from Sweden, I can give a short description and figure of this species.

D II (III) 7, A II 5, V I 6 — I 6, P I 8 — I 8, Cn 7 + 7 n.

Body normal elongate (Fig. 13) with the insertion of the dorsal fin closely to base of caudal than to tip of snout. Insertions of both dorsal and ventral fins generally on the same line. Head moderately long. Eyes a little close to tip of snout than to hind margin of operculum. Suborbital spine (Fig. 16) moderately long. The three pairs of barbels short. Mental lobes (Fig. 14) relatively large, a little longer than the lower (posterior) lip which is well folded. Sexual dimorphism : males have at the base of tke second pectoral ray, which is thickened and longer, a platelike process, which is wide with rounded margins (Fig. 15). Scales with a relatively large focal zone (Fig. 17) cover the whole body excepting the head.

Colour pattern in preserved specimens based on 13—21 dorsal dark brown spots. The four pigmentary zones of Gambetta normally developed as in Fig. 13. Lateral spots 11—20, generally 16—18. Pigmentation of head formed by numerous dark brown dots. There is a system of dark grey-brown stripes on the head (see Fig. 13) : a stripe comes from tip of snout to eye and then to nape, another from posterior part of eye to superior margin of operculum, a third one from the superior edge of preoperculum obliquely downward to the corner of mouth and the fourth from the mid part of opercular edge obliquely downward to inferoposterior angle of preoperculum. The jet black spot at the superior part of base of caudal extremely reduced but its presence is evident. Dorsal and caudal fins with 3—4 rows of brown dots.

It is extremely important to mention that the differences between the populations of different basins in Europe and Asia are sometimes apparently very minor, often based on only one character, but this feature can separate two species. For instance, *Cobitis vardarensis* Karaman, 1928, does not differ too much from the Danubian form. In *vardarensis* the body is a little higher than in Danubian form and the scales with a very reduced focal zone while in the latter the scales have a large focal zone these characters are sufficient enough to consider them as two different species. Of course, *vardarensis* seems to be the sister lineage of the Danubian form. Such examples are not too rare within the genus *Cobitis*.

3. THE STATUS OF *COBITIS MELANOLEUCA* NICHOLS, 1925

Examination of four specimens of *Cobitis melanoleuca*, ISBB 3230, three females 47.0—55.1 mm SL and one adult male 51.0 mm SL (see Figs 18—23) from China : Shanxi, Kwei-hwa, 1920, C. H. Pope coll.,

Third Asiatic Expedition of the American Museum of Natural History, agree very well with the original description and type figure of Nichols (6, 7). My material came from a relatively very close locality with the type locality, of *melanoleuca*. A comparison between these specimens and a series of twenty specimens of *Cobitis granoei* Rendahl, 1935 all adult males and females, 78.0—107.4 mm SL, Russia : Siberia, Ordinskoe Lake, Kuda basin, system of Ienisei, August, 1958, have revealed that both *melanoleuca* and *granoei* are conspecific. Having priority, we must retain for this species the name *melanoleuca*. The range of this species is presented in Figure 34. *Cobitis melanoleuca* has the northernmost distribution among the species of the genus.

4. THE POSITION OF THE DANUBIAN POPULATIONS OF *COBITIS*

Having the opportunity to examine large series of *Cobitis* ex gr. *taenia* from the Danube basin, I was able to compare them with other European species of *Cobitis* s. str., in the collections of ISBB such as *maroccana* Pellegrin, 1925, *zanandreai* Caviccioli, 1965, *bilineata* Canestrini, 1866, *taenia* Linnaeus, 1758 and *vardarensis* Karaman, 1928 and I had the possibility to evaluate all morphologic characters of each species. Therefore I agree the opinion of Bacescu (1) that "... *Cobitis taenia* notion collective", i.e. this is "a catch all" species. In this way, I found that in Balcans and in Anatolia there are many unknown species till now considered as *Cobitis taenia*.

Cobitis megaspila sp. nov.

Figs 24—28

Material : ISBB 4497, one adult male 69.3 mm SL, Romania : the Danube Delta, Caraorman, channel of a fish pond, September 1965, Drăgăşanu coll., as *holotype* (Fig. 24).

Paratypes : MINB 49925, five 56.0—75.1 mm SL, bearing same locality and data with holotype ; ISBB 4498, twenty (12 ♀♀ and 8 ♂♂) 51.0—72.3 mm SL, same data as holotype ; ISBB 4500, twelve 22.2—65.1 mm SL, Romania : the Danube Delta, Litcov channel, Caraorman, August 11, 1979, Nalbant coll. ; ISBB 4499, two 60.0 and 75.0 mm SL, Romania : Gurban Valley near Comana village, about 27 km south of Bucharest, May 11, 1983, Bănărescu coll.

Etymology : *mega*, Greek, meaning large, *spila*, Greek, meaning spot. The name must be regarded as a noun in apposition.

Diagnosis : D II—III 7, A II 5, V I 6 — I 6 (7), P I 8 — I 8 (9), Cn 7 + 7 n. Body relatively elongate, head moderately large, eyes close to the tip of snout than to the hind opercular margin. Dorsal insertion on the same line with ventrals insertion, being placed a little close to base of caudal fin. Barbels short. Lower (posterior) lip with the mental lobes a little longer than the rest of folded portion (Fig. 25). Suborbital spine long enough with no a conspicuous process on the outer edge of the spine (Fig. 27). In males lamina circularis is well developed with a relatively sharp distal angle (Fig. 26). Scales with large focal zone (Fig. 28).

Colour pattern (in alcohol preserved specimens) : ground colour whitish, creamy-yellow or yellow-brown ; on the dorsum 13—20, generally 15—18 dark brown blotches ; first and third Gambetta's pigmentary zones based on very fine (sandy) gray-brown dots, the second one being formed by dark brown lines or rounded small spots ; all three zones are anastomozing above the anal fin in a wide row of irregular small spots of same colour ; the fourth zone consists of large dark brown blotches, generally vertically elongated ; in some specimens the abdomen is pigmented with dark brown or dark gray dots ; the head is covered by a dense grayish-brown dots with darker stripes like in *Cobitis taenia taenia*. The jet black spot at the upper part of caudal fin case is a large one. Both dorsal and caudal with 3—4 rows of dark brown dots.

Remarks. The area of *Cobitis megaspila* is restricted till now along the Gurban valley near Bucharest and in the Danube Delta. In its pigmentation *megaspila* appear very close to *bilineata* but the latter has two black spots at the base of caudal fin. Same kind of colour pattern has the Greek species *trichonica* but it has two lamina circularis belonging to the subgenus *Bicanestrinia* Băcescu, 1961.

Cobitis taenia danubialis Băcescu, subsp. nov.

Figs. 29—33

Material : MINB 49923, one adult male 60.5 mm SL, Romania : Transilvania, Mureş river at Sărmaş, Harghita, August 3, 1991, Sárkány, Nalbant and Szombath coll., as *holotype* (Fig. 29).

Paratypes : MINB * 49924, three females 60.0—72.3 mm SL, bearing same data as holotype ; ISBB 4168, six 36.0—81.0 mm SL, bearing same data as holotype ; ISBB * 3021, five 60.0—94.5 mm SL, Romania : Banat, Timiş river, Rudna, September 12, 1975 ; ISBB 2902, five 60.0—97.2 mm SL, Romania Banat, Timiş river, Sânnicolaul Mare, September 9, 1975, Bănărescu coll. ; ISBB 4501, one 97.5 mm SL, Romania : Moldova, Suceava river, Liteni, November 16, 1958.

Etymology : the name came from the Latin Danubius (or Istros) meaning Danube, but the word is a noun in the genitive case meaning of (or from) the Danube river (Basin).

Diagnosis : D II—III 7 (6), A II 5, V I 6 — I 6 (5), P I 8 — I 8(9), Cn 7 + 7 n, but specimens with 7 + 6 or 6+6 were found in a few cases. Body moderately elongate being similar with *taenia taenia*. Eyes are a little close to the tip of snout. Dorsal fin placed in the second half of the body. Insertion of ventral fins generally under the second or third dorsal rays insertion. Mouth with reduced barbels. Mental lobes like in *taenia taenia* (Fig. 30). Suborbital spine generally well developed, similar in all respects with those of *Cobitis megaspila* (Fig. 32). Sexual dimorphism is evident the males having a large lamina circularis (Fig. 31). Scales with a large focal zone.

* *Abbreviations.* ISBB—Institutul de Ştiinţe Biologice, Bucureşti ; MINB—Muzeul de Istorie Naţională „Grigore Antipa", Bucureşti.

Colour pattern : similar in many respects with those of *megaspila* excepting the fourth Gambetta's zone in which the lateral spots are more reduced in their size and number than in *megaspila*. Jet black spot at the upper part of caudal fin large or very large.

Remarks. This form, characteristic for the Danube basin, was described by Băcescu (2) more than 30 years ago as *Cobitis taenia* var. *elongatoides*. But according to ICZN, 1985 (Third edition), Art. 16 "A scientific name proposed as the name of a *variety* or *form* after 1960, is infrasubspecific and excluded from zoological nomenclature". Therefore I was obliged to redescribe the variety *elongatoides* under a new name (Băcescu).

Acknowledgements. Special thanks go to Prof. Dr Mihai C. Băcescu for the long discussions and comments about *Cobitis* species.

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VALIDATION OF THE SUBGENUS *ODONTOCLADIUS* ALBU, 1974, AS A PART OF THE GENUS *BRYOPHAENOCLADIUS* THIENEMANN, 1934

VICTORIA TATOLE

Because the description given to the new subgenus *Odontocladius*, Albu, 1974 does not specify the generotype according with article 13 b of ICZN, it was invalidated. I consider the absence of the infrageneric taxonomical unity and validate this subgenus.

As part of the genus *Bryophaenocladius* Thienemann, 1934, Albu, 1974 has described from the chironomid fauna of Romania a new subgenus : *Odontocladius*, which contains two species, both of them being new : *Bryophaenocladius (Odontocladius) pectinatus* Albu, 1974 and *B. (O.) nigrus* Albu, 1974. Because in that paper P. Albu has not specified the generotype, according with article 13 b of ICZN, the subgenus *Odontocladius* becomes invalid.

Recently, working up the other eight species of the genus *Bryophaenocladius* — *flexidens* Brundin, 1947 ; *ictericus* (Meigen, 1830) ; *illimbatus* (Edwards, 1929) ; *inconstans* (Brundin, 1947) ; *nidorum* (Edwards, 1929) ; *scanicus* (Brundin, 1947) ; *subvernalis* (Edwards, 1929) and *tuberculatus* (Edwards, 1929) — that were found in Romania, I have ascertained the absence of the infragenerical taxonomical unity which was invalidated. At other times, Saether, 1973 wrote "The species seem to indicate that *Bryophaenocladius* is a heterogeneous genus and eventually may be divided into several genera". I do not go so far, yet, but I apply for the revalidation of the subgenus *Odontocladius* Albu, 1974.

Because the holotypes of both species are missing in the collection and only one paratype of the species *B. (O.) nigrus* Albu, 1974 was found, I consider that it can be considered as the type of subgenus *Odontocladius* Albu, 1974.

Therefore, in this paper, I give the original drawings (Plate I : 1, 2) for *Bryophaenocladius (Odontocladius) pectinatus* Albu, 1974, again for *B. (O.) nigrus* Albu, 1974 which I appoint the generotype of the subgenus *Odontocladius* Albu, 1974 as part of the *Bryophaenocladius* Thienemann, 1934. Now, I give a redescription of the paratype.

Bryophaenocladius nigrus Albu, 1974

Male. Head brownish black ; bristles on vertex ; postorbitals : 2 ; outer verticals : 3 ; inner verticals : 4 ; tentorium 110 μ long, maximum width 29 μ, width anterior of posterior tentorial pit 14.6 μ ; clypeus with 5 setae ; palp from 5 segments (in according with Saether, 1971) ; palp length (μ) ; 20 ; 31.8 (31) * ; 113 (106–135) ; 115 (100–106) ; 130 (100–

* The number in parentheses following the measurements refers to the holotype (after Albu, 1974).

135), third palp segment (the second, after Albu, 1974) with a 27μ long finger-like apical lobe (Plate I : 4).

Antenna brownish black ; with 14 segments ; AR = 1.75 (1.64—1.77).

Thorax brownish black, except for the pale disks at the basis of Dl hairs and a part of the pleurae, where the last Pa hairs are inserted ; halteres brownish black ; pronotum black, very well developed, broadly, totally split in the middle ; thoracic chaetotaxy : Dl : 11 (15—16) ; Pa : 7 (6—8) ; Sa : 1 (1) ; Sc : 9 (8—10) forming a single row.

Wing greyish with distinct microtrichia ; squama brown with 6 hairs (4—6) ; anal lobe well developed, rounded ; basal vein with 1 hair ; macrotrichia present on R and R_1 ; R_{2+3} in the midway between R_1 and R_{4+5} ; An ends shortly after fCu ; Cu_2 slightly curved ; VR = 1.18 (1.19—1.25) ; wing length (mm) : 1.32 (1.32—1.34) till the arculus, 1.65 (1.64—1.68) squama included.

Legs brown ; pulvilli absent ; tibiae with spurs as in all the other species of the genus ; t_2 has no comb ; length (μ) and proportion of legs :

	fe	t	ta_1	ta_2	ta_3	ta_4	ta_5	LR
P I	564(520)	641(680)	358(360)	194(200)	135(140)	100(100)	76(80)	0,53(0,53—0,56)
P II	617(600)	676(640)	280(280)	153(140)	123(120)	76(80)	76(80)	0,41(0,43)
P III	540(500)	765(760)	442(440)	206(200)	176(160)	80(80)	76(80)	0,58(0,58)

Abdomen brownish black with uniformly distributed bristles. Hypopygium (Plate I : 3) brownish black ; anal point broadly, triangular ; basal lobe of the basystyle not much produced ; the hairs on its surface with very light disks at basis ; distystyle more hairy.

Distribution. This species was found only once in Ieşelniţa (the Iron Gates region of the Danube).

Also, in the collection is one male specimen with the some finger-like lobe on the third palpl segment. The male was found in a light trap in Cornet (Olt river).

Finally, I consider that the presence of this character at a few species of the genus *Bryophaenocladius* is a matter of interest.

Subgenus *Odontocladius* Albu, n. s.g

This new subgenus belongs to the genus *Bryophaenocladius*, and its species have all the characters of the genus, as defined by Brundin (1956) as well as the diagnoses of Cranston and al., 1989 (5). Its characteristic feature is the presence of prolongations, teeth or squamae, on the inner side, distally of the second palpal segment.

Again, Saether, 1973 referring to *B. brincki* (Freem.) comb. nov. showed that the finger-like lobe of the third palpal segment "may possibly be of generic value". I remark only the necessity of validation of the *Odontocladius* Albu, 1974 with the specification of *B. (O.) nigrus* Albu, 1974 as a generotype of the subgenus and as a type-locality, Ieşelniţa (Iron Gates) Romania.

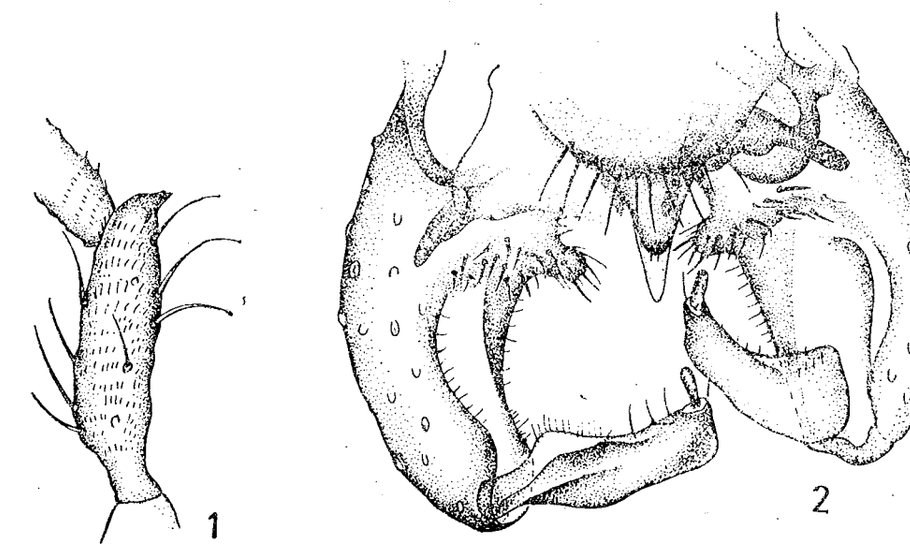

Plate I. — 1 Third palpal segment of *Bryophaenocladius (Odontocladius) pectinatus* Albu 1974 ; 2 Hypopygium of same spcies ; 3. Hypopygium of *Bryophaenocladius (Odontocladius) nigrus* Albu, 1974 (paratype) ; 4. Third palpal segment of the same species (paratype).

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THE REPRODUCTION OF *TRACHELIPUS DIFFICILIS ROTUNDATUS* RADU, 1950 *(CRUSTACEA: ISOPODA)*

SHARON ACCOLA *, N. TOMESCU *, CRISTINA BERCIU * and CARINELA RUSU **

The sex ratio, reproductive period and females fecundity of over 7 000 specimens of *Trachelipus difficilis rotundatum*, collected in the Cheile Turzii Natural Reservation, were studied.

INTRODUCTION

Terrestrial isopods, like the greatest majority of animal species, have a specific seasonal reproductive period. This period can vary according to species [1] as well as within the species and it is influenced by ecological factors [4], [9], [10], which interact with genetic factors, setting off reproductive activities [3], [6]. Isopod species can have a longer or shorter reproductive period, determined by the number of broods per year and age class differences of the females.

Trachelipus difficilis rotundatus is a subspecies which is endemic in the Romanian fauna. They live under the leaf litter of deciduous woods under rocks and under moss. It is very abundant in the Cheile Turzii Natural Reservation, where it was studied. The reservation is located in northwestern Romania, 30 kilometers south of Cluj. Its morphological aspects have been described but its reproductive aspects have not been studied. Over 7000 specimens were collected and on the basis of this rich material, we studied the sex ratio, reproductive period and the fecundity of the females in relation to their body length.

MATERIALS AND METHODS

Biological material was collected over a period of two years (1991 and 1992), using Barber traps, which we placed in five different ecosystems in Cheile Turzii. *T. difficilis rotundatus* was found in two of these ecosystems; a deciduous woods in which oak and hornbeam predominate, and along the banks of the Hasdate Brook, where poplar and willow grow. The traps were emptied every two weeks and the material from each trap was put in individual tubes. In the course of two years, 7769 adult specimens were collected, on which we studied their reproduction. In the laboratory the individuals of each collection were counted, measured and classed according to sex and age. The length of the gravid females was measured and the eggs or embryos in the marsupial pouch were counted. On the basis of the length of the female adults, nine class sizes were established, ranging from 8 to 17 mm. The average number of eggs for each class size was calculated in order to establish a relationship between fecundity and the length of the reproductive females.

REV. ROUM. BIOL.–BIOL. ANIM., TOME 38, N° 2, P. 115–121, BUCAREST, 1993

RESULTS AND DISCUSSIONS

Sex ratio

A study of the sex ratio of dioecious species provides valuable information regarding their reproductive potential. In the species in which the males mate only once or a few times, the reproductive potential is dependent on the numerical equilibrium of the two sexes in the population structure.

The number of adult males and females of *T. difficilis rotundatus* collected in 1991 and 1992 is rendered in Table 1. In our calculations we considered only the adult individuals, which are capable of participating in reproduction. In figure 1, the total percentage of males and females collected in the two years of study are displayed graphically (A) and the proportion of the two sexes for each individual collection (B). At the population level, the proportion of the two sexes was nearly equal in 1991 and unequal in 1992. The smaller number of females in the population of 1992 is explained by a greater mortality rate during pre- and postreproductive moulting. 1992 had an extremely dry period in the months of July and August. During the moulting process, isopods are much more susceptible to dessication and therefore the mortality rate is higher in drought conditions. In the period of biological activity (feeding and reproducing), which lasts from April until November, a variation in the proportion between the two sexes can be seen. In the first months, (May and June), which coincide with the period of maximum reproductive activity, the percentage of males captured in the Barber traps is greater. The number decreases toward the end of the reproductive period and oscillates slightly around the value of 50 : 50%. We did not take into consideration the results of the collections of March 1992 when only 2 and 3 individuals were collected.

Our results indicate that the sex ratio of *T. difficilis rotundatus*, which is approximately 1 : 1, is genetically determined. The variations which appear over a period of time can be determined by a number of factors. It has been shown that gravid females are less active during the reproductive period; they remain generally hidden and eat less [2; 13]. The greater percentage of males in the first half of the reproductive period is due, to a large extent, to the decreased mobility rate of the gravid females, resulting that fewer of the latter are captured in the Barber traps. In the following period there was a numerical equilibrium between the two sexes until the end of the period of activity (November). An increased rate of female mortality during the reproductive moulting also contributes to the numerical differences between sexes. The males of some other species, such as *Protracheoniscus politus*, die in large numbers after reproduction [12].

Reproductive period

The reproductive period of the *T. difficilis rotundatus* population of Cheile Turzii is from the beginning of June until the end of August. (Table 1; Figs. 2). The first gravid females (under 50%) were collected at the beginning of the month of June, from which we can presume that

Table 1

The number of adult males and reproductive and nonreproductive females of *Trachelipus difficilis roundatus* collected in 1991 and 1992 in the Cheile Turzii Natural Reservation

1991

Sample date	Total no. of animals	No.of males	No. of females	No. of gravid females	% of males	% of females	% of gravid females	x^2
May 1	28	7	21	0	25.0	75.0	0	7.000
May 16	209	122	87	0	58.4	41.6	0	5.860
May 30	335	221	114	0	66.0	34.0	0	34.176
June 13	408	240	168	29	58.8	41.2	17	12 705
June 27	454	344	110	72	75.8	24.2	65	120.600
July 10	248	103	145	135	41.5	58.5	93	7.112
July 26	157	106	51	45	67.5	32.7	88	19 260
Aug. 8	473	152	321	256	32.1	77.9	85	60.380
Aug. 23	390	153	237	86	39.2	60.8	36	24.330
Sept. 5	261	88	173	1	33.7	66.3	2	27.680
Sept. 18	224	119	105	1	53.1	46.9	1	0.875
Oct. 3	726	346	380	0	47.6	52.4	0	1.592
Oct. 18	265	111	151	0	43.0	57.0	0	5.162
Nov. 1	48	24	24	1	50.0	50.0	4	0.000
Nov. 15	66	33	33	0	50.0	50.0	0	0.060
Nov. 28	61	26	35	0	42.6	57.4	0	1.320
	4353	2198	2155		49.0	51.0		0.421

1992

1	2	3	4	5	6	7	8	9
Mar. 13	2	1	1	0	50.0	50.0	0	0.000
Mar. 27	3	3	0	0	100.0	0.0	0	3.000
Apr. 10	93	46	47	0	49.4	50.6	0	0.010
Apr. 21	66	22	44	0	33.3	66.7	0	7.330
May 5	79	42	37	0	53.1	46.9	0	0.310
May 22	409	304	105	5	74.3	25.7	1	96.820
June 5	245	118	127	46	48.1	51.9	10	0.330
June 19	1052	734	318	203	69.7	30.3	64	164.500
July 3	306	140	166	149	45.7	54.3	90	2.200
July 17	226	169	97	81	63.5	36.5	83	19.480
July 30	226	110	116	101	48.6	51.4	87	0.150
Aug. 31	91	27	64	40	29.6	70.4	62	15.040
Sept. 14	209	102	107	0	48.8	51.2	0	0.110
Sept. 25	48	26	22	0	54.1	45.9	0	0.330
Oct. 9	168	80	88	0	47.6	52.4	0	0.190
Oct. 23	56	26	30	0	46.2	53.8	0	0.142
Nov. 6	77	39	38	0	50.6	49.4	0	0.010
Nov. 20	36	22	14	0	61.1	38.9	0	1.770
	3432	2011	1421		54.0	46.0		101.420

Sex rate for 1991 and 1992

Total no. of indiv.	No. of males	No. of females	% of males	% of females	X^2
7785	4209	3576	51.5%	48.5 %	51.46

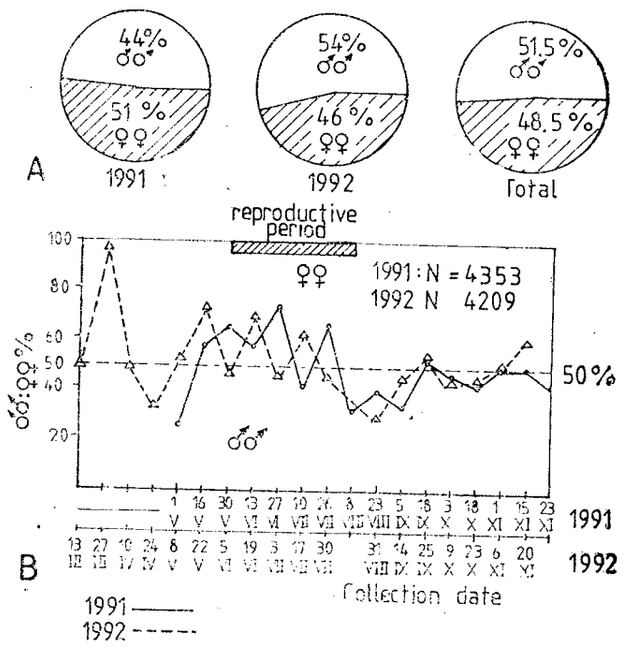

Fig. 1. — The percentage of male and female adults of the *Trachelipus difficilis rotundis* collected at Cheile Turzii in 1991 and 1992 (A) and the percentage relationship for males and females in each collection (B).

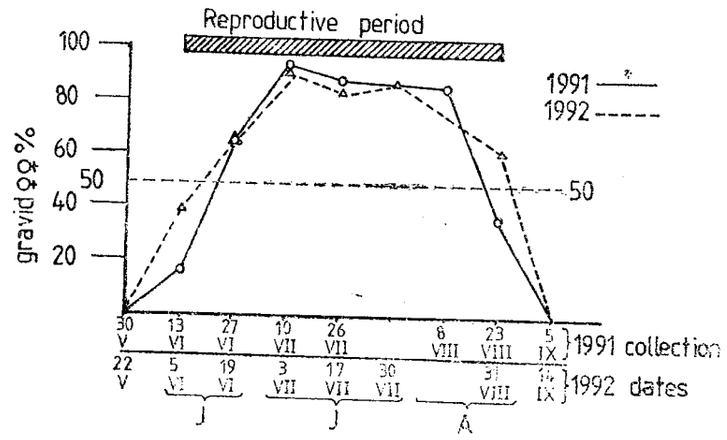

Fig. 2. — The percentage of gravid adult females in the reproduction period.

mating took place in the second half of the month of May. The last of the larger collections of gravid females was collected, during the two years, by the end of August. Sporadically, in the collections of Sept. 5th and 18th, gravid females were found : 4 and 1 individuals found among a number of 173 and 105 adult females, respectively (Table 1). We consider that the reproductive period of the species, in which at least 5% of the female adults are gravid, finished by the end of the month of August.

The peak reproductive period, in which about 90% of the females are gravid, is in July and the first half of August (Figs. 1).

The reproductive period of terrestrial isopods also depends on the number of broods produced by a female in a year. The species which produce two broods per year, as for example *Ligidium hypnorum*, *Hyloniscus transylvanicus*, *Trichoniscus pusillus*, *and Trachelipus balticus*, have a longer reproductive period for the population, extending from May until the end of August [8 ; 13 ; 15]. *T. difficilis rotundatus* also enters in this category. However, the beginning of the repoductive period is in June, later than of the other species, probably due to the lower temperatures in the ecosystem in which this species lives. The reproductive period of *Protracheoniscus politus* and *Porcellium conspersum*, which produce only one brood per year, is shorter, being in June—July [12 ; 14]. The beginning and the end of the reproductive period is determined by the length of the photoperiod and by temperature [4 ; 5]. There exists a synchronism between genetic and ecologic factors which determine the reproductive activity in the seasonal period with optimum conditions [1 ; 3 ; 10].

Fecundity

The fecundity of female isopods varies intraspecifically in relatively large limits according to the length of the female and the number of broods produced per year [1 ; 5 ; 11 ; 12—15]. The fecundity of *T. difficilis rotundatus* was studied on a number of 1153 gravid females which were collected in Cheile Turzii in 1991 and 1992. Female fecundity varies according to size (Figs. 3) and the number of broods produced per year. According to the number of eggs in the marsupial pouches and the collection date, we could ascertain that the females that are of 10 mm length or longer produce two broods per year. The first brood is produced in June and the first half of July and the second brood in the second half of July and August. Fecundity of the first brood is larger than that of the second, a phenomen which is also observed on other species of terrestrial isopods [8 ; 13 ; 15].

The average number of eggs calculated for the first brood shows a positive correlation between the length of the female and fecundity ($r = 0.98$) with a linear increase (Figs. 3, 4). Females of 10—14 mm represent the largest part of the population of this species (80%) and therefore make the most significant contribution to reproduction. The number of eggs per female in this size group varies between 32.7 and 58.5. Females of 8—9.9 mm body length make a much smaller contribution to reproduction due to the fact that they produce a smaller number of young, as well as the fact that their ponderance in the population is much smaller. The fecundity of reproductive females of *T. difficilis rotundatus* varies largely, being between 26.1 per female for the females in the 8—8.9 mm body length category and 81.7 per female for those in the 16—16.9 mm category.

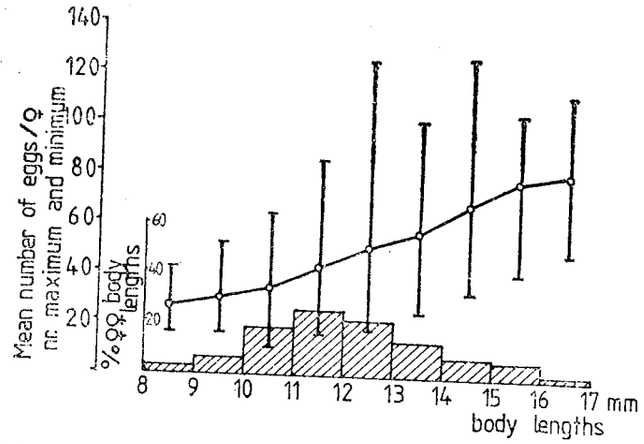

Fig. 3. — The relationship between the number of eggs per gravid female and the body length (r = 0.98).

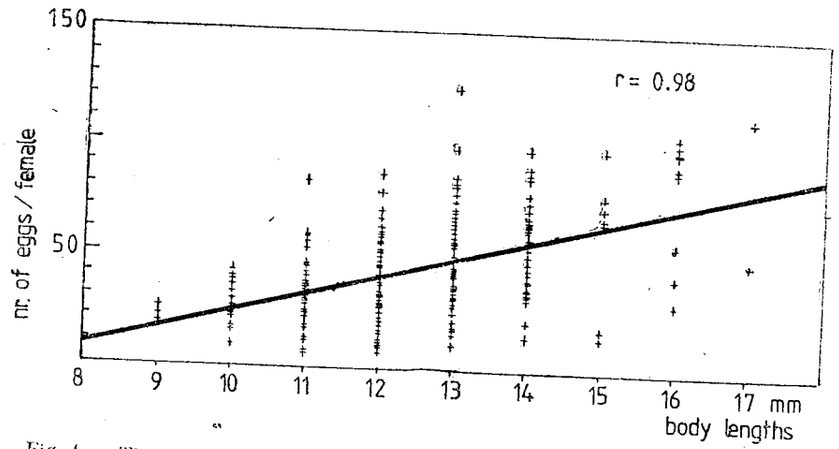

Fig. 4. — The regressions of the number of eggs per female on female body length.

CONCLUSION

The sex ratio of *Trachelipus difficilis rotundatus* indicates a numerical equilibrium between the two sexes of this population. In dry years the mortality of reproductive females during the pre- and postreproductive moults is greater, leading to a decrease in their number. The equilibrium is reestablished in the following generation.

The reproductive period of *T. difficilis rotundatus* is from June until the end of August. The peak of the reproductive period, in which over 90% of the females are gravid, is in July and the first half of August. Females with a body length of over 10 mm produce two broods per year.

The average number of eggs per female varies between 26.1 for the females with a body length of 8—8.9 mm and 81.7 for the females with a 16—17 mm body length. There exists a positive correlation between the number of eggs and the length of the females (r = 0.98).

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THE MORPHOLOGY OF ENDOCRINE RETROCEREBRAL GLANDS WITH *PALINGENIA LONGICAUDA* (OLIVIER), *INSECTA, EPHEMEROPTERA*, FAM. *PALINGENIIDAE*

A. SĂFTOIU

The retrocerebral endocrine glands are described by anatomical and histological methods, at terminal stages of development : nymph, subimago and imago with *Palingenia longicauda*.
Corpora cardiaca presents the most peculiar structure by extending its borders towards the ventral side and by achieving a perioesophagus "cardiac muff" in the median region. Ventrally lateral ; these cardiac borders have close contacts with the two symmetrical groups of vesicles belonging to corpora allata. This plurivesicle gland (c.al.) reaches a maximum of development and activity in subimago without showing any involutive signs.
The ventral glands reach a maximum volume at medium nymph disappearing completely at subimago.
These morphological particularities of the endocrine retrocerebral endocrine glands offer new discussions in the field.

Retrocerebral endocrine formations have been described even from 1913, when NABERT signals out corpora allata by *Cloëon dipterum* larva. B. HANSTRÖM described completely the endocrine glands by *Ephemera vulgata* and *Cloeön sp.* in 1940, printing out an uneven gland situated behind the brain and in contact with it (gland subsequently identified as corpora cardiaca) for the first time. This author also described the innervation of the retrocerebral system.

Later on, Pflügfelder [6] ascertains the number of four endocrine formations by Ephemeropteres describing the "pericardial glands" and the ventral ones situated at the cephalo-prothoracic limit.

P. Cazal, [3], resumes their study bringing along some completions. Thus, he describes the median uneven supraaortic gland calling it the "paracardiac body" printing out its dorsal position as a singular situation in the group of insects. Its histological structure resembling a nervous ganglion is described. The histologic structure of corpora allata is also described in details.

A perfecting of the situation of the endocrine glands is however made by L. Arvy and M. Gabe [1], who minutely describing the histology of the retrocerebral formations by Ecdyonuriidae, considers them the most primitive, as concerns the endocrine structure.

We held some apart morphological peculiarities of the endocrine glands from the study of the protocerebral neurosecretion of the *Palingenia longicauda* [7], that we are pointing in the present work.

MATERIAL AND METHODS

We have used terminal stages of development by *Palingenia longicauda*, in the last 15—20 days of the last year aquatic development. The studied stages were : young nymph, medium and advanced, subimago

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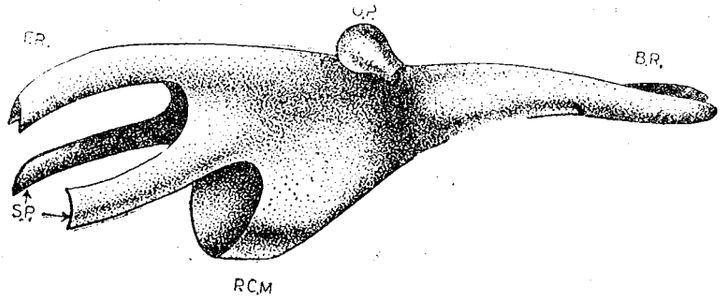


Fig. 1. — The scheme of the ensemble structure of corpora cardiaca with *Palingenia longicauda* : front region, binder region, side protuberances, perie-sophageous cardiac, muff, Palmen organ.

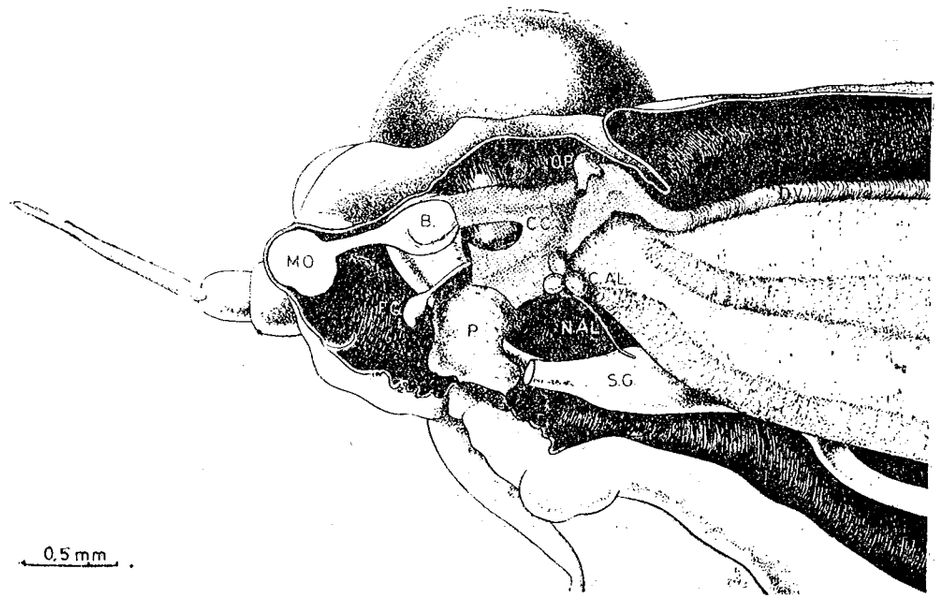


Fig. 3. — The reconstitution of cephalic area by sagital sections with subimago : dorsal pharyngeal muscle, dorsal vessel, pharynx (front intestine).

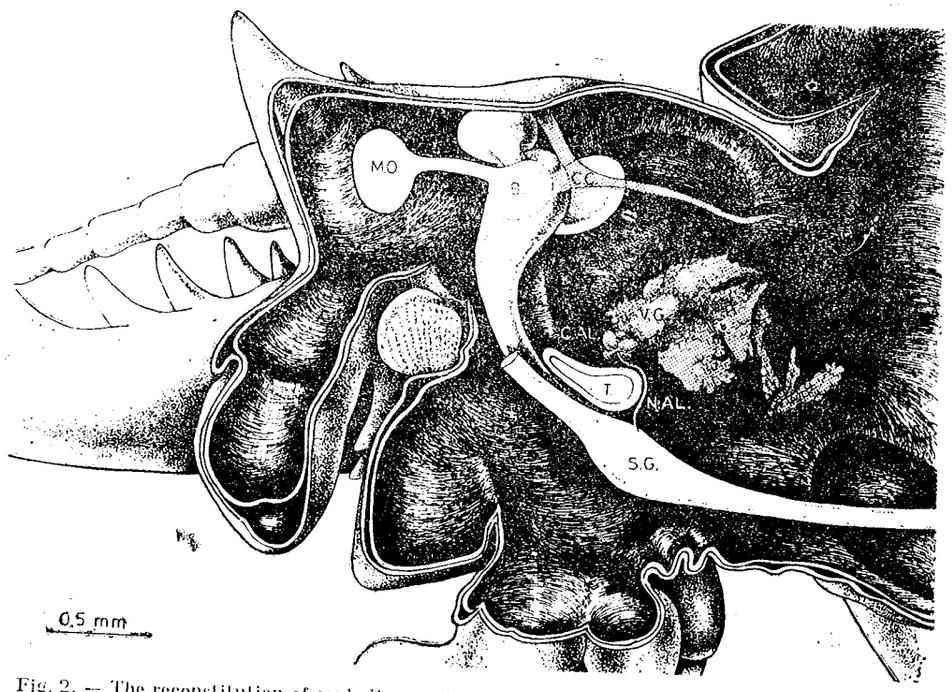


Fig. 2. — The reconstitution of cephalic area by sagital sections with advanced nymph : brain, protocerebron, c. allata, c. cardiaca, allat nerve, right ventral gland.

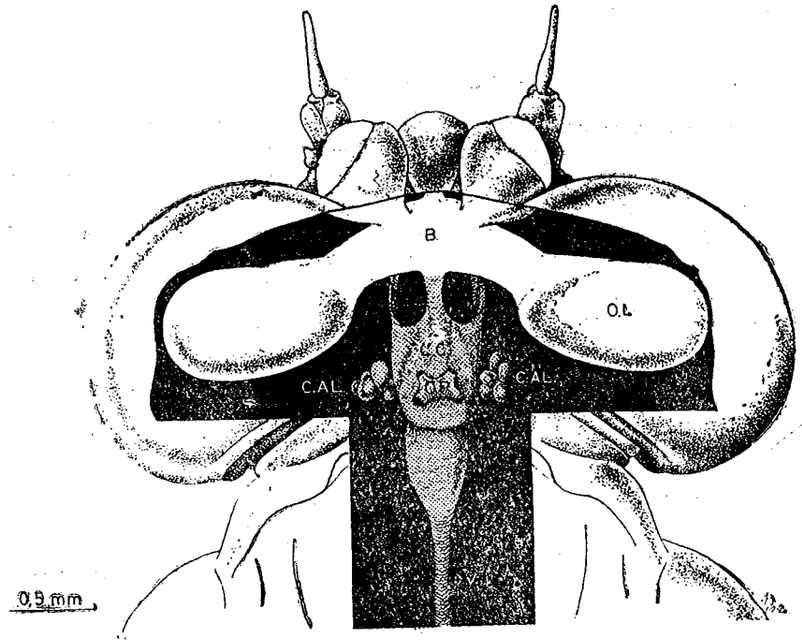


Fig. 4. — Dorsal view of cephalic area by subimago.

and imago of both sexes. They were collected and fixed during the spring swarming in the Danube, directly in nature as their transport and breeding in the laboratory proved to be most difficult.

We used as fixers: Bouin, neuter formol, Susa, Helly, Zenker for 1—3 hours. The insects were got ready for fixing by sectioning the digging mandibles, the feet by practising some incision into the thorax and abdomen leaving however some individuals intact for comparison. We ensured a good fixing by constantly maintaining the anatomical pieces in immersion.

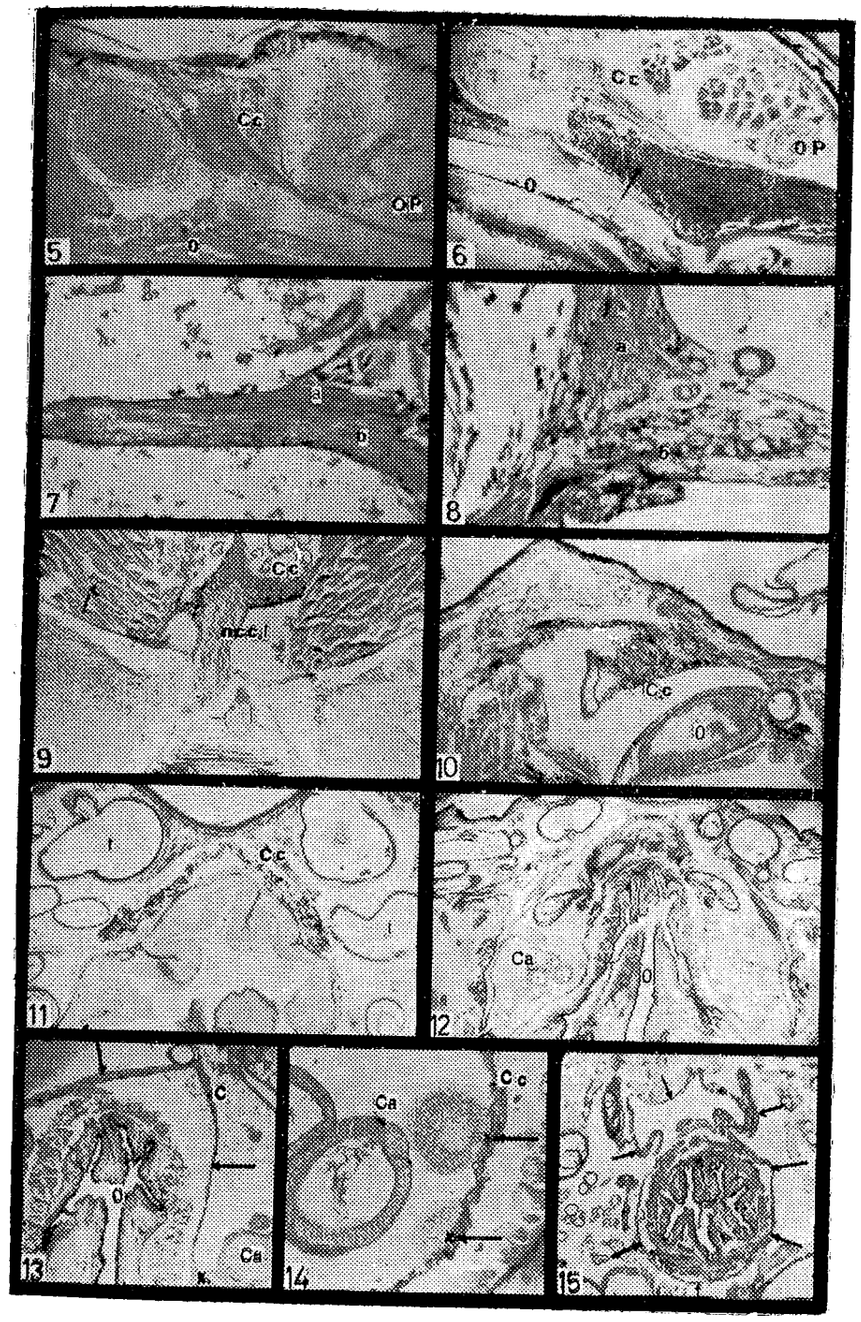
The paraffin inclusion was made in, the pieces were sectioned at 7—10—25 μ. The histological stainings were topographically usual: Hemalaun-erythrosine, or specific: Azan (Haidenhain), Paraldehyde-fuchsine (M. Gabe).

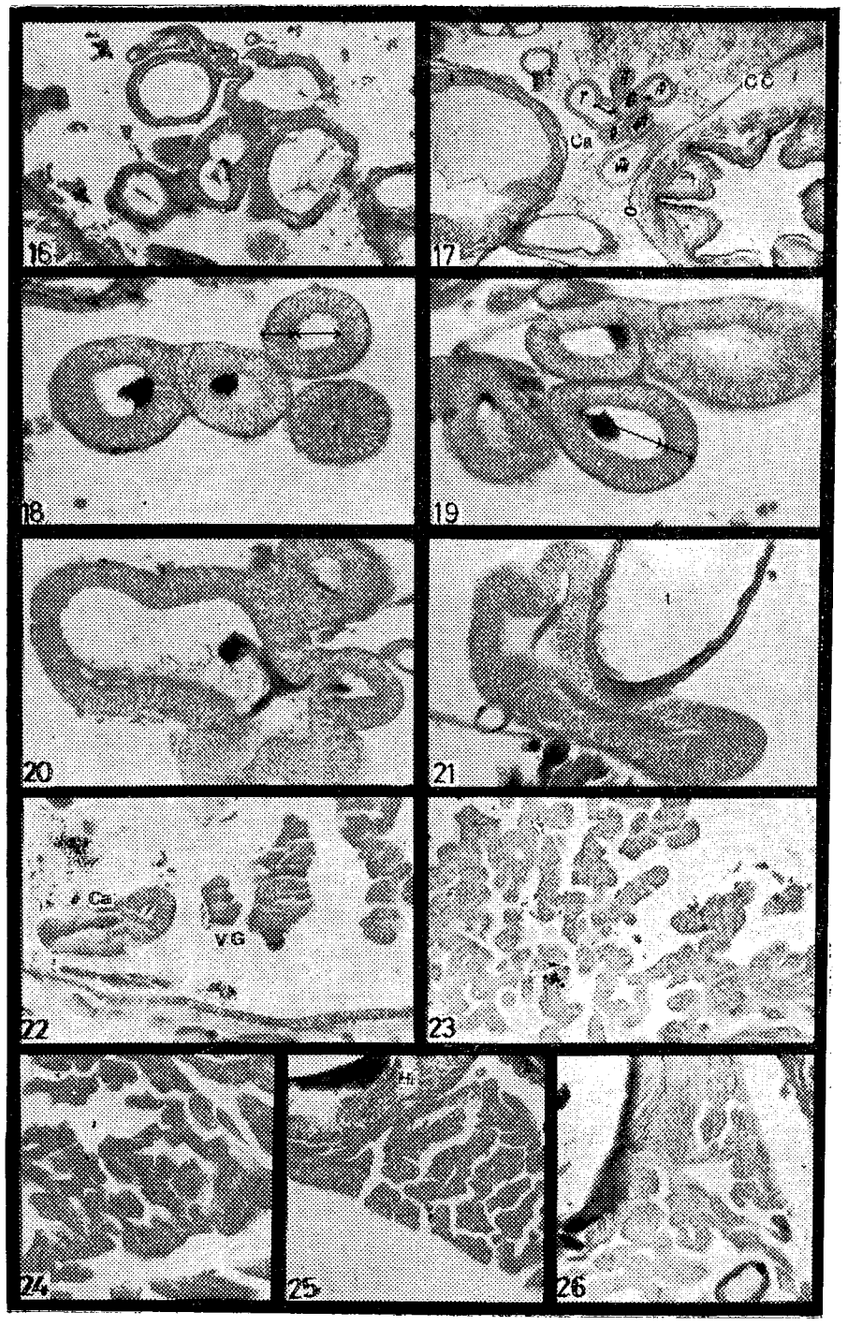
For the anatomical reconstitution, thick sectionings (25 μ), microdissections or gradual sectioning and dewaxing of some fragments by us were used. The dissections were made on living material fixed and dehydrated or material inserted in paraffin for separate inclusion of endocrine organs only, keeping the relations among them and the innervation. The aspects thus obtained were completed with the histological results (thick sectionings and sectionings of common thicknesses). Cutting by certain plans some of the pieces inserted in paraffin, we obtained fragments that after dewaxing may be observed as such or may be fixed in Canada balsam. The decortication of several plans of the parafined piece, especially of the massive tissues (muscular, fat) allowed the ideal approach to the aimed anatomical region.

RESULTS OBTAINED

1. *Corpora cardiaca.* Referring to the two internal cardiac nerves we noticed that their initial parts are short owing to the apart configuration of the two neuronal areas origin (the median isles of pars intercerebralis). These two cellular neurosecretory groups have a very front position and the axonal cone lowers deep ventrally where the initial axonic prolongation cf. n.c.c. I dispose like this: in a fan with a large basis. They describe immediately a slight side-external deviation and then start the vertical routes that reach in the rather law in the cerebral floor, following the semicircular routes, well known to all species of Ephemeropteres and other Insects. However, their last parts, still intracerebral, look like a "precardiac tank" where the axons outstrip, become winding and create thus lacunose distances where the neurosecretion granules increase their volume without reaching the dimensions of corpora cardiaca drops (Fig. 8,9). After these small final dilatations, the axons continue linearly, forming behind the brain an independent hilum that precedes the entering into the tank gland.

The most active periods of the n.c.c. I did not show a massive filling with lines of intraaxonic granules by Palingenia as presented by the small size species (Baëtidae, Caenidae). The granules keep their individuality and they seldom fuse into short traces separated by clear parts. Many of the ↔ axonic fibres remain empty owing to the inactivity of the unstudied terminal stages, of some of the neurosecretory median cells.





The beginning of the filling of these two cardiac nerves as well as their emptying are stages that may be concluded after disposing the granules of product and by the region (anterior or caudal) that they occupy at a certain moment. Minimum levels of the neurosecretory activity cannot pass unnoticed through these two cardiac nerves.

As concerns the external cardiac nerves (n.c.c. II), they have not been observed in a leading activity at any of the terminal stages.

Corpora cardiaca has a very special morphology representing, perhaps, an intermediary link between the *Prosopistoma foliaceum* type and the general type of gland anatomically independent of corpora allata. The maximum volume is reached by this gland (c.c.) at advanced stages, (subimago and imago) its peripherical anatomical limits being clearly marked, now, by neurosecretory stored granules that fill all the existing spaces.

It is a supraaortic organ whose margins lower much laterally, reaching, on the one hand, an intimate neighbourhood with the vesicles of corpora allata, and on the other hand, establishing a real anatomical perioesophagus continuity.

The front cardiac region, ventrally approached by the two hiles of n.c.c. I, leans upon the protocerebral neurilema in the posterior median ditch then it is caught between two strong muscles (dorsal pharyngeal) (Fig. 1, 9). This part continues caudally, constituting the most massive part up to the neighbourhood of the Palmen organ; in its thickness, the own cardiac area is to be found (Fig. 5). Even from the young nymph, the two cardiac internal areas distinguish themselves (Figs 7, 8); the dorsal areas constituted of a cellular massif with affinities grown for fuchsine and the ventral area with numerous blanks and with chromophobous cells bigger than in the first area.

The apex cardiac region, found immediately after the posterior edges of the two pharynged muscles, lowers laterally covering the upper

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Fig. 5–15. — Histological structure of corpora cardiaca by *Palingenia longicauda*.
5. Sagital section in the cephalic area of advanced nymph ♂. Zenker, Paraldehyde-Fucksine. 6/6,3 ×. 6. Deposit of figurated elements caught in the aorta sinus by young nymph ♀, sagitally sectioned. Z, P–F, 6/6.3. 7. Sagital section by medium nymph ♀. The areas of c. cardiaca. Z, P–F, 120 ×. 8. The same structure in areas of the fore part of c. cardiaca by young nymph ♀ in sagital section. Z., P–F, 250×. 9. The going out of n.c.c. I from the brain and its penetration in c.c. in the front section at subimago. Z., P–F., 10/6.3×. 10. Side-sagital section at subimago. The side borders of c.c. with grains of stored material may be noticed. Z., P.–F., 10/6.3×. 11, 12. Front sections in c.c. from imago ♂. Bouin, Z., P.–F., 10/6.3×. 13. Caudal borders of c.c. at subimago ♀ (cross section). Z., P.–F., 10/6.3×. 14. Relations c.c.–c.al. (vesicles) in the ventral side area (cross section at subimago ♂). Bouin–Z., P.–F., 200 ×. 15. Cardiac periesophagus muff in cross section at imago ♂. Bouin–Z., P.–F., 10/6.3×.
Fig. 16–21. *Histological structure of corpora allata (c.al.)*
16. Allate vesicle groups by young nymph ♀ in sagital section. Halmi–Zenker, P–F., 150 ×; 17. Some formation at subimago ♀. Halmi–Z., P.–F., 10/6.3×. 18, 19, 20. Allate vesicles with central body (residual chitin) by advanced nymph ♂ in sqgital section Z., P.–F., 200 ×. 21. Allate confluent vesicles (subimago ♂, front section). Halmi–Z., Azocarmin, 200 ×.
Fig. 22–26. *Histological structure of ventral glands.*
22. Close relations v.g.–c.al. by young nymph in sagital section. Z., P.–F., 150 ×. 23–26. The structure of belts of glandular cells and the relations with the hypodermis (Hi.) in sagital sections Z., P.–F., 120 ×.

third of the pharynx and presenting two protuberances (left and right), that, surrounding the bottoms of the two muscles, reach frontly near the brain.

On the whole, this first anterior third, immediately retrocerebral, represents a pipe with edges side-oesophagially much lowered, that leaves loose two large ovoid spaces through which the fascicles of the two muscles mount (Fig. 10).

Further, the cardiac edges lower more and more starting the constitution of the "cardiac muff" in the region of the second pharyngeal curvature. In this side ventral area, close contacts establish with the vesicles of the corpora allata, without clear nervous links or passages of substances rendered evident by the technics used (Figs 11, 12, 13, 14). Finally, the cardiac muff completes, circling completely around the ventral part of the pharynx (Fig. 15). This region of corpora cardiaca is, as the whole rest of the gland, superposed on the aortic sinus. In some cases, when a large quantity of elements found sanguineous were surprised by a histological fixing we may ascertain that the area of "cardiac muff" doubles, as a matter of fact the cerebral aortic sinus that, in its turn, in this place, also presents a muff that extends itself ventrally under the oesophagous, surrounding it completely (Fig. 6).

The perioesophageal ventral areas of the cardiac muff are rather thin, contain mainly blanks where the granules of stored neurosecretory product may be seen, and represent, as a matter of fact, the stakes of the tank gland, as well as scarce chromophobous cells.

The dorsal cardiac regions and the bottoms of the side walls in the neighbourhood of the Palmen organ have medium thicknesses compared to the front cardiac part and are lacking the cellular elements of the own secretory area.

The caudal part of corpora cardiaca extends up to the cephalo-protoracic chitinous fold (Figs 2, 3, 4) being, further on, at this level too, marked out by fuchsinophilous-paraldehyde granules but scarcer. In some cases, this last part forks, forming two side protuberances of the dorsal vessel.

At young nymph II, a corresponding stage of a first maximum, an important filling of corpora cardiaca takes place. Only a small front and dorsal cardiac region remains without a stored neurosecretory product but definitely framed at edges by paraldehyde-fuxinophilous granules.

At nymph III, where the neurosecretory activity stops from pars intercerebralis, there takes place a rapid emptying of the corpora cardiaca and afterwards, an important increase in volume of the gland starts. It increases its general volume extending more and more caudally, and in the inside, the achieving of the own area is continuing which now becomes evident to both sexes. At the end of this nymphal stage, we may ascertain that this endocrine gland reaches the maximum size by *Palingenia longicauda*.

Subsequently, by airy stages (subimago and imago), the volume of corpora cardiaca remains big but the first involutive signs appear in the cardiac own area (front-dorsal) materialized in nuclear alteration and a diminishing of the cellular tinctorial affinities. Opposed to the nuclei of chromophobous cells in the proper blank part those from the area with own secretion elongate and get a winding form.

Therefore, by *Palingenia longicauda*, the own cardiac activity begun in the nimphal stage II in the spring of the year of aquatic development is of a short period, ending as the volume and histological indexes show it, with the neighbourhood of subimaginal emergen afterwards diminishing involutively during the short airy life.

As an exception to this characteristic of corpora cardiaca, we met an apart lobulary derivation by the histological preparation with a single nymph; it was situated very dorsally under the hypodermic of the cephalic capsule. The oval lobe, apparently isolated from the rest of the gland, contained however paraldehyde-fuchsinophilous material of protocerebral origin and presented a blank characteristic structure. This case, without a special anatomical consequence, completes other anatomical-histological aspects of structural variability of the neuroendocrine system of some ephemers.

2. *Corpora allata*. This formation is constituted by *Palingenia longicauda* from two symmetrical groups of each 4—8 vesicles confluent in a common centre (Fig. 17) and disposed side-ventrally the pharynx. The most caudal parts of the vesicle groups lowers under the intestine edges (Fig. 3, 4).

With the medium nymph, the allate vesicles have another thin, unstratified epithelial wall, the inner cavity having a diffuse contents, homogeneous with slight affinities for the great majority of the used colourings. The vesicle cavities contain each a central body: lineary, slightly sketched at this stage (Fig. 16).

At the advanced nymph stage (Fig. 18, 19) the glandular epithelium thickens becoming pluristratified, the lumen gets sensibly smaller and the central body, considered by the great majority of the authors as a simple chitinous deposit, resulted from a repeated moulting process, gets bigger. There is a gradual increase of the allate vesicles that get to function more and more in the central region of the vesicle group marking the frontier of a unique increased cavity (Fig. 20); here, in these vesicles faint filamentous trama of a product with slight acidophily, partially preserved by the used histological fixings, may be observed. The central body in these increased cavities, intensely paraldehyde-fuchsinophilous, has no lamellar structure as found by other insects, but appears formed by agglomerations with granula structure.

At subimago, the allatal vesicle ensemble reaches the maximum development (Fig. 17) also establishing the largest direct contact with corpora cardiaca. The vesicle contents, also slightly colourable, fills now the whole lumen having though a delicately granulated and homogeneous structure. On the edges of this deposit, in the immediate neighbourhood of the vesicle epithelium, vacuolization that suggests a secretory activity of the allate cells may be observed. Their nuclei (Fig. 21) do not show the signs of a picnotic involution, but on the contrary, they have a normal aspect that expresses a glandular activity of an adult type, the innervation of corpora allata is of suboesophagous origin as by other Ephemeropteres species. The afferent allatal nerves innervate the vesicle group,

with middle, by short routes that leave then the corpora allata and penetrate the ventral glands after describing some posterior side routes.

3. *The ventral glands.* They reach the maximum development by young nymph II, too (Fig. 2) when presenting their complete structure. The symmetrical massifs composed of cellular stripes are disposed sidewards and medium-ventrally behind the tentorium and reach an impressing volume as they are the biggest by *Palingenia longicauda* compared to the other endocrine glands.

A single glandular massif, approximately globulose, presents protuberances on its entire surface. Caudally, two contorted protuberances that extend to the initial prothorax cavity may be observed. The cellular massif, convex on its external side, presents internally a concavity penetrated by two deep ditches: one is oblique, dorsal-ventral and another one, more delicate, horizontal and front-posterior. These hollows show the marks of some cephalic organs (trachea trunks) on which the proliferative massif moulds gradually by increase. They mark frontiers among distinct regions. Thus, the front part as well as the posterior one are bilobed. The two posterior lobes of the ventral gland, as we look at the internal side, are unequal compared to the front lobes, the ventral lobe being larger and having a clear globulous shape.

Deriving from the hypoderm, the two cellular massifs still keep connections with it, under the shape of some large hils placed side-median (Fig. 25, 26). From these hils caudal protuberances with sinuous routes from cellular strings detach.

The cell strips that make the two massifs of the ventral glands contain reduced sinuous spaces among them and this allows an eventual drainage. The internal front-ventral angle of each massif that finds itself in the close neighbourhood of the corresponding corpora allata presents the area where the allatal nerves of suboesophagous origin address to these formations, too.

As concerns the volume evolution by young nymph I, the ventral glands have a medium size presenting normal cellular and nuclear aspects. But in short time, up to the stage of young nymph II or III, a rapid cellular increase is taking place, the proliferations leading to a maximum volume of the endocrine formations.

With medium nymph having browny pterotheca they start their involution that develops rather slowly in the first part. By the advanced nymph, however, the nuclear degenerescence is produced in an accelerated rhythm in the cellular strings while the glandular massifs reabsorb.

The so much extended area they occupied remains in the end in the subimago stage, completely emptied. Seldom, only with female subimago, the ventral glands with stagnant cellular aspect and not degenerative, may be still observed. But in these cases, the involution will be extremely accelerated developing during a few hours.

This disappearance of the ventral glands with adults is signaled out in the special literature for Insects. By Ephemeropteres it produces with *Palingenia longicauda* but by other species (among which Caenidae and Baetidae), the glands persist till the end of the adult life.

DISCUSSIONS

Willey [8] described by Blattidae the existence of two massive small bridges between corpora cardiaca and corpora allata calling them "the nerves of corpora allata I", without specifying, however, if they are pure nervous formations and do not contain blank spaces or specific cardiac cells. Their massiveness allows them to be considered as a cardiac-allatal anatomical contiguity rather than individualized nerves. Rather similar to this situation is the close contact and of a simple proximity between these two endocrine glands, observed by *Palingenia longicauda.*

The "anatomical small bridges" of Blaberus-Periplaneta and the intimate contact of Palingenia suggest a red primitiveness of the anatomical situation of the area under discussion, previous perhaps to the configuration of *Prosopistoma foliaceum* (Ephemeroptera) where no suboesophagous innervation exists.

By Blatidae studied by Willey, there is a strong innervation, of suboesophagous origin of corpora allata (n.c.al.2), a partially similar situation to Palingenia where there is only one suboesophagous innervation of this gland.

Taking into account other species of Ephemeropteres that possess two distinct systems between them: the cerebro-cardiac system on the one hand and the suboesophagous-allatal system on the other, we may better understand the special importance of the close contacts between the two endocrine glands (c.c.—c.al.) from *Palingenia longicauda.*

This continuous anatomical way (cerebro-cardiac-allat-suboesophagous) approaches somehow to the situation, a lot more determined, however, of *Tomocerus minor* (Collembolae) where a true continuity has been described by Cassagnau and Jubertie [2].

Trying to frame the endocrine retrocerebral system with *Palingenia longicauda* into one of the morphological types established by P. Cazal [3] with Insects, we meet with difficulties because it would be necessary to correct the nearest semi-centralized system: the cardiac-allat link is achieved by this species not by individualized nerves but by simple anatomical contacts.

As concerns the ventral glands, Pflügfelder showed that a maximum of their development corresponds to the stage of subimago (for some species of insects) where the cells contain secretions under the form of acidophilous granules, then, with imago stage, the glands suffer a clear atrophy without disappearing.

L. Arvy and M. Gabe observed with Ephemeropteres, secretory phenomena described by Pflügfelder, placing them nonetheless by Ecdyonuriidae, in a more precocious stage (advanced nymph). From this point, the involution of the ventral glands becomes clear: the nuclei fold and the chromatine, rather lax under normal conditions, condense completing the picnosis image that is previous to karyolysis. The chondrioconts, during this involution, thicken and divide into fragments. At subimago, the involution of ventral glands is most advanced and at imago they completely disappear.

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SPECIFIC EFFECTS OF SOME FLAVONOIDIC COMPOUNDS ON THE MEMBRANE POTENTIAL IN VARIOUS ELECTROLYTIC MEDIA *

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The effects of escin and rutacyl (diacetylrutin) flavonoids (alone or in association) upon the membrane potential of striated muscle fibers in normal medium, either with high $-K^+$ or Ca^{2+} –free medium, were studied. Flavonoids evidence a specific membranotropic action, resulting in different bioelectrical effects, as depending on the extracellular ionic ratios. In normal medium, flavonoids induce membrane's hyperpolarization, which is ampler and more persistent in the case of rutacyl. In a high $-K^+$ medium, the agents intensity depolarization through K^+ (having therefore a similar action with K^+) and delay repolarization in a normal medium, the effects of escin being stronger in the depolarization phase, while those of rutacyl – in the repolarization stage. In a Ca^{2+} –free medium flavonoids reduce the depolarization induced by the absence of Ca^{2+}, having a similar action with this ion. Escin evidences stronger effects, yet it is more weakly bound in the membrane, compared with rutacyl which induces, therefore, membrane's hyperpolarization on re-establishing the normale concentration of Ca^{2+}. Association of the two flavonoids does not modify the direction of their separated effects, but only their amplitude and stability.

Flavonoids are known as biologically-active agents with properties of the vitamin P and synergetic actions similar to those of vitamin C, as well as specific effects on the permeability of sanguine capillaries, on the properties of the cell membrane and on some metabolic processes, being therefore applied in the therapy of certain vascular diseases (6), (15), (18), (20).

The natural products are quite varied, depending on their chemical structure, represented by aglycon and associated glucides, as correlated with their biological activity (2), (4), (7).

Their bioelectrical actions have been little investigated (11), (12), (13), which determined us to concentrate the present study on the effects of two products – escin and rutacyl – as such or associated, on the membrane potential, in physiological solutions, either normal or with a modified $K^+ : Ca^{2+}$ ratio, for obtaining data on their mechanism of action at the cell membrane level, either under normal conditions or on the membrane depolarization through increased K^+, or in the absence of Ca^{2+} (1), (5), (9), (10), (16).

Escin is a natural flavonoid, extracted from *Aesculum hippocastanum*, having a complex chemical structure and therapeutical properties in vascular diseases (2), (4), (7); rutacyl is a hydrosoluble diacetylated derivative of the natural flavonoid rutin, possessing interesting pharmacological properties (14).

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MATERIAL AND METHODS

Experiments have been performed *in vitro*, on the membrane of striated muscle fibres of frog (*Rana ridibunda*, Pall.) sartorius muscle taken over from 5 animals for each experimental batch. The membrane potential (MP) has been determined by the technique of glass intracellular microelectrodes.

Normal Ringer solution (NR) has been employed with bicarbonate buffer and pH = 7.2, or having modified ionic ratios (with K^+ increased from 2.5 mM to 30 mM, or Ca^{2+}-free).

Concentrations of flavonoids have been equivalent to those applied in therapeutics (4), on using similar preparations: escin 0.2 mg/100 ml; rutacyl 6 mg/100 ml; maintained too, when associating the two agents. MP measurements were made at room temperature (22°C), at time intervals of 10 minutes, for one hour, for each electrolytical medium employed.

RESULTS

In a normal medium, the flavonoids considered in our study induce MP increase (hyperpolarization) (Fig. 1). Thus, escin (0.2 mg/100 ml) determines an average hyperpolarization of 2.65 mV, representing 2.95%

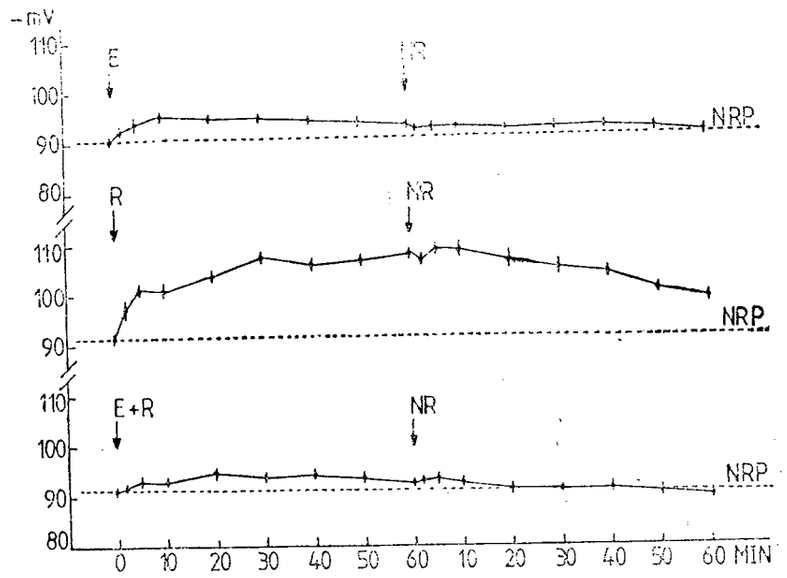

Fig. 1. — The effects of flavonoids on membrane potential in normal physiological medium (NR: normal Ringer): E: escin (0.2 mg %); R: rutacyl (6 mg %): E + R: escin (0.2 mg %) associated with rutacyl (6 mg %); NRP: normal resting potential.

of the values of the normal resting potential (NRP), hardly reversible on washing the muscle with NR without agent. Rutacyl (6 mg/100 ml) induces a stronger hyperpolarization — of 12.45 mV (13.62% from NRP), very persistent, after washing with NR for 60 minutes the hyperpolarization being of 8.21 mV (8.97% from NRP). Association of escin with rutacyl leads to a more reduced hyperpolarization — of 1.39 mV (1.59% of NRP), easily reversible bringing back the muscle in NR.

Control experiments with 30 mM K^+ (Fig. 2—C) have evidenced that increased K^+ induces an ample and rapid reduction of MP (depolarization), presented in others papers, too (1), (10), (16), (19), with an amplitude of 40.48 mV (44.58% of NRP) after 10 minutes and of 48.07 mV (52.93% of NRP) after 60 minutes. In NR there occurs NRP recovery (repolarization), nevertheless the phenomenon is slower than depolarization, due to the functional asymmetry of the two sides of the membrane (8), (19). Thus, depolarization is reduced to 35.77 mV (39.39% from NRP) after 10 minutes and to 5.77 mV (6.35% of NRP) after 60 minutes.

The action of flavonoids in high—K^+ medium leads to stressing of membrane depolarization as compared to that determined by K^+ alone (Fig. 2). Thus, in the case of escin, the amplitude of depolarization after 10 minutes is of 48.61 mV (51.84% from NRP), while after 60 minutes, it is of 55.94 mV (59.66% from NRP). In the case of rutacyl, the effect is weaker, depolarization being equal to that of the control sample in the

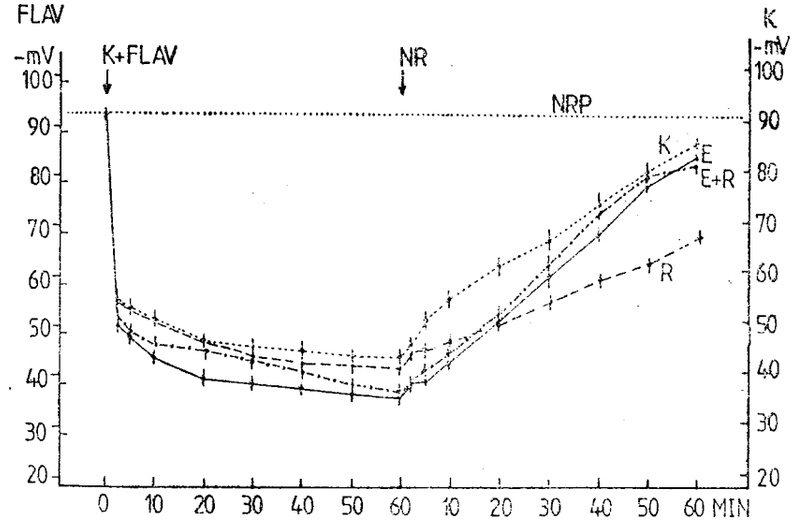

Fig. 2. — The effects of flavonoids on membrane potential in high—K^+ medium (30 mM). C: control (30 mM K^+): K + FLAV.: high—K^+ + flavonoids. Other explanations — see Fig. 1.

first 20 minutes and of 49.81 mV (54.47% of NRP) after 60 minutes. Association of the two flavonoids evidences a depolarization of 44.89 mV

(48.50% of NRP) after 10 minutes and of 54.51 mV (58.90% of NRP) after 60 minutes.

Repolarization of membranes in NR without agents is nevertheless delayed, as compared with that of the control experiments. Thus, after 60 minutes washing with NR the membranes remain depolarized with 8.23 mV (8.78% from NRP) in experiments with escin; with 23.38 mV (25.46% of NRP) in those with rutacyl, which, actually evidences a stronger retarding effect of repolarization, and with 8.30 mV (8.96% of NRP) in those with associated agents which have, nevertheless, a stronger effect upon repolarization in the first 20 minutes.

In the Ca^{2+}-free medium (RCaF) a membrane depolarization takes place, as observed in other studies, too (1), (5), (9), (10), (16), (17), with an average amplitude of 5.40 mV (5.84% from NRP), easily reversible when re-establishing the normal concentration of Ca^{2+} (Fig. 3). This depolarization is similar to that determined by high—K^+ as, in both cases,

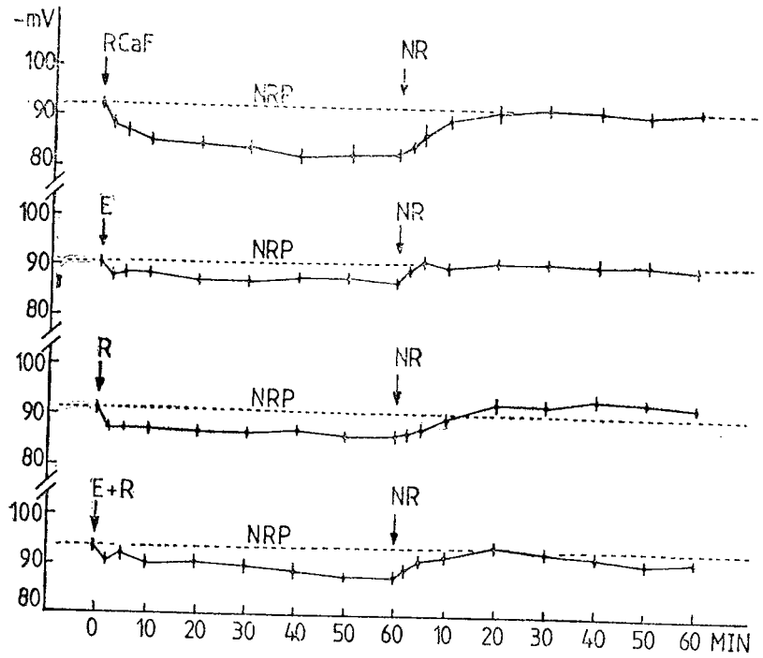

Fig. 3. — The effects of flavonoids membrane potential in Ca^{2+} — free medium (RCaF: Ringer Ca^{2+} — free). Other explanations — see Fig. 1.

an increase of the $K^+ : Ca^{2+}$ ratio occurs, yet the amplitude and development of this phenomenon is different as depending on the modification of the two ions (K^+ increase or Ca^{2+} decrease) (1), (5), (9), (10), (16), (17).

In RCaF, flavonoids determine an obvious reduction of the depolarization induced by the absence of Ca^{2+} (Fig. 3), up to 2.80 mV (3.09% of NRP) in the case of escin, up to 4.10 mV (4.47% of NRP) in the case

of rutacyl which has, therefore, a weaker effect than escin, and up to 3.47 mV (3.71% from NRP) on the association of the two agents. Depolarization is easily reversible on bringing the muscle back into NR, yet, in the case of rutacyl the recovery of NRP is followed by a relatively stable hyperpolarization, with an average amplitude of 2.54 mV (2.77% from NRP) determind by a stronger binding of this agent to the membrane in RCaF.

DISCUSSIONS AND CONCLUSIONS

The obtained results evidence that, in a normal electrolytic medium, the specific effect of the two flavonoids, alone or associated, consists in the hyperpolarization of the cell membrane. The amplitude of hyperpolarization is characteristic of each agent, the strongest effect being induced by rutacyl. The effects of both agents are highly stable when bringing the muscles back in NR without flavonoids, which evidences their relatively strong binding to the structure of the cell membrane. The hyperpolarization effect expresses a specific interaction of the flavonoids with the membrane's structure, more probably with membranary phospholipids, if considering the lipophilia of these agents, which are thus inserted into the membranary structure. This phenomenon might lead to a reduction of the membrane opening degree, and to lowering of the passive permeability, concomitantly with a stimulation of the $Na^+—K^+$ pump, as observed in other studies (3). Such an effect seems to be the most characteristic one for the properties of vitamin P of the flavonoids studied.

On increasing the $K^+ : Ca^{2+}$ ratio by high—K^+ or by the lack of Ca^{2+} in the external medium, by the action of K^+ in excess, reduction of the membrane packing degree occurs, in parallel with a modification of the ionic flows and membrane permeability (17), having a depolarizing effect (1), (5), (8), (9), (10), (16), (17), (19). Recovery of the normal concentration of K^+ leads to the recovery of the structure, of the membranary packing degree, of the normal ionic flows and of NRP, through the action of Ca^{2+}, whose ratio *versus* K^+ becomes normal again (1), (9), (10).

The membranotropic action of studied flavonoids depends on the composition of the electrolytic medium in which they manifest. Thus, in high—K^+ medium, the flavonoids intensify the membrane depolarization, with a similar effect to that of K^+, i.e. increase of the degree of the membrane opening, as opposed to that occurring in a normal electrolytic medium. More than that, being bound to the depolarized membrane the flavonoids delay NRP recovery on placing the muscle in NR, thus hindering the action of Ca^{2+} of re-establishing the structure and properties of the membrane, characterizing the normal $K^+ : Ca^{2+}$ ratio (1), (9), (10). Thus, the flavonoids intensify the membrane functional asymmetry (8), (19). The escin effects are stronger upon the depolarization stage, while those of rutacyl — upon the membrane repolarization stage.

The flavonoids under study evidence, therefore, specific membranotropic actions, having different effects upon the membrane potential, as depending on the ionic ratios from the extracellular medium.

Experiments performed in RCaF have evidenced that these flavonoids induce, under such circumstances, effects similar to those of Ca^{2+}, whose role they are supplying, to a certain extent, thus reducing the depolarization determined by its absence. In such situations, their effects have the same direction with those manifested in a normal electrolytic medium (Fig. 1), determining increases of the membrane potential value. Also, the observation is to be made that association of escin with rutacyl, in various electrolytic media, does not modify their separated effects, yet their amplitude and stability are diminished, which may be applied in the elimination of the negative effects of escin in therapeutics (lysis) (4), (7).

The results obtained suggest that the investigation of the specific pharmacological effects of flavonoids has necessarily to consider also the electrolytical conditions of the organism in the physiological situation given.

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IMMUNOMODULATING EFFECTS OF SOME LOW MOLECULAR WEIGHT AGENTS

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The effects of the acetylated products Pagosten-2 (hexaacetylmanitol) and Pagosten-3 (hexaacetyl-sorbitol) on the dynamics of total leucocytes, neutrophils, eozinophils, basophils, lymphocytes and monocytes of rabbits (Chinchilla) have been studied, comparatively to Rodilemid (immunostimulator) and Antifolan (immunosuppressor), taken as reference agents. The treatments have been applied through intramuscular injections, for six weeks, in doses of 2.5 mg/kg body/day for the acetylated products and in therapeutical doses for the reference agents. The analyses have been performed at intervals of two weeks. The observation has been made that the acetylated products evidence immunomodulator effects, influencing the dynamics of total leucocytes, as well as their types, as depending on the agent's nature, type of leucocyte and duration of treatment, the recorded effects of Pagosten-2 being generally more stable. Most of the effects manifested on the types of leucocytes are generally similar to those of Rodilemid; effects similar to those of Antifolan are manifested, too, depending on the duration of treatment.

Study of the possible control on the immune response by various techniques, methods and substances (immunomodulating agents) represents a major problem today, as to a possible utilization either for the induction, stimulation and amplification of the immune response (immunostimulating agents), or for diminishing or inhibiting of the response (immunosuppressing agents), as depending on the experimental or therapeutical requirements (5), (7), (9), (14), (16), (17). Special attention is paid to immunomodulating agents with low molecular weight, that may intervene in either activation or inactivation of the immune system, stimulating or inhibiting the synthesis of antibodies and their release in circulation (the humorally mediated immune response) or the formation of immunocompetent cells (the cellularly mediated immune response).

The present paper analyzes the immunomodulating effects *versus* the cellularly mediated immunoresponse, of some original products, such as acetylated polyolic derivatives Pagosten-2 (hexaacetyl-manitol) (Pag-2) and Pagosten-3 (hexaacetyl-sorbitol) (Pag-3), with significant pharmaceutical properties and special implications in various biochemical processes (10—12). In order to evidence the effects of the products studied on the cellularly mediated immune response, we followed their influence on the dynamics of total leucocytes, as well as on the leucocyte type (namely, neutrophils, eosinophils, basophils, lymphocytes and monocytes), compared with effects of some reference agents, that is Rodilemid as immunostimulator and Antifolan as immunosuppressor (2), (3).

MATERIAL AND METHODS

All experiments have been made on laboratory rabbits (Chinchilla), grouped in five batches, each of them containing five individuals, subjected to different treatments, as follows: batch I—taken as reference,

was not treated; batch II was treated with Pag-2 2.5 mg/kg body/day; batch III — treated with Pag-3 2.5 mg/kg body/day; batch IV — treated with Rodilemid 10 mg/kg body/day and batch V — with Antifolan 0.083 mg/kg body/day (with batches IV and V, doses close to the therapeutical ones, usually administered to humans, have been given). All products have been administered through intramuscular injections, in a volume of 0.4 ml physiological salt, at intervals of two days, for six weeks. All batches have been analyzed before beginning the treatments, for the establishment of the normal initial values, as well as at intervals of two weeks, for the whole duration of the treatment. For evidencing the effects on the cellularly mediated immune response, the leucocytary formula has been determined on blood with 1% EDTA as anti-coagulant. The total number of leucocytes has been determined hemocytometrically while the number and ratio of leucocyte types was established by microscopic numbering, on plates containing smears coloured by the May Grünwald-Giemsa method and, respectively, by calculation (6).

RESULTS

1. Effects of the Pagosten-2 product

Total leucocytes (Fig. 1—TL) are observed as decreasing numerically as compared with the initial value, up to 86.40% after two weeks, 81.31% after four weeks and 86.87% after six weeks. With the reference batch, the maximum fluctuation does not exceed a value of 9.00% against the initial value. At the Rodilemid treated batch, a continuous increase of the number of total leucocytes, of 23.50%, is recorded, while at the Antifolan treated batch, a gradual numerical decrease of 15.84% of the initial value is observed after six weeks.

Neutrophils (Fig. 1—N) remain relatively unmodified in the first four weeks (101.41% of the initial value), decreasing up to 84.67% after six weeks. At the reference batch, the maximum fluctuation is of 8.40%, while, for all animals treated with both Rodilemid and Antifolan, an increase of 14.80% and 5.49%, respectively, is observed after six weeks.

Eosinophils (Fig. 1—E) evidence a slight decreasing tendency up to values of 93.18%, 93.18% and 95.45% as compared to the initial value, after two, four and, respectively six weeks of treatment. With the reference batch, the maximum fluctuations recorded are of 6.50%, quite similar with the values determined for Pag-2. Yet, Rodilemid induces a final decrease of eosinophils up to 70.98%, while Antifolan produces no significant effects.

Basophils (Fig. 1—B) decrease continuously, as compared with the initial value, up to 93.30%, 73.78% and 65.24% after two, four and, respectively, six weeks of treatment. With the untreated animals the maximum fluctuation is of 16.59%. Rodilemid induced a marked final decrease, with 60.14%, while Antifolan provoked a final increase of basophils, of 37.65%.

Lymphocytes (Fig. 1—LY) show an oscillating, although continuous increase, compared with the initial value, of 5.72%, 21.98% and 13.84% respectively, after two, four and six weeks of treatment. With

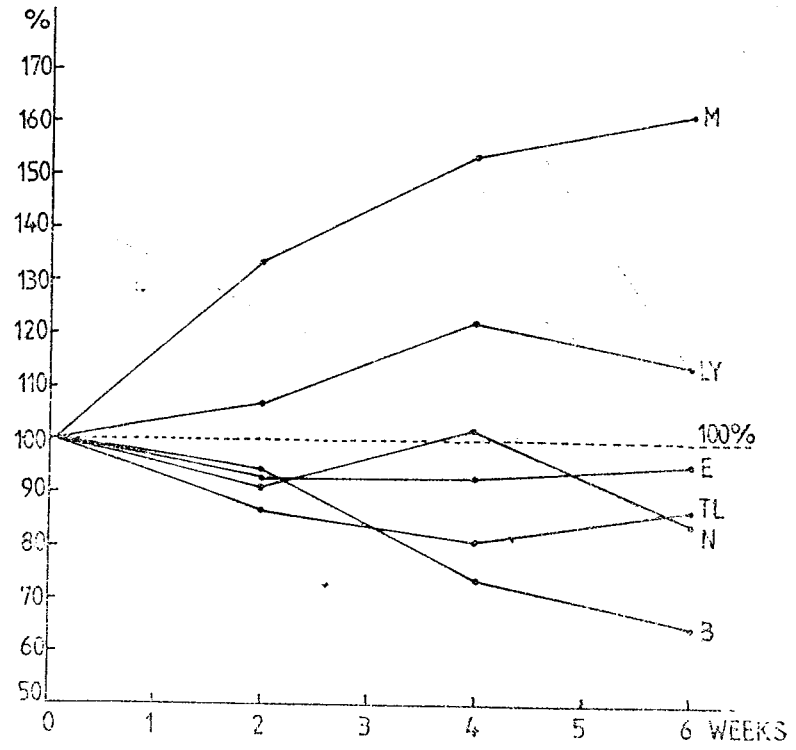

Fig. 1. — Effects of Pagosten-2 on the dynamics of leucocytes. TL — total leucocytes; N — neutrophils; E — eosinophils; B — basophils; LY — lymphocytes; M — monocytes.

the control batch the maximum fluctuation recorded is of 8.56%; Rodilemid caused a marked increase of lymphocytes with 32.40% compared to the initial normal value, while Antifolan — a final decrease with 46.51%.

Monocytes (Fig. 1—M) increase significantly, with 32.82%, 52.37% and 60.56%, compared to the normal values, after two, four and six weeks, respectively. With the control batch, the maximum was of 9.28%. Rodilemid induced a very marked increase of monocytes, with 177.28% over the initial value, while the increase provoked by Antifolan was weaker, the final value being 25.00% higher than the initial one.

2. Effects of Pagosten-3 product

Total leucocytes (Fig. 2—TL) record initially a decrease up to 98.26% against the initial value, after two weeks of treatment, and up to 94.49% after two weeks of treatment, followed by a final increase with 11.30% over the initial value. (With the control batch, fluctuations of 9.00% are recorded, with Rodilemid — an increase with 23.50% and with Antifolan — a decrease with 15.84%).

Neutrophils (Fig. 2—N) decrease significantly compared with the

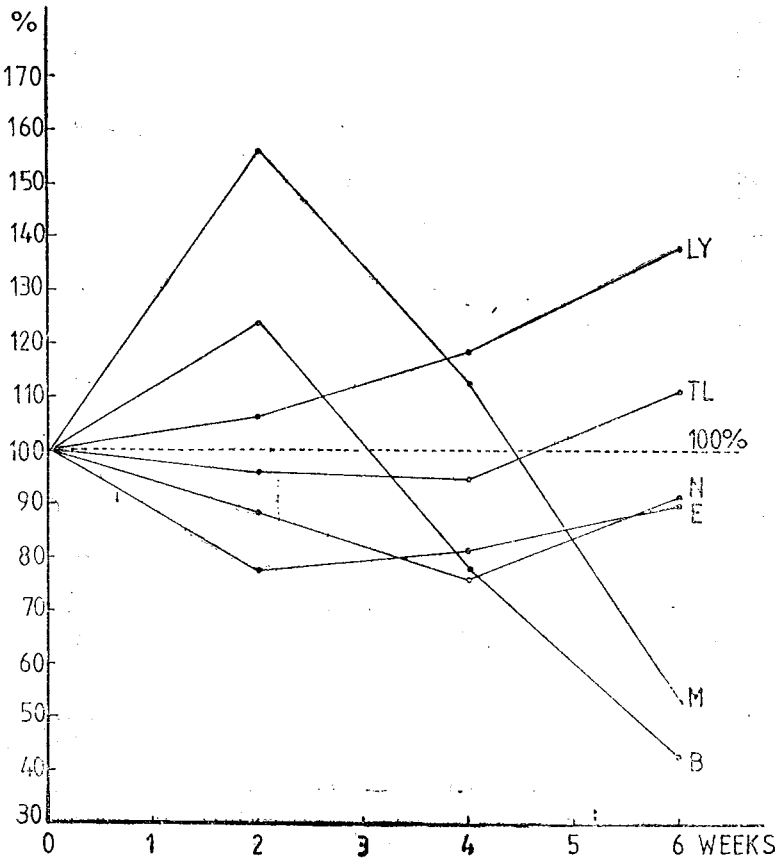

Fig. 2. — Effects of Pagosten - 3 on the dynamics of leucocytes. Other explanations — see Fig. 1.

respectively six weeks of treatment. (Control batch — fluctuations of 8.40%; Rodilemid — increases of 6.92%, 11.48% and 14.84%, respectively, after two, four and six weeks of treatment; Antifolan — increase of 5.49%).

Eosinophils (Fig. 2—E) record initially a quite strong decrease, gradually reducing during the treatment, the values recorded being of 77.50%, 81.65% and 90.00% — compared with the normal ones — after two, four and, respectively, six weeks of treatment. (Sample batch — fluctuations of 6.50%; Rodilemid—decrease of up to 70.98%, Antifolan — no significant modifications).

Basophils (Fig. 2—B) increase after two weeks with 23.81%, compared with the initial value, then decrease considerably, up to 77.76% and 42.86% after four and, respectively six weeks of treatment. (Reference batch — fluctuations of 16.59%; Rodilemid — 60.14% decrease; Antifolan — 37.65% increase).

Lymphocytes (Fig. 2—LY) increase continuously, with 5.82%,

pectively, six weeks of treatment. (Control sample — maximum variations of 8.56%; Rodilemid — 32.40% increase; Antifolan — 46.51% decrease).

Initially, monocytes (Fig. 2—M) increase considerably, with more than 56.06% of the normal value, after two weeks of treatment, then decrease up to 12.12%, after four weeks, and up to 53.03% of the initial value after six weeks of treatment (Sample batch — variations of 9.28%, Rodilemid — 177.28% increase; Antifolan — 25.00% increase).

DISCUSSIONS AND CONCLUSIONS

The general idea is that the immune response represents an assembly of reactions specific to a certain organism, or to a certain cellular system, as induced through the contact with a foreign substance having high molecular weight (proteins, polysaccharides, etc.) or with bacteria, viruses or various foreign cells (antigenes) (8), (13). Nevertheless, the observation has been made that some low molecular weight substances, too (such as antibiotics, alkaloids etc.) may intervene either in the activation or inactivation of the immune system (immunomodulators), stimulating or inhibiting the humorally or cellularly mediated immune response (8).

It is generally accepted that one of the ways through which substances with low molecular weight acquire antigenic properties lies in their capacity of combining themselves with certain reactive groups of tissular proteins (8), although the mechanism is not sufficiently clear. The nature of the immunomodulating effect depends on the agent's dose, and on the conditions of its administration, an immunosuppressing agent being capable of evidencing, in certain cases, immunostimulating effects, as well (8). On the other side, the activation, proliferation and differentiation of immunocompetent cells, as well as the formation of antibodies are phenomena involving both the cell membrane (with its specific receivers) and the cellular metabolism, so that the immunomodulating agents may influence both the cellularly and the humorally mediated immune response (8).

As to the immunomodulating effects of the acetylated polyolic derivatives employed in our experiments, they have been found as influencing, too, the cellularly mediated immune response, inducing modifications of the number of the various types of leucocytes — quite similarly as the reference agents — Rodilemid and Antifolan — do. Thus, the general action of Pag-2 and Pag-3 products is manifested, after a four week treatment, by a reduction of the total number of leucocytes (Figs. 1 and 2), the effect of Pag-2 being more pronounced (81.31%) compared to that produced by Pag-3 (94.49%). After six weeks, nevertheless, an increase of the total leucocytes is recorded — more pronounced in the case of Pag-3, the final value exceeding by 11.30% the normal one, while with Pag-2, it remains below the normal value (86.87%), which permits the conclusion that, for shorter duration of treatment, such products evidence an immunosuppressing effect similar to that of Antifolan, taken as reference agent (84.16%). On increasing

than that, after six weeks, an immunostimulating effect is to be observed in the case of Pag-3, as well as in that of Rodilemid — the immunostimulating agent taken as reference, although weaker (11.30% compared to 23.50%).

As to the action of Pag-2 and Pag-3 on the various types of leucocytes, differentiated effects are to be observed, depending on the agent's nature, on the type of leucocytes and duration of treatment. Thus, a general depressing effect is manifested on neutrophils, eosinophils and basophils, along with a stimulating effect on lymphocytes and monocytes, with both products, although somehow differentiatedly. Generally, these effects are more clearly expressed and more stable with Pag-2, in the case of which the stimulating and depressing effects are generally manifested on the whole duration of treatment; with Pag-3, nevertheless, the effects manifested upon monocytes and basophils are stimulative only at the beginning of the treatment, being then transformed into depressing effects; only with lymphocytes, the stimulating effects are observed on the whole duration of treatment. The observation to be made as to the doses employed in that the stimulating effects are manifested on some types of leucocytes for a short duration of treatment with Pag-3, followed by depressing effects, while, in the case of Pag-2, the specific effects are maintained on the whole duration of treatment. Mention must be also made that the actions of these agents on the total leucocytes are firstly influenced by those manifested on neutrophils and leucocytes, whose proportion is higher within the leucocytary formula. Also, it is to be observed that both the stimulating and suppressing effects are generally weaker than those induced by the reference agents. Nevertheless, the influence of Pag-2 and Pag-3 on leucocytes is obvious, and they may be considered as immunomodulating agents, most of their effects upon the various types of leucocytes being generally similar with the effects of the immunostimulating agent Rodilemid although, depending on the duration of treatment, effects similar to those of immunosuppressing agent Antifolan may also be observed.

Acetylation may lead to the obtainment of drugs with increased bioavailability, with special implications in a series of biochemical processes, at various levels in the organism (1), (4), (16). In this respect, glucides and polyols may be employed as carriers of acetyl radical (5—6 radicals on one molecule), acetylated derivatives with special metabolic characteristics and significant pharmacological effects, such as Pag-2 and Pag-3, being thus obtained (10—12).

It might be thus possible that the effects of the studied products on the leucocytary dynamics should result not only from their direct effects on the mechanism of cellularly mediated response, but also from their actions on certain correlated metabolic aspects. As a matter of fact, no immunostimulating or immunosuppressing effects of the reference products upon all leucocytary forms may be evidenced, alongwith the observation that their nature depends on the duration of treatment, as well.

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EFFECT OF CUTANEOUS TREATMENT WITH FLUOCINOLONE-ACETONIDE-N CREAM UPON THE MUSCULAR GLUCOSE UPTAKE AND INSULIN-SENSITIVITY IN YOUNG WISTAR RATS

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In 45-day-old male Wistar rats a cutaneous treatment with Fluocinolone-acetonide-N cream was applied for 10 days, by smearing in a thin-layer 50 mg cream/day in the interscapular region on previously shaven skin (2 cm²). After this intervention, vs. the control, a marked hyperglycemia (+ 106 %) and decreased "in vitro" sensitivity to insulin (−76 %) of the isolated diaphragmatic muscle were observed. The conclusion is drawn that in the hyperglycemia elicited by cutaneously absorbed Fluocinolone-acetonide-N in young organism a high muscular insulin-resistance is essentially involved.

Fluocinolone-acetonide, highly active fluorated glucocorticoid, is widely used for local treatments in human dermatology, although its side-effects under such conditions are not fully clarified yet. Our recent preliminary data indicate that in young rats the cutaneously applied Fluocinolone-acetonide-N cream leads to a diabetic state, characteristic for glucocorticoid excess during steroid-diabetes (5), (6). On the other hand, it is well established that in white rats striated muscles are major insulin-dependent consumers of blood glucose (1), (2), (13), which react in a rapid and sensitive manner by modification of glucose consumption with glycemia changes in response to exogenously induced glucocorticoid excess (2), (4), (7), (8), (9), (10), (11), (12).

Taking into account the above facts, in the present study the "in vitro" glucose uptake and the insulin-sensitivity of isolated diaphragm-muscle pieces were correlated with the glycemia level, after cutaneous treatment of young Wistar rats with Fluocinolone-acetonide-N containing cream.

MATERIALS AND METHODS

For experiment intact control and Fluocinolone-treated groups of young male Wistar rats from the stockfarm of our laboratory were used, being 45-day-old at the beginning of treatment, maintained under standardized laboratory and feeding conditions before sacrifice.

Commercial Fluocinolone-acetonide-N cream ("Antibiotice S.A."—Iași, containing 25 mg glucocorticoid/100 g excipient) was applied for 10 days by smearing 50 mg cream/day in a thin-layer on the interscapular region of skin, shaven previously on a surface of 2 cm².

24 hrs after cessation of the treatment and after 18-hr starvation, the animals, together with the control ones, were sacrificed by cervical dislocation and sectioning of carotid vessels.

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Blood glucose level was assayed from 0.1 ml deproteinized blood-supernatant, according to the enzymatic method of Werner (14) and glycemia was expressed in mg%.

After sacrifice, the diaphragms were quickly excised and immersed for 20 minutes into ice-cold Krebs-Henseleit saline (without glucose, pH = 7.4) and sectioned in approximately equal hemiorgans. From each animal a hemidiaphragm was used for testing the "in vitro" basal glucose uptake (BAS) in the absence of insulin, while on the other half the global glucose uptake in the presence of insulin (INS) was tested.

The incubation was carried out for 2 hrs at 37.6°C in 1.0 ml glucose containing (16.7 mM) Krebs-Henseleit bicarbonate solution (pH = 7.4) without or with insulin ("Calbiochem" 10^{-3} I.U. ml medium), under aerobic condition (gas phase = 95% O_2 + 5% CO_2), using an original device (1) and our procedure (3), (8).

The initial and final glucose content of the incubation medium was determined enzymatically with GOD-Perid-Kit ("Boehringer", GmbH, Mannheim, Germany). The basal glucose uptake as well as the global glucose uptake in the presence of insulin by the corresponding hemi-diagram-pairs were calculated in micromoles/100 mg fresh tissue per 2 hrs. The insulin-sensitivity of hemiorgans was evaluated by calculating the insulin-stimulated net glucose uptake over the corresponding basal values, i.e. by estimating the Δ(INS−BAS).

The results were statistically checked for the homogeneity of the means using Chauvenet's criterion. Mean values were compared according to Student's t test, the modifications at $P < 0.05$ being considered statistically significant.

RESULTS AND DISCUSSIONS

The data summarized in Table 1 show that by cutaneous treatment with Fluocinolone-acetonide-N containing cream for 10 days in young rats there appears a marked hyperglycemia, the blood-glucose level being elevated with 105.79% ($P < 0.001$) as compared to the control value. Under this hyperglycemic condition the "in vitro" basal glucose uptake by isolated hemidiaphragms markedly increases (+ 63.74%, $P < 0.001$), while the insulin-stimulated net glucose uptake over the basal glucose consumption of the corresponding hemidiaphragm-pairs significantly decreases under the control level (− 76.42%, $P < 0.001$). The present observation referring to Fluocinolon-acetonide induced hyperglycemic state underline our recent preliminary data (5), (6), which indicate also an elevated blood glucose level, associated with hypercholesterolemia (+ 22%), marked thymus involution (63%), adrenal atrophy (21%), enhanced hepatic glycogen production (+ 170%), as well as decreased growth rate (− 17%) in Fluocinolon-treated young rats. Such modifications strongly suggest a glucocorticoid excess, characteristic for steroid-diabetes in young rats (1), (5), (6). On this basis it is pertinent to assume that the percutaneously absorbed Fluocinolone-acetonide-N containing cream induces glucocorticoid excess, leading to a steroid-diabetic state in young organism, in which muscular insulin-resistance is essentially involved.

Table 1

Glycemia level, „in vitro" basal glucose uptake (BAS), global glucose uptake in the presence of insulin (INS) and insulin-stimulated net glucose uptake (ΔINS−BAS) by isolated hemidiaphragms of control and with Fluocinolone-acetonide N-unguentum treated groups of young rats

Glycemia	micromole glucose uptake by 100 mg diaphragm per 2 hrs		
mg%	BAS	INS	(ΔINS−BAS)
Normal control group			
69±1.90 (25)	4.06±0.26 (8)	6.27±0.24 (8)	2.21±0.23 (8)
Fluocinolone treated group			
142±7.80 (15) +105.79% P<0.001	6.65±0.31 (8) +63.74% P<0.001	7.17±0.60 (8) +14.33% P>0.05	0.52±0.21 (8) −76.42% P<0.001

(The values represent means ± standard errors (S.E.). The number of experiments is given in brackets, per cent modifications and P are calculated against the values obtained in control-group)

In conclusion, in young male Wistar rats the cutaneously applied Fluocinolone-acetonide-N containing cream induces muscular insulin-resistance associated with hyperglycemia.

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A SELENIUM TREATMENT INDUCED MODIFICATIONS OF CARBOHYDRATE METABOLIC PARAMETERS IN THE CHICKENS

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Cornish-Rock chickens aged 5 days at the beginning of the experiment were subjected to a treatment with Selenium (Huhtamäki Oy Novamed; 0.2 mg/kg of fodder) that lasted for 21 days. We found increases of glycemia after 3, 11 or 18 days of Selenium treatment, and those modifications were correlated with increases of liver glucose-6-phosphatase activities. Skeletal muscle glycogen content increased by Selenium treatment. Glycogen phosphorylase *a* activity was increased in the liver and decreased in the skeletal muscle after that treatment. In our opinion some of the above metabolic modifications might be due to an activated gluconeogenic pathway. Ten days after Selenium treatment (which lasted for 21 days) cessation, we observed decreases in glycemia, liver and skeletal muscle glycogen content, as well as in liver glucose-6-phosphatase activity. About those persistently modifications, we can conclude that ten days is an insufficient period to remove all the effects of a Selenium treatment which lasted for 21 days.

Since the discovery of Selenium (Se) as an essential micronutrient, the molecular role of the element in the mammalian organism has attracted much interest. One of the aspects of Se defficiency is the extraordinarily wide variety of organs which are affected in different species by this abnormal nutritional state. It was established a discrete organotropy, e.g., the pancreatic degeneration and edema in poultry, the skeletal muscle dystrophy of cattle, cardiac muscle degeneration in pigs (11).

A relationship between low serum Selenium concentrations and increased risk of death from acute coronary heart disease and from myocardic infarction has been recently suggested (6, 13).

It was established, too, that physiological function of Se in mammals and humans is to form the catalytic site of the enzyme glutathione peroxidase, that plays a major role in cell's antioxidant defence by chemically reducing the very different hydroperoxides to alcohols (3).

Of a particular interest for us was a recent report which indicated a significant increase of gluconeogenic capacity of hepatocytes obtained from rats subjected to a Selenium treatment (1).

The objective of our investigation was to observe a possible effect of a Selenium treatment upon some of liver carbohydrate metabolic parameters in chickens.

MATERIAL AND METHODS

Our experiment was performed on Cornish-Rock chickens aged 5 days at the beginning of the experiment. The chickens were divided in two groups: the control group (C) and Selenium treated group (Se). Selenium was administered in the food in a dose of 0.2 mg/kg of fodder (organic Selenium from Huhtamäki Oy Novamed). The number of chi-

ckens in both C and Se groups was 60. The treatment with Selenium lasted for 21 days, after which Se group received only normal fodder as group C.

The chickens were sacrified by decapitation between 8 and 9 hours in the morning, after a previous fasting for 16 hours, at 3, 11, 18 and 31 days from the beginning of experiment. The sacrification at 31 days from the beginning of experiment was made after 21 days of Se treatment and 10 days after Selenium treatment cessation.

After sacrification the following metabolic parameters were performed: glycemia (8); skeletal muscle and liver glycogen content (7), liver glucose-6-phosphatase activity (4), as well as skeletal muscle and liver glycogen phosphorylase *a* activities (5; see for other explanations 2 and 12).

Statistical processing of the results included the means of calculation; the control of homogeneity of mean values by Chauvenet's criterion, aberrant values being eliminated (10); comparison of the means (Se treatment groups versus the correspondingly C group) using Student's "t" test (9). The differences were considered statistically significant for $p < 0.05$.

RESULTS

As we can see from Table 1, a treatment with Selenium for 3, 11 or 18 days caused a significant increase of glycemia (+ 43.94%; +54.69% and + 21.94%). Liver glycogen content was increased after 3 days of Selenium treatment (+ 31.15%; $p < 0.05$). When this treatment lasted for 11 or 18 days, only insignificant modifications of liver glycogen content (+ 0.64% and − 9.07% respectively) were observed. On the other hand, skeletal glycogen content was increased after 3 (+ 28.09%; $p<0.01$), 11 (+ 35.89%; $p < 0.001$) and 18 (+ 31.14; $p < 0.01$) days of treatment with Selenium. Glucose-6-phosphatase, one of those four key gluconeogenic enzymes, was significantly increased in the liver of chickens treated for 3 (+ 64.17%; $p < 0.01$), 11 (+ 44.78%; $p < 0.02$) or 18 (+ 44.66%; $p< 0.001$) days with Selenium (see Table 2). Liver glycogen phosphorylase *a* activity, as glucose-6-phosphatase activity, was increased, too, in chickens receiving Selenium, and those increases were observed at 3 (+ 28.84%; $p < 0.01$), 11 (+19.25%; $p<0.05$) and 18 (+14.99%; $p < 0.01$) days from the beginning of the treatment (see Table 2). Muscle glycogen phosphorylase *a* activity was decreased when the period of Selenium treatment lasted for 11 (− 38.85%; $p < 0.01$) or 18 (−40.25%; $p < 0.02$) days and insignificantly modified (+ 8.96%) when that period lasted only 3 days (see Table 2).

Ten days after the cessation of Selenium treatment (which lasted for 21 days) we observed decreases in glycemia (− 8.72%; $p < 0.05$), liver (− 32.41%; $p < 0.001$) and muscle (− 25.16%; $p < 0.001$), glycogen content as well as in liver glucose-6-phosphatase activity (− 20.69%; $p < 0.001$) (see Tables 1 and 2). After that period when chickens did not receive Selenium, only insignificant modifications in the liver (+ 7.21%) or skeletal muscle (+ 4.23%) glycogen phosphorylase *a* activities were found (see Table 2).

Table 1

Glycemia (mg%), as well as liver and skeletal muscle glycogen content (mg/g tissue) in chickens subjected to a selenium treatment

Group		Glycemia	Liver glycogen	Muscle glycogen
C1	$\bar{X} \pm SE$	86.67±7.45	3.98±0.33	2.10±0.11
	n	8	8	8
Se_1	$\bar{X} \pm SE$	127.63±2.21	5.22±0.39	2.69±0.12
	n	9	8	8
	p<	0.001	0.05	0.01
	± C1 %	+43.94	+31.15	+28.09
C2	$\bar{X} \pm SE$	81.00±5.46	4.68±0.34	2.73±0.14
	n	8	8	8
Se_2	$\bar{X} \pm SE$	125.30±3.41	4.71±0.22	3.71±0.17
	n	8	8	8
	p<	0.001	NS	0.001
	±C2 %	+54.69	+0.64	+35.89
C3	$\bar{X} \pm SE$	86.58±4.45	5.51±0.16	3.05±0.17
	n	8	8	8
Se_3	$\bar{X} \pm SE$	105.08±2.64	5.01±0.73	4.00±0.22
	n	8	8	8
	p<	0.01	NS	0.01
	±C3 %	+21.94	−9.07	+31.14
C4	$\bar{X} \pm SE$	128.14±3.46	6.88±0.17	4.65±0.22
	n	8	8	8
Se_4	$\bar{X} \pm SE$	117.03±3.88	4.65±0.16	3.48±0.12
	n	8	8	8
	p<	0.05	0.001	0.001
	±C4 %	−8.67	−32.41	−25.16

Note: $\bar{X} \pm SE$ = mean ± standard error; n = number of individual values; p = signification limit; ± C1, C2, C3 or C4 % = percentage differences versus controls; C1 and Se_1 groups sacrified at 3, C2 and Se_2 groups at 11, C3 and Se_3 groups sacrified at 18 days from the beginning of the experiment; C4 and Se_4 groups sacrified ten days after the Selenium treatment; NS = not significant.

DISCUSSION

When the treatment with Selenium lasted for 3, 11 or 18 days, increases of glycemia were found. On the other hand Selenium treatment caused increases of liver glucose-6-phosphatase activities. It is possible that those increases of glycemia are due to an intensified gluconeogenesis in the liver. Our opinion is supported by the observation of Bell et al. (1) who demonstrated an intensified gluconeogenesis in hepatocytes obtained from rats which were sacrified after 180 minutes post-treatment with a single dose of Selenium. It comes out that an intensified gluconeogenesis is correlated with an amplified storage of glycogen in the liver. We found

Table 2

Liver glucose-6-phosphatase (G6 Pase) activity, as well as liver and skeletal muscle glycogen phosphorylase *a* (GPa) activities in chickens subjected to a Selenium treatment

Groups		G6Pase	Liver GPa	Muscle GPa
C1	$\bar{X} \pm SE$	6.00±0.45	12.93±1.10	28.02±2.97
	n	8	8	8
Se_1	$\bar{X} \pm SE$	9.85±0.95	16.66±0.56	30.53±4.67
	n	8	8	8
	p<	0.01	0.01	NS
	±C1 %	+64.17	+28.84	+8.96
C2	$\bar{X} \pm SE$	9.02±0.42	16.00±0.65	49.10±4.18
	n	8	8	8
Se_2	$\bar{X} \pm SE$	13.06±1.32	19.08±1.02	29.08±3.02
	n	8	8	8
	p<	0.02	0.05	0.01
	±C2 %	+44.78	+19.25	−38.85
C3	$\bar{X} \pm SE$	7.35±0.22	19.48±0.64	39.35±5.35
	n	8	8	8
Se_3	$\bar{X} \pm SE$	10.78±0.54	22.40±0.65	23.51±1.61
	n	8	8	8
	p<	0.001	0.001	0.02
	±C3 %	+46.66	+14.99	−40.25
C4	$\bar{X} \pm SE$	8.65±0.35	22.60±0.79	26.68±1.06
	n	8	8	8
Se_4	$\bar{X} \pm SE$	6.86±0.25	24.23±1.81	27.81±1.32
	n	8	8	8
	p<	0.001	NS	NS
	±C4 %	−20.69	+7.21	+4.28

Note: In Table 2 the enzyme activities were expressed in nanomoles of inorganic phosphate liberated /mg protein/ minute. For other explanations see Table 1.

that correlation when Selenium treatment lasted for 3 days. When Selenium treatment lasted for more than 3 days, the metabolic pathway of glycogen storage was insignificantly modified versus the control.

Liver glycogen phosphorylase *a* activity, the key enzyme implied in glycogen breakdown, was increased after three, eleven or eighteen days of Selenium treatment. It seems that the treatment with Selenium induced an activation of gluconeogenesis as well as an intensification of glycogenolysis in the liver.

Skeletal muscle glycogen content increased by Selenium treatment. It is possible that an increase of muscle glycogen storage is due to a decrease of glycogen phosphorylase *a* activity in this tissue or / and an increase of glycogen synthase system activity.

Ten days after Selenium treatment cessation, all the modifications which we found were decreases. We are not able to give a reasonable explanation about those modifications, but we can say that ten days is an insufficient period to remove all the effects of a Selenium treatment which lasted for 21 days.

We conclude that Selenium treatment caused a disturbance of glucose metabolism in the chickens, such as: increases of glycemia, liver glucose-6-phosphatase and glycogen phosphorylase *a* activities, muscle glycogen content, as well as a decrease of muscle glycogen phosphorylase *a* activity. In our opinion some of those metabolic modifications might be due to an activated gluconeogenic pathway.

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BIOCHEMICAL MODIFICATIONS IN THE THYMOCYTES AND BURSOCYTES OF CHICKENS AFTER A SELENIUM AND E VITAMIN ADMINISTRATION

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Administration of selenium and selenium and E vitamin in the fodder (organic selenium on 0.2 mg/kg fodder and E vitamin on 79.2 mg/kg fodder) of different age chickens produced biochemical modifications in the bursocytes or thymocytes which are dependent on the age of animals, the nature and duration of treatment and on the cell type.

Selenium and E vitamin appear to affect significantly the function of all components of the immune system, the cellular and humoral immunity (2, 12). The selenium supplementation in mouse diet increase the primary immune response and its effect is dependent on the E vitamin administration (1). The actions of selenium and E vitamin include their role as cellular antioxidants, the maintenance of membrane integrity by increasing the anti-oxidant activity of glutathione peroxidase (3, 8, 13, 15). Selenium and E vitamin supplementation remove the depression of immune response and increase the IgM and IgG synthesis, which determine an increase of antibody production (13).

The present investigation aimed to determine the *in vivo* effect of selenium and E vitamin upon the thymocytes and bursocytes function in the ontogenetical development of chickens.

MATERIAL AND METHODS

The experiments were performed on broiler Studler-Cornish chickens, aged 5 or 21 days. The housing conditions were similar to those in an avian farm, fodder and water were given *ad libitum*. The chickens were distributed in three experimental groups, for both ages: control group (C); selenium-treated group (Se), and selenium and E vitamin-treated group (SeE). Each group contained 10 individuals. Organic selenium and E vitamin (Huhtanaki Oy Novamed) were administered in a dose of 0.2 respectively 79.2 mg/kg fodder. The periods of treatment were 21 days (for the 5 days-old chickens), and 23 days (for the 21 days-old chickens). The chickens were sacrificed by decapitation, after a previous fasting period of 16 hours, as follows: at 5, 11 and 21 days of treatment, for those aged 5 days at the beginning of the experiment; at 8, 15 and 23 days of treatment, for those aged 21 days at the beginning of the experiment.

The bursa Fabricii and the thymus were immediately collected, weighed on a torsion balance, then bursocytes and thymocytes were isolated. The cells in the suspension were counted and their number expressed per ml of suspension. Samples of the suspension were used to

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determine the total protein content (6), glycogen (9) and glucose (10) concentrations. Another part (aliquot) of the cell suspensions were incubated for 1 hour in a Warburg apparatus, at 42°C. The oxygen consumed by the cells was read at 15, 30, 45 and 60 min; at the end of the incubation period, total proteins (6), glucose (10), glycogen (9), nucleic acids (14) and gammaglobulins (6, 16) were determined. The obtained values for each parameter were reported to the number of cells in each ml of suspension. Mean values were calculated and tested for homogeneity by Chauvenet's criterion. Student's "t" test was used to check the statistical significance of differences between means; significant threshold level was taken at $p = 0.05$.

RESULTS AND DISCUSSION

The modifications induced by Se or SeE administration are dependent on the nature and the period of treatment, on animals age and the nature of immunocompetent cells (bursocytes or thymocytes).

The age-dependent differences are obvious, for both bursocytes and thymocytes, which testify the role these cells play in several stages of the ontogenetical development. In the chickens that were only 5 days old at the beginning of the experiment (when they are in the stage of organisation of the immunobiological process, and they have not reached neuro-endocrine maturity), (11), Se and SeE induced modifications were higher in bursocytes than in thymocytes immediately after administration, but they were reversed as the treatments went on. In the older chickens, bursocytes also showed a more intense answer to Se administration than thymocytes, after 8 days of treatment. After 15 and 23 days of Se treatment, we did not notice any reversibility of the process. The direction of modifications, which was dependent on the duration of the treatment, emphasized an inhibition of the oxygen consumption in younger chickens, for both cell types, and an increase of the parameter in older chickens. Most of the tested parameters showed age-dependent inverse effects. Our results demonstrated that in chickens of both ages the effects of the continuation Se + E vitamin are higher than those of Se alone — a fact previously confirmed on sheep lymphocytes by Finch and Turner (5). Kiremidjian-Schumacher and Stotzky (8) have noticed that food supplementation with low, nontoxic levels of Se apparently maintains the integrity of biological membranes through augmentation of the antioxidant action of glutathione peroxidase and this effect may be further potentiated by the simultaneous administration of E vitamin, which determines the enhancement of immunocompetent cell function. It is reasonable to assume that at least some of the biological functions of Se can be linked to the activity of glutathione peroxidase (1, 2, 7, 15). This enzyme contains selenocysteine at its catalytic site, and the lack of Se leads to a deficiency in the activity of the enzyme and, consequently, to a decrease of ability to degrade H_2O_2 (4). By contrast, supplementation with Se can prevent the structural damage and disruption of cellular integrity caused by peroxidation of membranes and other cell components by enhancing the action of glutathione peroxidase on hydroperoxides of unsaturated fatty acids (12). Supplementation with Se has been shown to stimulate the random migration, chemotaxis and bactericidal activity of neutrophils; the production of antibodies; the proliferation of both T and B lymphocytes in response to mitogen; the production of lymphokines; the cytotoxic function of non-killer cells; delayed-type

Table 1

The number of bursocytes and thymocytes and the effects of Se and SeE upon them in 5 days old chickens in the *in vivo* experiments

Sacrif.:	I			II			III		
Groups:	C	Se	SeE	C	Se	SeE	C	Se	SeE
				INITIALLY — BURSOCYTES					
Nr.10^8	3.31	5.31	5.75	10.53	4.41	6.64	4.88	4.74	4.81
TP	23.84±10.68	−54	−40	29.24±0.93	−58	−37	17.01±0.16	−26	+57
Gl.	7.46±0.04	−42	−27	4.98±0.05	−32	−68	2.76±0.01	−36	−32
Gs.	50.11±3.72	−26	−41	44.70±0.27	+73	+43	12.03±0.71	+28	+39
				FINALLY — BURSOCYTES					
TP	0.37±0.05	−13	−22	0.19±0.09	−1	−11	0.33±0.12	−36	−59
Gl.	5.47±0.96	−72	−49	1.19±0.03	+93	+85	1.78±0.46	−36	+2
Gs.	21.38±0.08	−34	−45	11.60±0.16	+60	+4	5.85±0.50	−55	−53
RNA	1.33±0.26	−39	−45	0.33±0.05	+1332	+1234	2.11±0.43	+238	+390
DNA	1.50±0.14	−50	−52	0.58±0.09	+750	+165	0.97±0.17	+159	+30
Gg.	0.24±0.02	−55	−71	0.03±0.02	+129	+386	0.74±0.16	+204	+23
O_2C	40.31±4.34	−98	−98	41.42±6.41	−58	−81	45.62±1.79	−30	−43
				INITIALLY — THYMOCYTES					
Nr.10^8	4.14	16.60	9.12	7.19	6.30	3.92	6.70	2.25	2.04
TP	16.72±3.67	−52	−14	15.02±0.2	+76	+297	12.08±1.91	−89	−71
Gl.	5.92±0.007	−78	−83	1.88±0.16	+27	+37	1.98±0.19	−4	−38
Gs.	51.50±3.20	−80	−64	5.85±0.64	−0.8	+93	9.71±0.86	+309	−268
				FINALLY — THYMOCYTES					
TP	0.19±0.04	−73	−46	0.18±0.02	+8	−27	0.77±0.03	+244	−564
Gl.	1.83±0.30	−65	−8	1.76±0.17	+41	+131	0.63±0.08	+622	−455
Gs.	18.97±0.38	−73	−62	2.18±0.23	−63	−22	3.78±0.22	+113	−6
RNA	1.25±0.28	−81	−64	0.56±0.06	+117	+37	1.20±0.23	+157	−140
DNA	1.50±0.50	−82	−60	0.86±0.22	+23	+54	0.75±0.03	+149	−217
Gg.	0.10±0.009	−12	−42	0.05±0.01	+25	+270	0.01±0.004	+380	−290
O_2C	46.40±1.07	−20	−34	31.61±3.69	+64	−94	6.89±0.55	+144	+224

TP = total protein; Gl = glycogen; Gs = glucose; Gg. = gammaglobuline; O_2C = = oxygen consumption; Nr. = number of cells. Sacrifications: I — at 5 days of treatment; II = at 11 days of treatment; III = at 21 days of treatment. In C group mean values ± ± standard error; in Se and SeE groups percentage differences versus C group. The statistical significant differences versus control group are underlined. The values for each parameter are reported to cells number. Other explanations in the text.

Table 2

The number of bursocytes and thymocytes and the effects of Se and SeE upon them, in 21 days old chickens in the *in vivo* experiments

crif.:	I			II			III		
roups:	C	Se	SeE	C	Se	SeE	C	Se	SeE
				INITIALLY — BURSOCYTES					
$\cdot 10^3$	7.15	1.54	3.62	3.27	3.80	6.96	5.36	2.09	4.92
f	0.11±0.04	+278	+157	0.17±0.05	+1556	+47	0.14±0.008	+121	+92
.	0.40±0.04	+127	+370	4.66±0.20	−0.8	−22	1.11±0.009	+115	+2
s.	26.47±1.71	+1000	+154	71.89±0.37	−22	−54	47.60±6.39	+260	+38
				FINALLY — BURSOCYTES					
.	0.65±0.08	+168	+38	0.26±0.05	+47	−28	0.11±0.01	+100	+81
l.	1.80±0.27	+694	+194	5.88±1.84	−38	−61	1.52±0.15	+150	+20
s.	4.70±1.31	+744	+311	6.01±0.28	+25	−25	11.81±2.08	+213	+16
NA	0.46±0.14	+6943	+934	3.53±0.36	−42	−68	1.09±0.21	+262	+21
NA	0.51±0.08	+7733	+2403	4.58±0.59	−73	−65	1.38±0.005	+200	+3
g.	0.83±0.09	+1253	+148	1.07±0.12	+685	+57	0.67±0.04	+191	+157
C	26.48±0.81	+199	+40	0.73±0.42	+876	+231	7.17±0.42	+124	+72
				INITIALLY — THYMOCYTES					
r.10^3	4.76	2.98	4.60	8.11	7.97	9.97	7.96	8.66	11.0
P	0.16±0.03	+406	+87	0.20±0.005	+80	+160	0.15±0.009	+180	+46
l.	0.56±0.10	+46	−46	2.81±0.09	−55	+2	1.07±0.33	−36	−50
s.	61.00±1.50	+135	+61	29.51±1.80	−13	−23	38.81±1.84	−9	−30
				FINALLY — THYMOCYTES					
P	0.64±0.07	+77	+30	0.16±0.03	−28	−5	0.08±0.004	−10	−23
l.	3.98±0.39	+43	+59	2.39±0.05	−17	−34	0.97±0.06	−10	−20
s.	14.82±1.56	+59	−5	0.84±0.07	+865	+240	10.30±1.67	−14	−13
NA	0.59±0.23	+1486	+250	1.28±0.14	−3	−50	0.73±0.34	+939	+30
NA	1.32±0.19	+1212	+1551	1.66±0.96	−30	−74	1.26±0.40	−63	−53
g.	1.31±0.18	+360	+91	0.75±0.13	+376	+229	0.22±0.01	+268	+377
C	16.74±0.74	−8	+38	1.92±0.47	+1071	+1294	5.03±0.23	−35	+724

Explanations in Table 1 and in the text

ypersensitivity reactions and allograft rejection; and the ability of a ost to reject transplanted malignant tumors. We considered that the bility of Se to interact with cell membranes may represent only one of s many possible regulatory mechanisms. The results indicate the neces- ity for further studies in this problem.

In conclusion, the Selenium and Selenium + E vitamin treatment f different age chickens produces biochemical modifications in the tested ells, which are dependent on the cell type — bursocyte or thymocyte —

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STRUCTURAL AND ULTRASTRUCTURAL ASPECTS OF THE COLLAGEN IN THE BOVINE CORNEA

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Corneal transparency is dependent upon the maintenance of an organized extracellular matrix containing small diameter collagen fibrils. We studied the organization of collagen fibrils in the bovine cornea by light and transmission electron microscopy. Our electronmicrographs have shown in the Bowman's layer the collagen fibrils are irregularly disposed. In the stroma, the collagen fibrils form characteristic lamellae bundles. Each collagen lamella is composed of uniform-sized fibrils arranged in parallel and the lamellae are perpendicular one to another. Keratocytes were interposed between collagen lamellae only in the cornea stroma.

The outer fibrous tunic of the eye is formed in the anterior region by a transparent membrane-cornea that continues at limbus with sclera. Limbus corresponds to the abrupt optical change from the transparent cornea to the opaque sclera.

Although both of these structures consist mainly of collagen fibrils, their optical properties are different. The cornea is transparent, the sclera is not.

Transparency is the most essential characteristic of the cornea and has been the subject of research mainly in man (12), (13), (16), (18), (19), rabbit (2), (4), (16), rat (11), dog and cattle (16).

Transparency of the cornea results from minimal absorption and scattering of light. The cornea does not absorb in the visible spectrum (7). However, scattering causes a slight diminution of the intensity of visible light when it traverses the normal cornea (1), (2). The collagen fibrils which give to the corneal stroma its mechanical strength are the primary scattering elements in normal corneas.

To clarify the histologic bases of this transparency many investigators studied the cornea and sclera by focusing on the patterns of collagen fibrils arrangement by light microscopy (3), (16), transmission electron microscopy (TEM) (3), (12), (13), light scattering (4), (10), X-ray diffraction (6), (14) and scanning electron microscopy (SEM) (12).

We studied the organization of collagen fibrils in the bovine cornea by light and transmission electron microscopy.

MATERIALS AND METHODS

The bovine corneas from adult (two years old) animals were provided from slanghter house.

For light microscopy corneas were fixed in Bouin's fixative, embedded in paraffine, sectioned at 7 μm and stained with Gomori and Azan.

For electron microscopy the corneas were immersed in a fixative containing 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for six days. After this fixation the cornea was

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into small pieces of about 1 × 2 mm and postfixed with 1.0% OsO_4 0.1 M cacodylate buffer at 4°C for 2 hours. It was dehydrated in a gra- l series of ethanol and embedded in Epon 812.

Ultrathin sections were cut with LKB ultratome, stained with uranyl tate and lead citrate ad then examined in transmission electron micro- pe (JEOL 7C).

RESULTS AND DISCUSSION

The histological composition of the cornea is uniform. The consti- nt layers listed in an anterior to posterior direction are : epithelium, vman's layer, stroma, Descement's membrane and endothelium (Fig. 1).

We will concentrate our description only on Bowman's layer and oma which contain collagen fibrils.

Bowman's layer is an acellular layer measuring approximately µm in thickness. It lies immediately beneath the epithelium trough- the entire extent of the cornea and terminates abruptly at the limbus. s layer consists of randomly arranged short collagen fibers and fine ils (Fig. 2). These fibrils were not ordered in bundles.

Previous TEM studies (9), (13), (15) showed that Bowman's layer sists of uniform-sized collagen fibrils running in random directions. ddition, Komay and Ushiki (12) observed the presence of a honeycomb tern and small, presumbly penetrating, pores on the anterior surface. s honeycomb pattern appears to reflect the contour of the base of the helium and the attachment of anchoring fibrils to the basal laminae he hemidesmosomes (5).

Collagen fibrils in the bovine stroma run parallel to each other with ewhat regular spacing to form flat lamellar bundles (Fig. 3). In the nior one third of the stroma collagen lamellae were thin and ran mostly quely to the corneal surface. Moving posteriorly most of the collagen ellae tended to be arranged parallel to the corneal surface (Fig. 5).

At a higher magnification (Fig. 4) each lamella was composed of thin agen fibrils running in parallel. The collagen lamellae are perpendicu- one to another (Fig. 6).

Keratocytes were enterposed between collagen lamellae (Fig. 7).

The keratocyte is an extremely flat cell that projects stellate pro- es over a wide area. The cytoplasm consists of an electron-dense matrix hich a moderate number of membranous organelles are present.

Previous TEM studies (8), (13) showed that collagen lamellae in rficial one third of the human corneal stroma are much narrower and e irregularly interwoven than these in the deeper two thirds.

Using silver impregnation (16) the lamellar bundles were found to s irregularly and form interlaced meshworks in the central area of human, dog and cattle cornea.

SEM studies (12) showed the three-dimensional features of lamel- criss-crossing at various angles to form interlaced meshworks in the rior and posterior stroma.

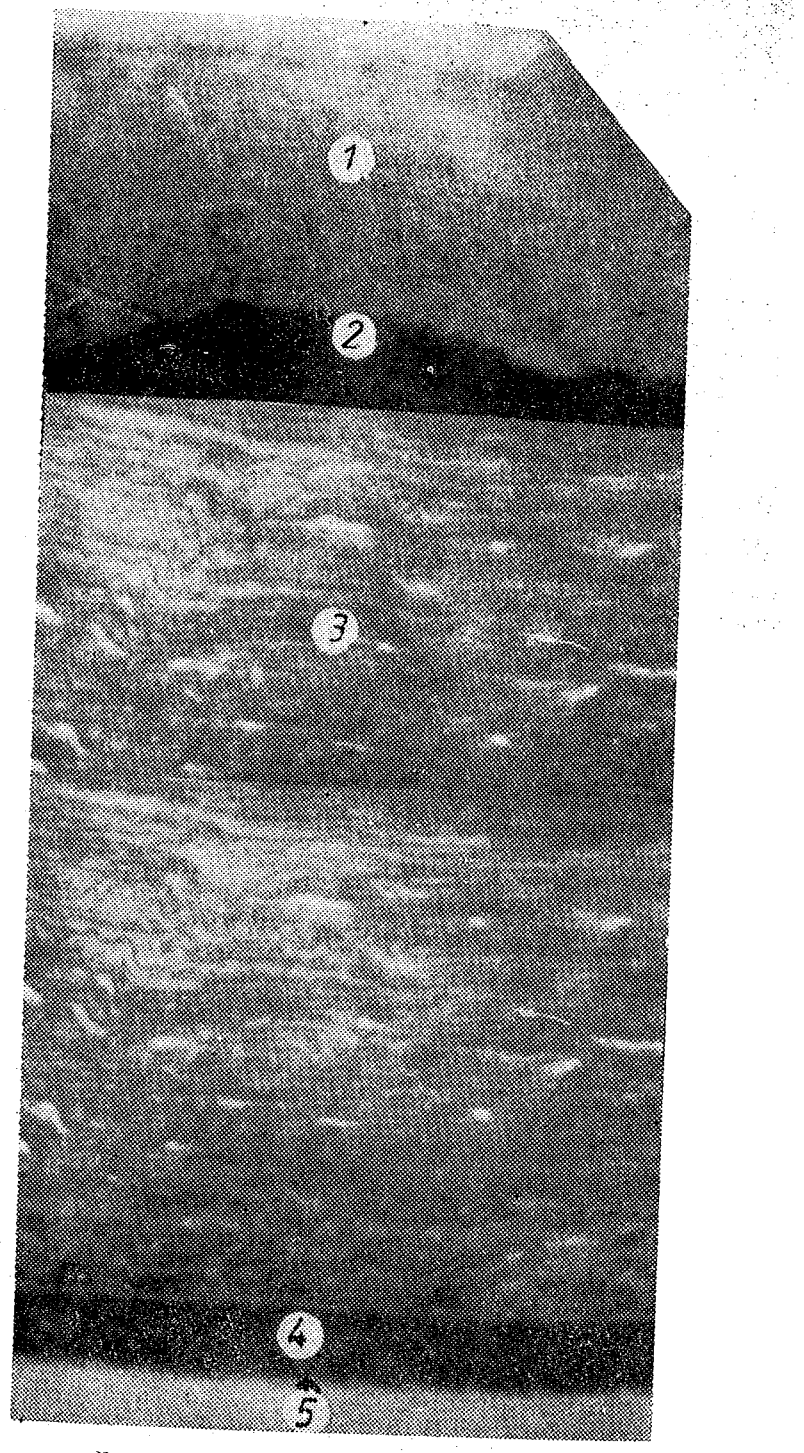

Fig. 1. — Light micrograph of bovine cornea
1— epithelium ; 2 — Bowman's layer ; 3 — stroma ; 4 — Desce-

Fig. 2. — Electron micrograph of Bowman's layer. This layer consists of randomly arranged short collagen fibers. These fibrils were not ordered in bundles, (× 45 000).

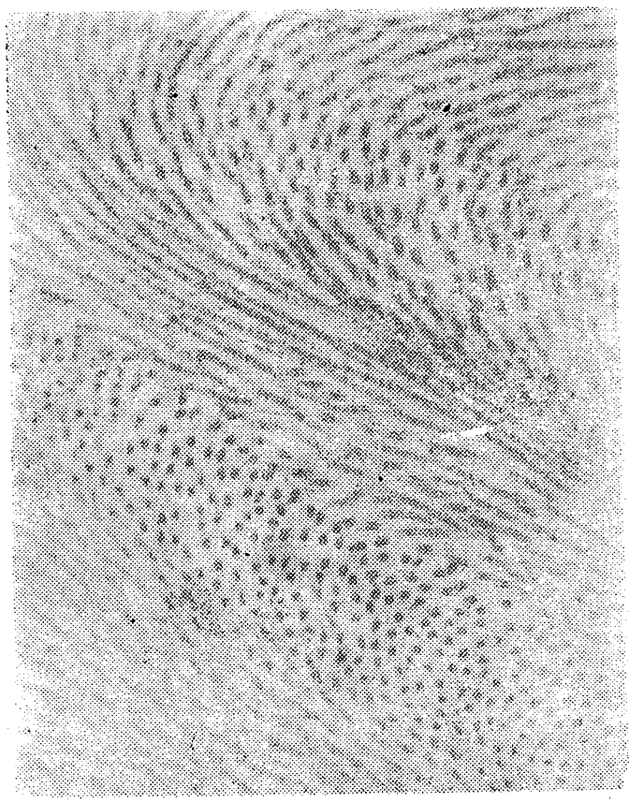

Fig. 3. — Electron micrograph of collagen lamellae from bovine stroma. Note the flat lamellar bundles of collagen fibrils. In the anterior one third of the stroma collagen lamellae run mostly obliquely to the corneal surface. (× 45 000).

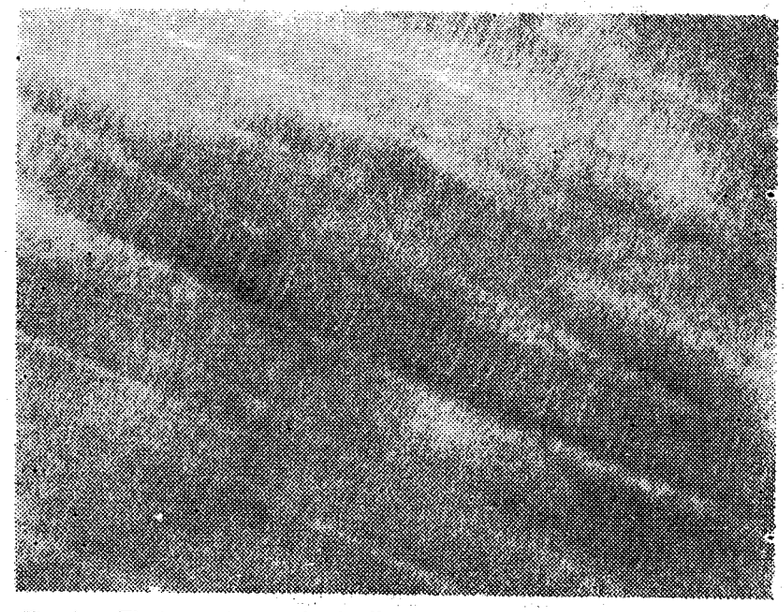

Fig. 4. — Electron micrograph of collagen fibrils from lamellae. Each lamella was composed of thin collagen fibrils running in parallel, (× 90 000).

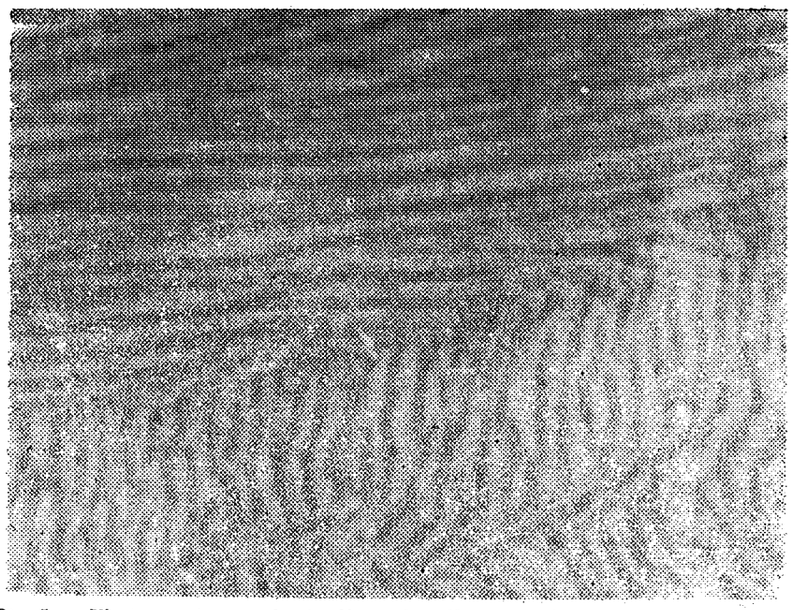

Fig. 5. — Electron micrograph of collagen lamellae from the posterior region of the stroma. The collagen lamellae tended to be arranged parallel to the corneal surface, (× 60 000).

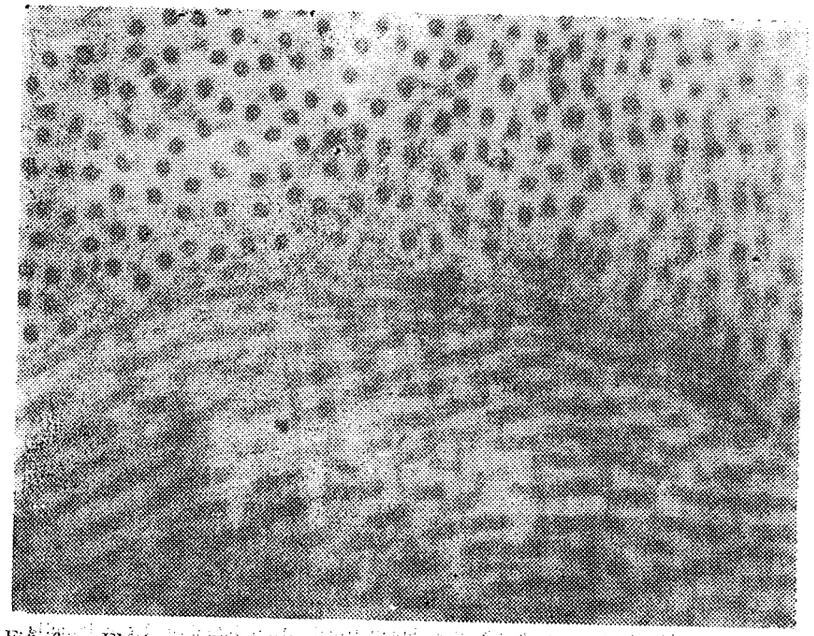

Fig. 6. — Electron micrograph of two collagen lamellae which are perpendicular, (× 80 000).

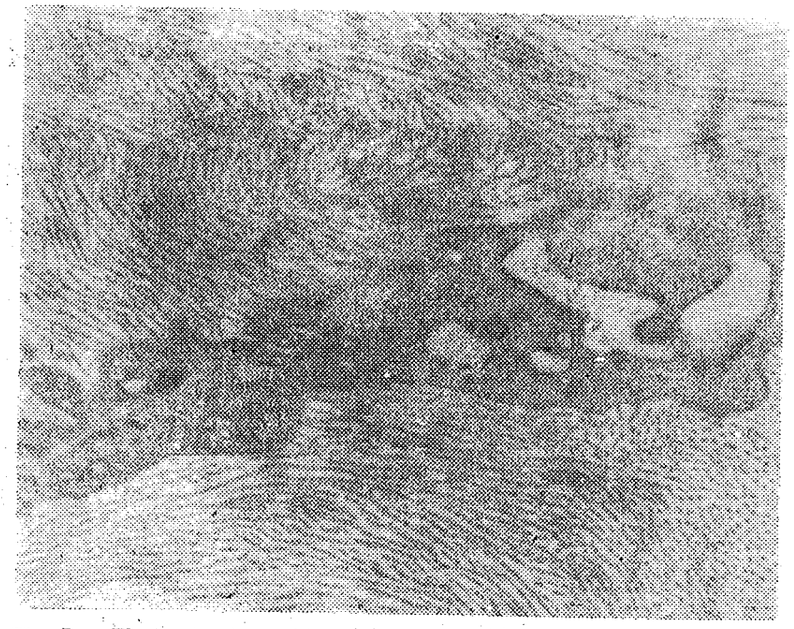

Fig. 7. — Electron micrograph of keratinocyte process. Note the electron-dense matrix in which a moderate number of membranous organelles are present, (× 20 000).

Corneal transparency has been explained basically in two different ways: one is based on the assumption that the collagen and intervening ground substance have uniform refractive indices and the other is the pattern of spatial arrangement of collagen fibrils which are responsible for distructive interference of the light except in the forward direction (12).

Some observations indicated (12) that the collagen lamellae are attached closely to each other with interlamellar spaces smaller than the wavelength of light. Therefore, the stocked lamellae as a whole optically act as a single uniform sheet causing no significant reflections at the surface of the individual lamellae.

However, composition and concentration of proteoglycans from extracellular spaces are considered to contribute to the organization of collagen fibrils (17).

As shown in our study the bovine corneal stroma consists of characteristic lamellae of collagen fibrils, each collagen lamella is composed of uniform-sized fibrils running parallel to each other. This arrangement of uniform-sized fibrils might be important in the corneal transparency.

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CHANGES OF THE STRUCTURE AND FUNCTIONING OF THE BENTHIC OLIGOCHAETE COMMUNITIES FROM THE DANUBE DELTA AQUATIC ECOSYSTEMS (1976–1982) 1. DYNAMICS OF THE STRUCTURE OF THE COMMUNITIES AND THEIR DOMINANT POPULATIONS

I. DIACONU, A. VĂDINEANU and GETA RÎȘNOVEANU *

The research activities have focused on the structure of benthic communities, especially on the dominant oligochaete populations. The spatial and temporal dynamics of oligochaete populations and their spatial distribution were investigated in some aquatic ecosystems of the Danube Delta, during 1976–1982. The criteria of dominance accepted by us, criteria that are based on the numerical and gravimetrical abundance level, were fulfilled by one (Porcu lake), two (Matita, Merhei lakes) or three (Roșu, Puiu lakes) populations of oligochaete, belonging to five different species (*Potamotrix hammoniensis*, *Branchiura sowerbyi*, *Limnodrilus hoffmeisteri*, *Tubifex tubifex* and *Ilyodrilus templetoni*). Most of these populations had a clumped spacing.

INTRODUCTION

The aim of our research work on some aquatic oligochaete populations, on a wide subject area, was to find out the laws that govern the structural and functional organization of several aquatic ecosystems in the Danube Delta, in order to evaluate their bioproductive potential.

The paper includes the results obtained by the authors during the 1976–1982 interval, in the Danube Delta aquatic ecosystems: Puiu-Roșu–Porcu and Matita–Merhei (Figs 1, 2), regarding the populations of the benthic limnicol oligochaete in order to establish the structural and functional characteristics of the studied ecosystems.

The benthic fauna represented and still continues to represent the object of several hydrobiological or ecological research works due to the importance of the respective organisms in fish food or as biological indicators of aquatic pollution.

1. MATERIAL AND METHOD

The aquatic ecosystems taken into study are placed in the maritime Delta (Puiu, Roșu and Porcu lakes) and in the river Delta (Matita and Merhei lakes) (Figs 1 and 2).

The research activity took place in situ and in the laboratory so as to ensure the information requested for solving the problems approached in this work.

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Structural and functional parameter evaluation for a real ecosystem aracterization was strictly conditioned by the working schedule regard- ; location and transect orientation, their number and number of sam- ng stations, the size of samples, their number and frequency (Figs. 1 and 2).

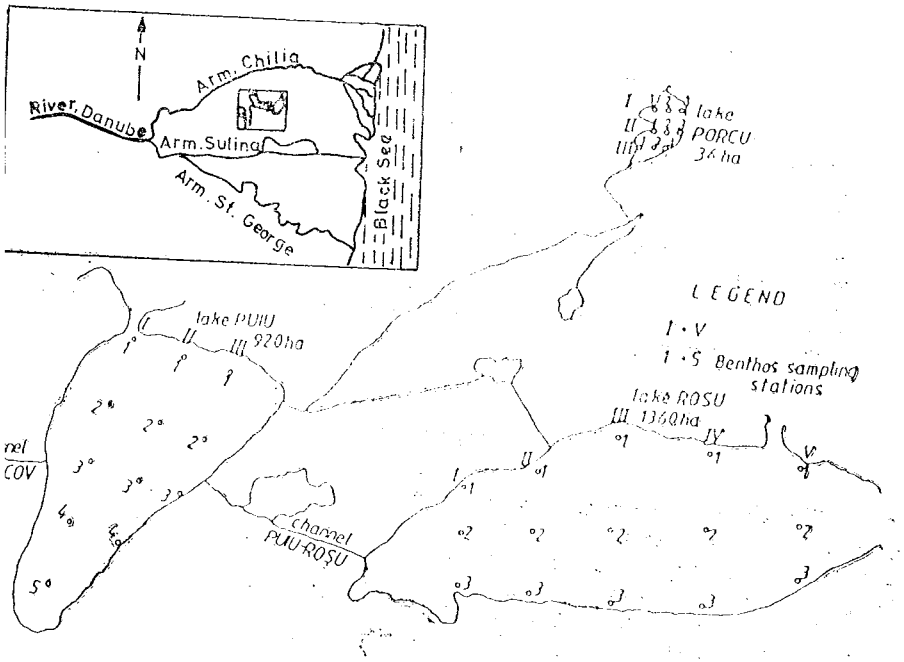

Fig. 1. — General diagrams of the Puiu, Roşu, Porcu lakes.

An amount of 2922 units of samples have been sampled with a corer with the height 25 cm and 50 sq cm in area. They have been pro- in the laboratory, thus obtaining the primary data, whose processing, ethods of ordinary statistic analysis (4), have led to the structural eters estimation of the oligochaete association and of dominant tions.

The place occupied by the oligochaete association within the ben- una structure was established by using as parameters the number nponents (groups of organisms) and their numerical (A_1%) and metric (A_2%) abundance.

t is stated that, in order to evaluate the share of representation group and especially of the oligochaetes within the benthic fauna, ne annual average values of the mentioned parameters were taken count.

he research methods for the physical and chemical parameters were lescribed in the current specialized literature being generally on d laboratory methods: gravimetic, calorimetric and titrimetric 2, 3).

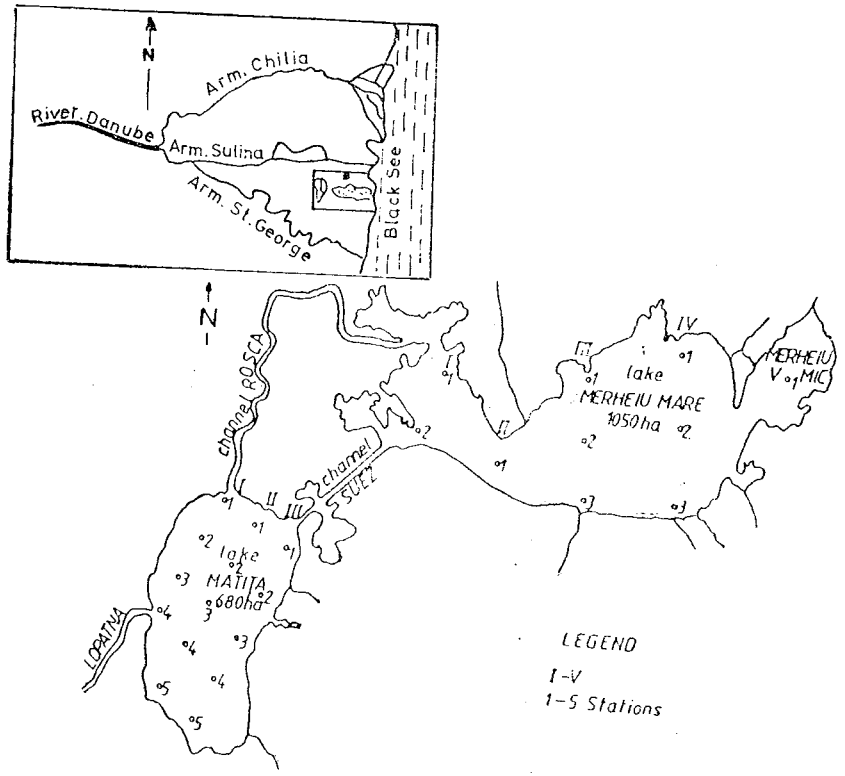

Fig. 2. — General diagrams of Matiţa, Merhei lakes.

2. RESULTS AND DISCUSSION

2.1. The contribution of the oligochaete asscciation to the structure of the benthic fauna

In the light of the criterion according to which a component is dominant if $A_1 > 10\%$ and $A_2 > 5\%$, the following aspects were differentiated: the oligochaete association was integrated in the fauna of all the ecosystems taken into analysis and represented a major component both by biomass A_2 (18—36%) and numerical representation A_1 (18—28%). Unlike chironomids larvae, that are by far dominant in all the ecosystems (5), the oligochaetes were in the greatest proportion represented in the benthic fauna of the lakes Puiu and Porcu and to a lesser extent in the lakes Roşu, Merhei, Matita.

2.2. Dynamics of the structure of the oligochaete association

The study of the structure dynamics of the oligochaete association, for each ecosystem taken separately, emphasized the fact that the reorganizations that occurred at the group level had been oriented to

simplification of the stucture, simplification manifested under the form of the disappearance of certain populations and also of the reduction of the representation share (numerical and gravimetrical) of others (Figs. 3—6)

2.3. Structure and dynamics of the dominant benthic oligochaete populations

Due to the simplification process of community structure, the function of an energy carrier for the entire group of species was taken over by one population (*Potamothrix hammoniensis*) in lake Porcu, two populations (*Branchiura sowerbyi*, *Limnodrilus hoffmeisteri* in lake Puiu, *Tubifex tubifex*, *Ilyodrilus templetoni* in lake Matita, *L. hoffmeisteri*,

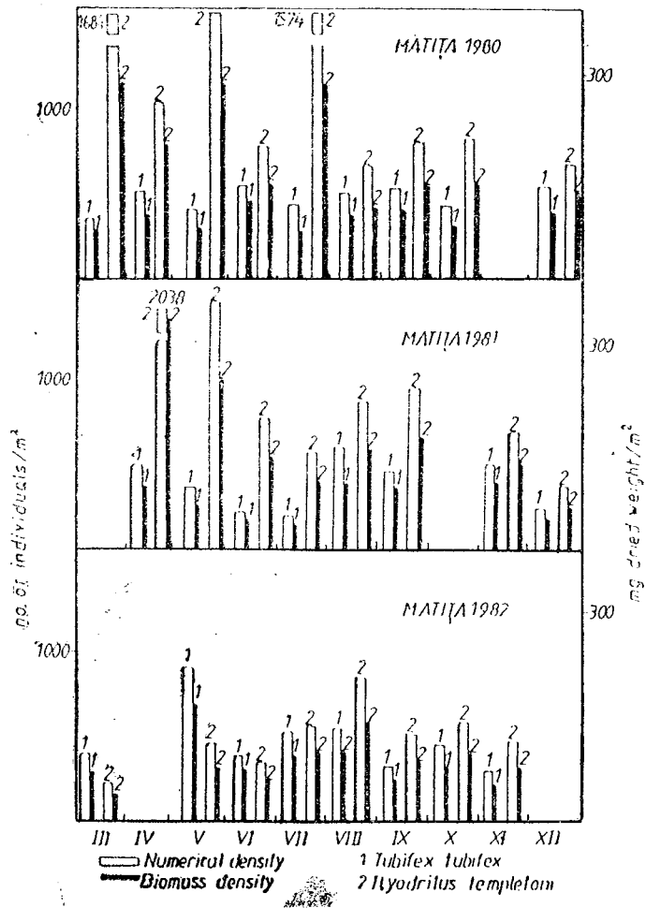

Fig. 3. — Dynamics of the oligochaete populations density in Matiţa lake.

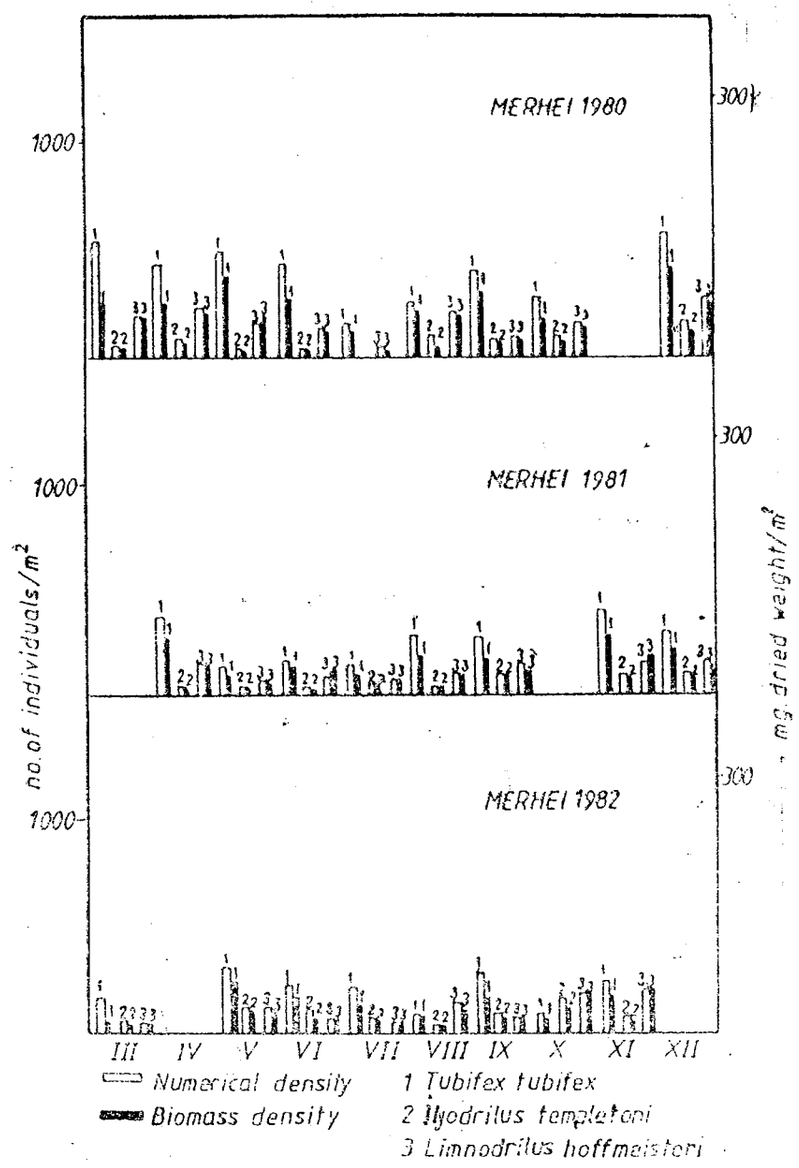

Fig. 4. — Dynamics of the oligochaete populations density in Merhei lake.

T. tubifex in lake Merhei) and respectively, three populations (*P. hammoniensis*, *B. sowerbyi*, *L. hoffmeisteri*) in lake Roşu (Table 1).

This fact points out to the important role which the respective populations have had in the development of the fundamental ecological

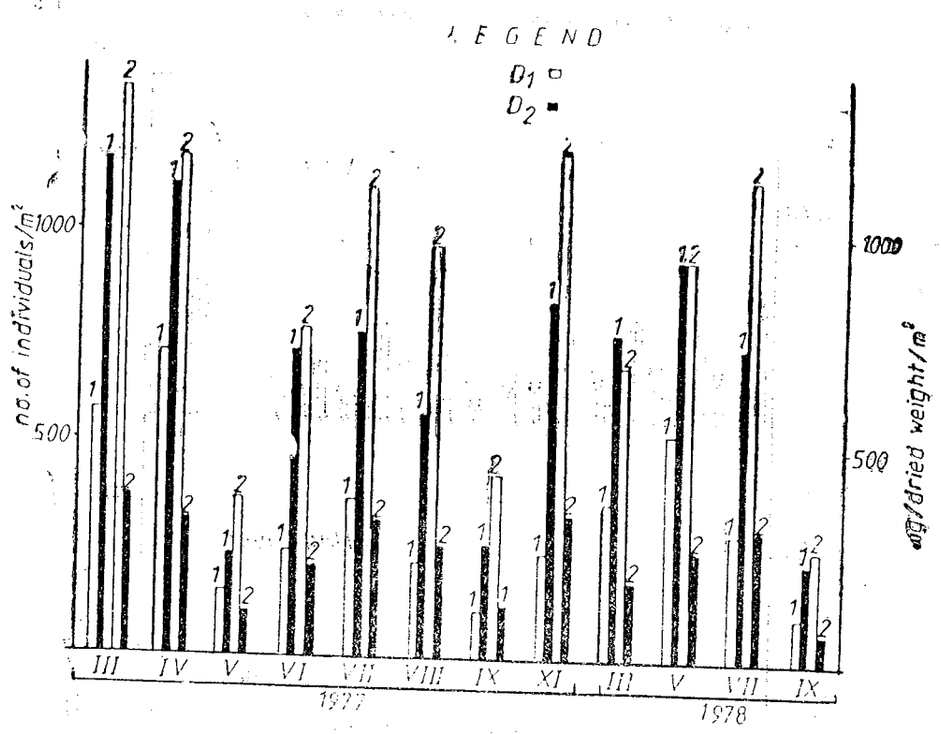

Fig. 5. — Numerical (D_1) and gravimetrical (D_2) density of *B. sowerbyi* (1) and *L. hoffmeisteri* in lake Puiu.

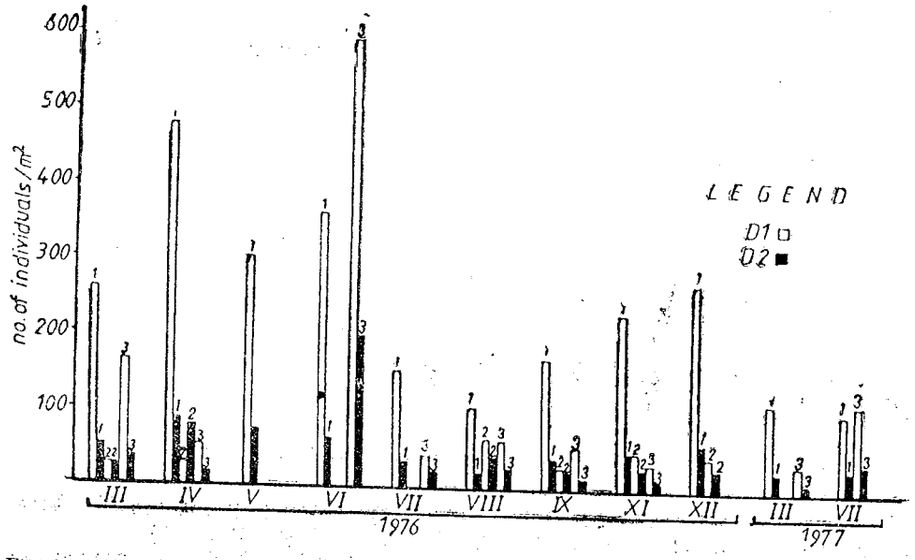

Fig. 6. — Numerical (D_1) and gravimetrical (D_2) density of *P. hammoniensis* (1), *B. sowerbyi* (2) and *L. hoffmeisteri* (3), in lake Roşu.

Table 1

Dominant populations in the structure of benthic oligochaete community

Population	ROŞU		PUIU		PORCU		MATIŢA		MERHEI	
	A_1%	A_2%	A_1%	A_2%	A_1%	A_2%	A_1%	A_2%	A_1%	A_2%
Potamothrix hammoniensis	27—79	21—93	—	—	66—91	73—94	—	—	—	—
Branchiura sowerbyi	7—14	21—40	11—26	63—72	—	—	—	—	—	
Limnodrilus hoffmeisteri	5—15	7—64	37—71	16—31	—	—	—	—	21—38	12—66
Tubifex tubifex	—	—	—	—	—	—	17—66	19—68	21—92	13—88
Ilyodrilus templetoni	—	—	—	—	—	—	34—82	53—81	—	—

processes within the analyzed ecosystems and especially, (taking into account the place occupied within the tropic structure) to their functioning as main links, in the transfer of the energy stored by the sedimented detritus to benthivorus fishes. Thus, it becomes imperative to evaluate the role played in energy transfer by these dominant populations during the whole period of research. In order to fulfill this task, it was necessary that a previous analysis of the evolution (in structural terms) should be applied to each of them (Table 1, Figs. 3—6).

The primary data obtained from the analysis of the sample units have ensured the estimation of two structural parameters: population size, expressed by numerical density (D_1) and gravimetric density (D_2) (Figs. 3—6) and spacing. The data regarding the share of the two age categories in the sample were used in obtaining the cumulated energy budget and for evaluating the number of generations per year.

The *B. sowerbyi*, *P. hammoniensis* and *I. templetoni* populations (in 1981 and 1982) had only one generation per year, the *T. tubifex* populations and *I. templetoni* ones had two generations per year (1980) and *L. hoffmeisteri* populations had 2—3 generations per year.

2.4. Spatial distribution

The clumped spacing of most of the dominant oligochaete populations was interpreted as a response to a complex of factors: the structural heterogeneity of the sediments, the quantity of organic matter, the de-

gree of covering (on the bottom of the lake) with submerged vegetation (Puiu, Roşu, Porcu lakes), water circulation and the degree of water agitation and the persistence of hypoxic condition. In order to estimate the type of spatial distribution of the dominant oligochaete populations the Fisher (J) and Green (C_x) indexes were used (1).

The *L. hoffmeisteri* populations had a random distribution in Roşu and Merhei lakes (the Fisher index is not significatively different from the value one — as the standard deviation "d" shows — and the Green values are towards zero — see Table 2). In Roşu lake the *P. hammoniensis* populations changed their spacing from a typically clumped spacing (in 1976) to a random one (in 1977). This happened due to the changes of the populations size. As can be seen in Table 2 all the other populations had a clumped spacing.

Table 2

The spatial distribution of oligochaete populations

Lake	Species	Year	J	d	C_x
PUIU	*L. hoffmeisteri*	1977	5.7	11.0*	0.035
		1978	7.2	12.8*	0.04
	B. sowerbyi	1977	14.7	19.4**	0.07
		1978	13.0	17.4**	0.062
	T. tubifex	1977	7.7	15.0*	0.033
		1978	9.0	11.4**	0.045
ROŞU	*L. hoffmeisteri*	1976	0.96	1.85	0.00
		1977	1.08	1.93	0.0002
	B. sowerbyi	1976	11	16.5**	0.078
	P. hammoniensis	1976	6.5	12.7*	0.00
		1977	1.15	2.8	0.00
PORCU	*P. hammoniensis*	1976	12.0	18.9*	0.085
		1977	8.8	16.2*	0.057
MATIŢA	*I. templetoni*	1980	11.5	21.0**	0.105
		1981	20.6	32.4**	0.156
		1982	10.7	19.7**	0.094
	T. tubifex	1980	5.7	11.0*	0.025
		1981	6.4	12.1*	0.03
		1982	7.8	14.0*	0.042
MERHEI	*L. hoffmeisteri*	1980	1.2	1.8	0.0005
		1981	0.98	1.6	0.0007
		1982	1.31	2.1	0.001
	T. tubifex	1980	6.2	11.6*	0.027
		1981	3.8	7.4*	0.018
		1982	3.0	7.0*	0.015

* $P > 0.95$
** $P > 0.99$

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Professor BOGDAN STUGREN

1928—1993

Professor Bogdan Stugren was born on September 9.1928, in the city of Reghin, in the Mureș District. He graduated from the Faculty of Natural Sciences at Cluj in 1952. He began his didactic career as a laboratory assistant at the Zoology Department in Cluj, working his way up to the position of a university professor. Professor Stugren distinguished himself, during more than 40 years of activity, through his didactic and research activity, both in the country and abroad.

In the didactic domain, his activities centered around the disciplines of ecology, general biology and vertebrate zoology. He wrote the first Romanian book of General ecology which was published in 1965 and later revised and reprinted in several editions. In 1972 he published *Grundlagen der allgemeinen Ökologie* with the VWB Gustav Fischer Verlag, Jena Publishing Company. This German version was revised and reprinted in 1978 and 1986. In his last years of activity he wrote *Zoologia și anatomia comparată a vertebratelor* (*The Zoology and Comparative Anatomy of Vertebrates*).

Professor Stugren also contributed to the development of elementary education in Romania. He wrote an ecology textbook for 8th year pupils and the chapter on evolution for the 12th year textbook.

An exceptionally erudite man, Professor Stugren also wrote books about the history and the philosophy of biological sciences, *Știința evoluției* (*Science of Evolution*) (1965) and *Evoluționismul în secolul XX* (1969). He also collaborated in the writing of *Mica enciclopedie de biologie și medicină* (*The Little Encyclopedia of Biology and Medicine*) (1976) and was a coordinating editor of the books *Probleme moderne de ecologie* (*Problems of Modern Ecology*) (1982) and *Ocrotirea naturii, tradiții, actualitate, perspective* (*Protection of Nature, Traditions, Present, Prospects*) (1988).

His entire journalistic activity in the field of biological education is a source of bibliographical reference which offers students a wealth of information.

Professor Stugren's scientific research activity was carried out in the field of vertebrate zoology, the field in which he wrote his doctorate thesis, entitled "Variabilitatea și evoluția unor amfibieni și reptile din fauna României". In 1962 he received his doctoral degree in biology.

In his researches Professor Stugren studied the complex aspects of faunistics, zoogeography and intraspecific variability. He clarified the problem of some subspecies of amphibians and reptiles, exaplaining the

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causes of intra- and interpopulational variabilities, as well as the evolution of the areas of herpetologic fauna in relation to the glaciers of the Quaternary Period. He was the initiator of an original method, determined statistically, of explaining the evolution of the areas on the basis of similarities and differences among herpetologic fauna.

After 1980, his research was extended to the uncompleted scientific study of the herpetologic fauna of Greece. Professor Stugren formed his conclusions using a wealth of material and an exceptional capacity of analyzing and synthesizing.

The results of a life of research are found in the more than 60 scientific articles published in the country and abroad. They will remain reference articles in the field of Romanian and European herpetology.

Through his participation at international Congresses and Symposiums — for example, The 16th International Congress of Zoology in Washington, 1963; The 11th Congress of Pacific Sciences at Tokyo in 1965 and The Conference of the Herpetologic Society in Prague in 1985, etc. Professor Stugren's worth as a man of science was recognized both in the country and abroad.

In the country he participated in numerous scientific meetings, organized on the local and national level. He presented valuable papers, sharing his encyclopedic knowledge in the field of science.

As an acknowledgement of his scientific merits, he was received as a member of the Ecological Society in Paris in 1964, a member of the Herpetologic Society of Kansas (1968—1969) and is the collaborator for Romania in the International Union for the Conservation of Nature. In Romania, Professor Stugren was awarded the "Meritul ştiinţific" medal and the "Emanoil Teodorescu" premium of the Romanian Academy. He was also a member of the Romanian Ecological Society.

Nicolae Tomescu

AVIS AUX AUTEURS

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

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