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RHAGIO MEDEAE n. sp. (DIPTERA — RHAGIONIDAE)

PAR

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In the present work a new species named *Rhagio medeae* n.sp. (Diptera — Rhagionidae) is described.

In order to emphasize the characters of this new species the author made a comparative study of the coloration, the form of palps, antennules and feet and of the abdominal apex between *Rhagio medeae* n.s.p. and the species *Rhagio latipennis* Lw. and *Rhagio scolopaceus* L. with which it has resembling characters.

L'étude de la Fam. Rhagionidae (Diptera — Brachycera) a été récemment abordée en Roumanie [1].

C'est le genre *Rhagio* qui a, dans cette famille, le plus grand nombre d'espèces. On en connaît 45 dans la Paléarctique, dont 38 en Europe. En Roumanie on a identifié jusqu'à présent 10 espèces appartenant au genre *Rhagio*.

Au cours de l'année 1969, nous avons étudié 6 exemplaires femelles, capturés à Eşelniţa, Cozla — Piatra Neamţ et Butucoasa — Focşani. Tous ces exemplaires présentaient des caractères qui, selon la clef des espèces et la diagnose [2], [3], les faisaient appartenir à l'espèce *Rhagio latipennis* Lw. Mais de la diagnose apparaissaient certaines confusions dans la délimitation des caractères de la femelle.

Après avoir capturé en 1970, à Herculane, un nombre de 35 ♂♂ et 12 ♀♀ de *Rhagio latipennis* Lw., nous nous sommes convaincus que les exemplaires femelles de l'année précédente n'appartenaient pas à cette espèce. De plus, nous avons établi que les exemplaires de Herculane, déterminés sur la base d'une étude de l'armure génitale, ne présentaient pas de dimorphisme sexuel.

La possibilité d'attacher les 6 exemplaires femelles à *Rhagio scolopaceus* L., dont le habitus était ressemblant, entrait également en discussion. Mais, tant la diagnose que l'analyse des exemplaires disponibles de *Rhagio scolopaceus* L. nous ont démontré que notre matériel ne pouvait être encadré ni dans cette espèce.

Vu cette situation, nous sommes arrivés à la conclusion que nous nous trouvons devant une nouvelle espèce. Nous l'avons dénommée *Rhagio medeae* comme preuve de respect et de reconnaissance envers Mme Medeea Weinberg, qui a guidé nos premiers pas dans la recherche, nous a accordé — et continue de le faire — une aide sans réserve pour notre spécialisation.

E. Lindner [2] indique comme premier critérium de détermination des espèces du genre *Rhagio*, la coloration des ailes. L'un des principaux groupes est celui des espèces aux ailes tachées, où s'encadrent tant *Rh. latipennis* Lw. et *Rh scolopaceus* L. que *Rh. medeae* n.sp. Selon la clef de détermination c'est la coloration des coxes qui sépare les espèces aux ailes tachées. *Rh. latipennis* Lw. appartient au groupe ayant les coxes jaunes, tandis que *Rh. scolopaceus* L. appartient à celui aux coxes noires ; nos exemplaires ont les coxes d'un jaune uniforme, sans s'encadrer toutefois ni dans *Rh. latipennis* Lw., ni dans son antithèse *Rh. mellinus*.

Rh. medeae n.sp. a le corps d'une couleur jaune-brun ; la tête aussi large que le thorax ; les antennes et les palpes jaunes, à pilosité noire ; la pilosité de la tête blanche-jaunâtre ; la zone occipitale à pilosité, courte, noire ; le thorax brun à raies grises ; le callus huméral et le bout du scutellum, jaunes, poudrés de gris ; la pilosité du thorax est noire. Les pattes sont jaunes, aux trochanters noirs et sur f_1 il y a toujours une tache bien délimitée, noire, sous-apicale, qui laisse jaunes l'apex et la base. Les ailes, larges, sont plus longues que le corps (12—16 mm), tachées de brun en forme de bandes, dont celle qui lie la ptérostigme à la cellule discale est continue ; la ptérostigme est grande, noire. Les haltères sont jaunes, au capitulum brunâtre. L'abdomen est jaune, avec des taches centrales et latérales noires sur les tergites, taches qui s'unissent — à partir du 4e tergite — et forment des bandes ; l'apex abdominal est noire ; les sternites, à l'exception des deux premiers, qui sont jaunes, ont une couleur noire, avec des bandes jaunes étroites à la partie postérieure. Longueur : 14—18 mm.

Pour mettre en évidence les différences qui existent entre les trois espèces, nous avons fait une étude comparée de la couleur et de la forme des palpes, des antennes, des pattes et des ailes, ainsi que de l'apex abdominal.

En analysant les photos* de ces importants composants taxonomiques des espèces *Rh. latipennis* Lw., *Rh. medeae* n.sp. et *Rh. scolopaceus* L. on voit que :

— *Rh. latipennis* Lw. et *Rh. medeae* n.sp. ont les palpes de couleur identique, jaune, mais différents en ce qui concerne la forme et les dimensions (Pl. I, A, B, C).

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* Les photos ont été exécutées par Şerban Boicescu, que nous remercions une fois de plus à cette occasion.

— *Rh. scolopaceus* L. a les palpes ressemblants — quant à la forme et aux dimensions — à ceux de *Rh. medeae* n. sp., avec la seule différence que leur base est noire sur à peu près la moitié de leur longueur.

— *Rh. latipennis* Lw. et *Rh. medeae* n.sp. ont les antennes de la même couleur, jaune, mais bien différentes comme forme et dimensions (Pl. I, A, B) ; les articles basaux ont la même largeur chez *Rh. latipennis* Lw., l'article 2 étant un peu plus court que le premier ; la largeur de l'article 3 ne dépasse pas celle de la base de l'article 2 ; chez *Rh. latipennis* Lw. le filament terminal est plus long que les trois articles de l'antenne ; chez *Rh. medeae* n.sp. les articles 1 et 2 plus longs que ceux des autres espèces ; la base des articles 1 et 2 est plus étroite et l'article 3 a un aspect bulbiforme, avec une tendance d'élargissement vers la partie externe ; le filament terminal est un peu plus long que l'antenne.

— L'antenne de *Rh. scolopaceus* L. est différente de celle des deux autres espèces quant à la couleur et à la forme (Pl. I, A, B, C). Les articles basaux (1 et 2) sont noirs, le troisième est jaune ; les deux premiers articles sont un peu plus larges que ceux de *Rh. medeae* n.sp. (Pl. I, C et B), le premier étant d'un tiers plus long que le second ; le troisième article est bulbiforme-allongé, avec la partie externe excavée, et est presque aussi long que les deux premiers ; le filament terminal de l'antenne est bien plus long que les articles de l'antenne.

Voici les différences constatées aux pattes : les coxes sont jaunes chez *Rh. latipennis* Lw. et *Rh. medeae* n.sp., mais brunes-noires chez *Rh. scolopaceus* L., avec de rares cas où la procoxa est jaune ; les fémurs sont jaunes chez *Rh. scolopaceus* L. et *Rh. latipennis* Lw., tandis que chez *Rh. medeae* n.sp., f_1 a constamment une tache noire sous-apicale (Pl. II, A, B, C).

Les ailes ont, chez toutes ces espèces, le dessin caractéristique en forme de bandes, mais les dimensions et l'intensité de la couleur en sont variables. Leur couleur est brun pâle chez *Rh. latipennis* Lw. et brun-noir chez les autres deux espèces ; chez *Rh. medeae* n.sp. la bande unissant la ptérostigme à la cellule discale est continue, tandis que chez *Rh. latipennis* Lw. et *Rh. scolopaceus* L. elle est discontinue (Pl. II, A, B, C).

Nous soulignons le fait que, bien qu'étant capturés en des localités et années différentes (3 ♀♀ Eşelniţa 14—18.V.1969 ; 1 ♀ Cozla — Piatra Neamţ 24.V.1966 ; 2 ♀♀ Butucoasa — Focşani 3.VI.1965), les 6 exemplaires présentent des caractères de morphologie externe constants.

Nous avons effectué une étude comparative de l'armure génitale, notamment du sternite VIII, nommé valve sternale, qui a une forme caractéristique, sa partie apicale étant bilobée, spécifique (Pl. III, A, B, C). Chez *Rh. latipennis* Lw. et *Rh. medeae* n.sp. la forme générale en est triangulaire, tandis que chez *Rh. scolopaceus* L. elle est plus proche de celle d'un quadrilatère. Si les lobes apicaux sont ovales-allongés chez *Rh. latipennis* Lw. et chez *Rh. medeae* n. sp., chez *Rh. scolopaceus* L. ils sont plus courts et arrondis. Un autre caractère de nette différenciation est constitué par la zone formée de groupements denses de petits poils, qui s'étend sur les côtés externes des lobes, ayant une forme et une surface différentes.

Le second élément caractéristique de l'apex abdominal est le tergite 9 et les cerques (Pl. III, A, B, C). Ces derniers, biarticulés, ont l'article basal deux fois plus large que celui apical ; la forme et le rapport entre ces

deux articles sont nettement différenciés chez ces trois espèces, ainsi qu'il résulte de la Planche III, A,B,C.

Voici comment s'intègre la nouvelle espèce dans la clef indiquée par E. Lindner [2]:

5. . . . . *Rh. latipennis* Lw.

— Longueur plus de 14 mm; dessin caractéristique de l'aile (Pl. II, B); antennes et palpes de couleur jaune, à pilosité noire; pattes jaunes, aux coxes jaunes, trochanter noir et f_1 toujours avec une tache noire sous-apicale, laissant jaunes l'apex et la base (Pl. II, B) . . . . . .
. . . . *Rh. medeae* Iacob.

Les types sont déposés au Muséum d'Histoire Naturelle « Gr. Antipa» de Bucarest; le Holotype est une femelle (N° inv. 12. 130), capturée à Eșelnița le 18.V.1969, leg. Maria Iacob.

Paratypes : 2 ♀♀ Eșelnița 14 et 17.V.1969, leg. Ion Drăghia et Maria Iacob; 1 ♀ Cozla — Piatra Neamț 24.V.1966, leg. Marin Voicu; 2 ♀♀ Butucoasa — Focșani 3.VI.1965, leg. Carol Nagler.

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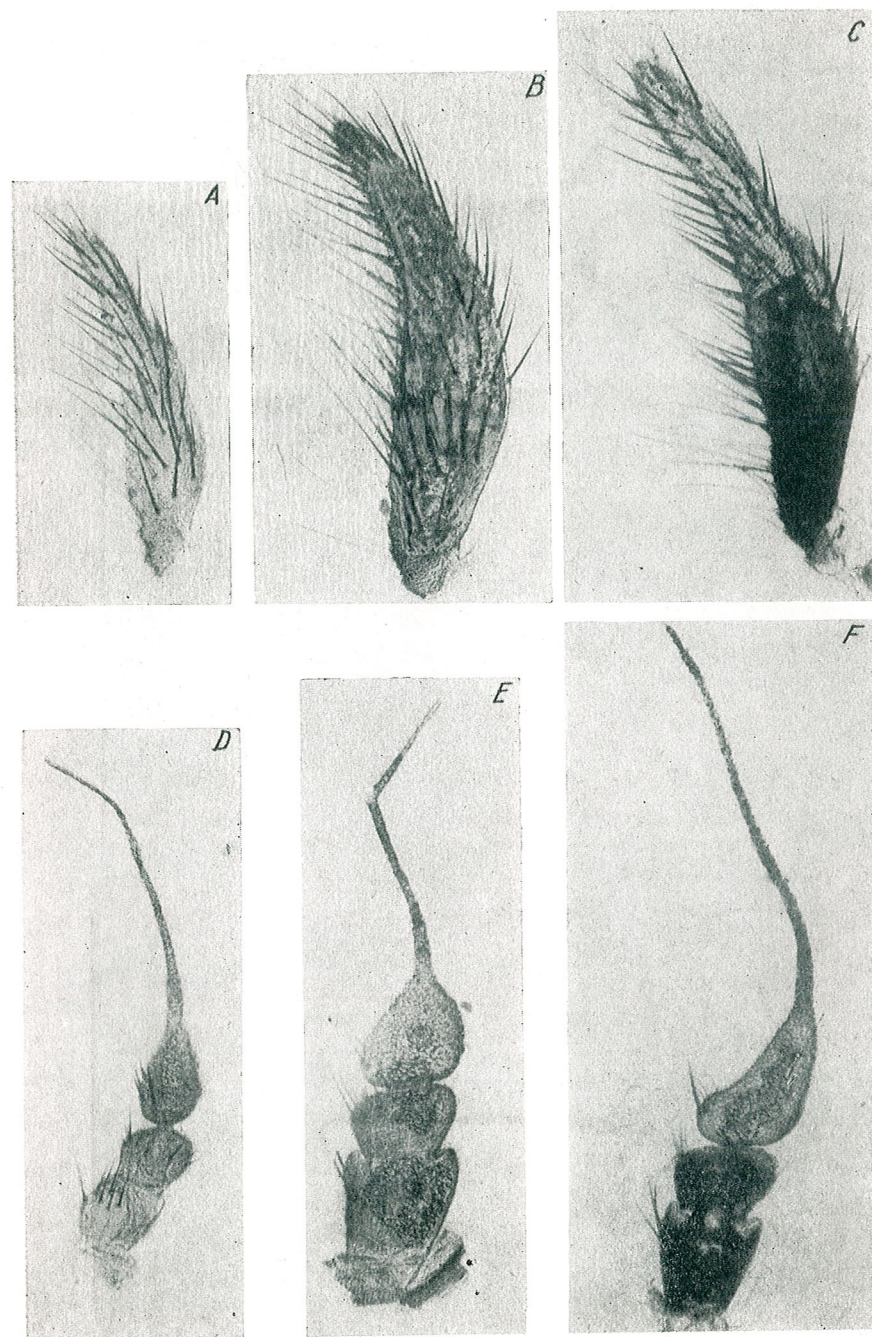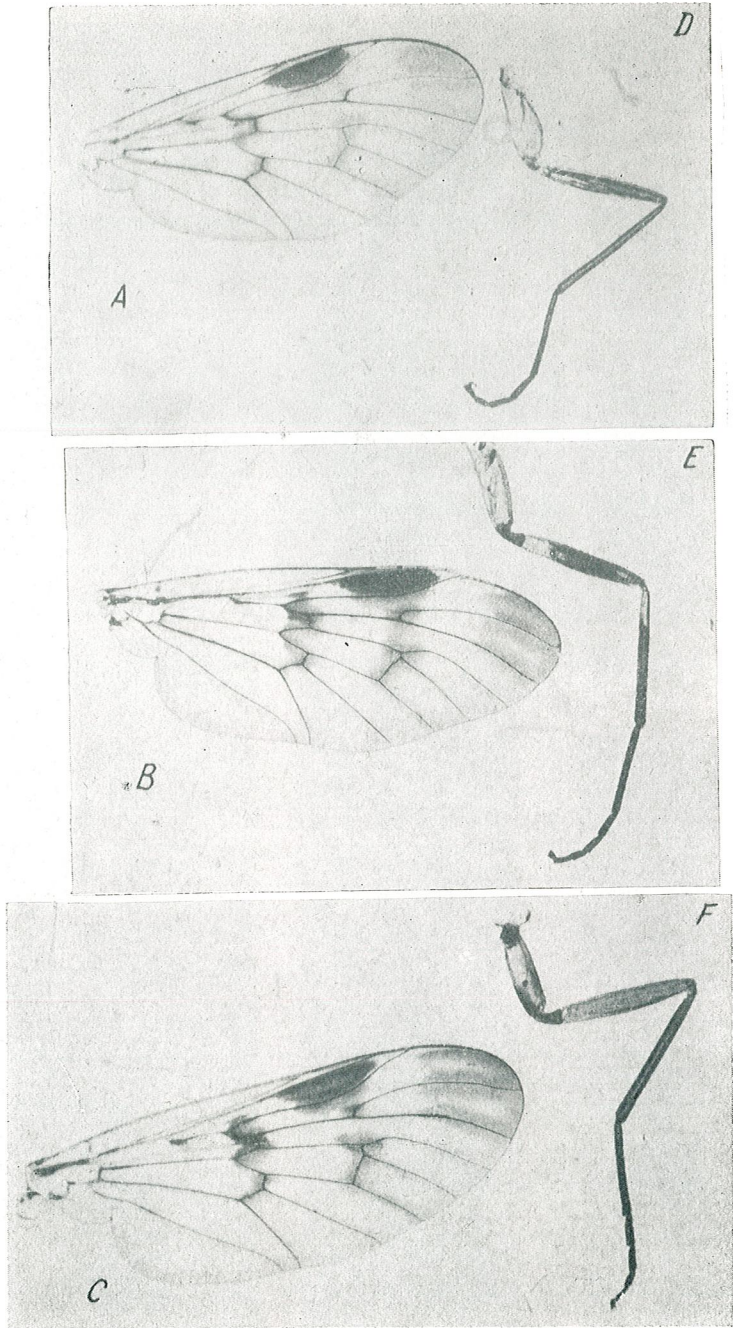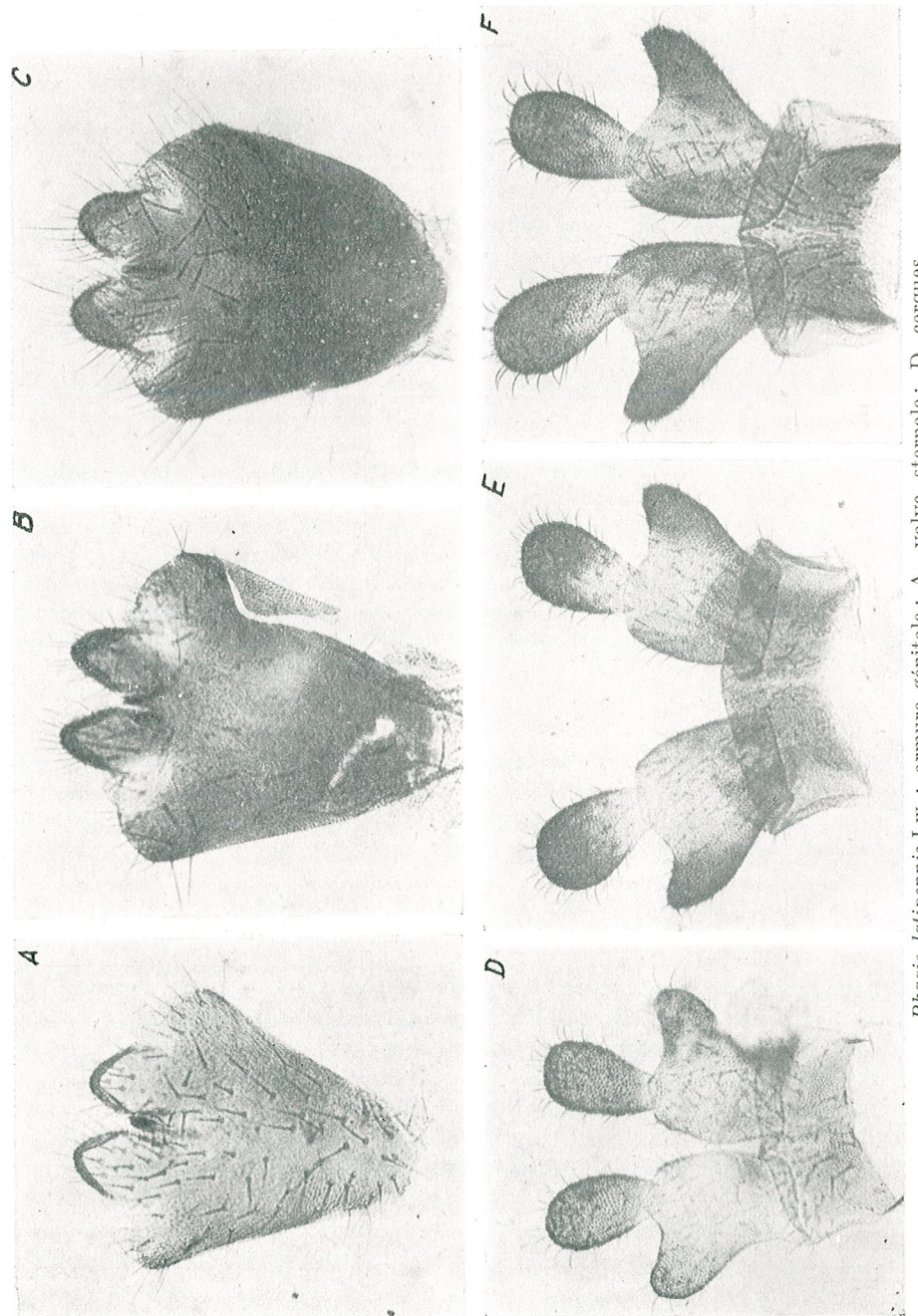
Rhagio latipennis Lw. : A, palpe; D, antenne.
Rhagio medeae n.sp.   : B, palpe; E, antenne.
Rhagio scolopaceus L. : C, palpe; F, antenne.

Rhagio latipennis Lw. : A, aile ; D, patte.
Rhagio medeae n.sp. : B, aile ; E, patte.
Rhagio scolopaceus L. : C, aile ; F, patte.

Rhagio latipennis Lw. : armure génitale : A, valve sternale ; D, cerques.
Rhagio medeae n.sp. : B, valve sternale ; E, cerques.
Rhagio scolopaceus L. : C, valve sternale ; F, cerques.

REVISION OF THE *ONYCHOSTOMA*—SUBGENUS *SCAPHESTES* (PISCES, CYPRINIDAE)

BY

PETRU BĂNĂRESCU

Scaphestes is considered a subgenus of *Onychostoma*, including the five species in which the last simple dorsal ray is always slender : *macrolepis*, *roulei* (both closely related and representative), *tamusuiensis*, *shansiensis* (both closely related and representative) and *barbata*. A biometrical analysis of large series of specimens (Table 1 and Figs. 1 and 2) demonstrated that the so-called species *robustus* is a synonym of *tamusuiensis*. The genus *Onychostoma* (with two subgenera and 16 species) has a typical East Asian range ; it reaches the Hwang-ho and centers in the Hsikiang and Song-Koi drainages.

As shown in a recent paper [1], the species of the East Asian genus *Onychostoma* having the last simple dorsal ray always slender can be ascribed to a distinct subgenus : *Scaphestes* Oshima, 1919. The present paper is the continuation of a previous one, dealing with the subgenus *Onychostoma* s.str. [1].

The specimens referred to belong to the following collections : American Museum of Natural History, New York (A.M.N.H.), Academy of Natural Sciences in Philadelphia (A.N.S.P.), Institutul de Biologie "Tr. Săvulescu", Bucharest (I.B.T.S.), Field Museum of Natural History Chicago (F.M.N.H.), Muséum National d'Histoire Naturelle, Paris (M.N.H.N.), United States National Museum, Washington (U.S.N.M.)

SYSTEMATIC ACCOUNT

Onychostoma (Scaphestes) macrolepis (Bleeker, 1871)

Specimen examined : Holotype of *Barbus macrolepis*, M.N.H.N. 5064, Yangtzekiang, 151.0 mm.

D 3/8; L. lat. $51\frac{9}{5}52$; Sp. br. 25; D. phar. 4.3.2—2.3.4.

Body depth 19.8% of standard length; least depth 9.3%; caudal peduncle length 17.9%. No barbels.

Yangtze; apparently no more recorded since its original description by Bleeker [2].

Having no barbels, this species does not fit in the definition of *Scaphestes* by Oshima [6]; yet I think the slender last simple dorsal ray is a more important character than the presence or absence of barbels (in *O. sima* this character is subject even to individual variation) and that *O. macrolepis* actually is closer to *O. tamusuiensis* and *O. shansiensis* than to the *Onychostoma* s. str. species lacking barbels.

Onychostoma (Scaphestes) roulei (Wu, 1931)

No specimen available.

D 3/8; L. lat. $47 \frac{7\,1/2}{4}$; D. phar. 4.3.1—1.4.5.

Body depth 26.3% of st. length; least depth 7.7%; caudal peduncle length about 22.6% (according to the illustration).

Foochow, lower Minkiang river.

Nichols [5] synonymizes *Barbus roulei* Wu with *Varicorhinus robustus*; yet in the original description of *roulei*, Wu [11] does not mention barbels and points out the similarity of this species with *macrolepis*, another species without dorsal spine and lacking barbels. I too consider *roulei* a species of *Onychostoma* closer to *macrolepis*, which may eventually prove to be a subspecies of the latter.

Onychostoma (Scaphestes) tamusuiensis (Oshima, 1919)

Synonyms : *Scaphestes tamusuiensis* Oshima, 1919; *Varicorhinus robustus* Nichols, 1925; *Varicorhinus tamusuiensis* auct.

Specimens examined :

From Taiwan (= Formosa) island : Holotype of *Sc. tamusuiensis*, F.M.N.H. 59091, Tamusui r., 186.0 mm st. length; A.N.S.P. 76423, Mokin r., 1 spec., 135.5 mm; U.S.N.M. 161710, Taiko r., 1 spec., 55.0 mm; I.B.T.S. 1344, I-lan, North Taiwan, 1 spec., 123.2 mm (received from Prof. J. Chen).

From Minkiang drainage (Fukien province, SE continental China) : U.S.N.M. 87981 & 130561, Foochow, mouth of Minkiang r., 11 spec. in all, 77.0—167.0 mm;

A.M.N.H. 11061, 10689 & 11124, Chungan Hsien, 36 spec. in all, 39.0—183.5 mm;

A.M.N.H. 12181, no locality, 12 spec., 51.2—166.0 mm;

A.M.N.H. 10691, Fukien (no locality), 6 spec., 61.2—110.0 mm (all three series determined *tamusuiensis* by Nichols).

Holotype of *Varicorhinus robustus*, A.M.N.H. 8424, near Nanping (= Yenping), middle Minkiang r., 105.0 mm;

Paratypes of the same, A.M.N.H. 10686, same locality, 11 spec., 69.2—88.2 mm;

A.M.N.H. 11634 & 10693, same locality, 12 spec., 52.0—78.0 mm (determined *robustus* by Nichols);

A.M.N.H. 10684 & 11637, same locality, 14 spec., 46.8—124.0 mm (determined *tamusuiensis* by Nichols);

A.M.N.H. 10696, Fukien (no locality), 8 spec., 52.0—69.3 mm;

A.M.N.H. 10690, Fuching, Fukien, 10 spec., 47.5—117.5 mm;

A.M.N.H. 12170, no locality, 8 spec., 44.0—141.0 mm (all three last-mentioned series were determined *robustus* by Nichols).

D 3/8; L. lat. (44) $45 \frac{6-7\,(8)}{3-4} 48$ (49).

Two pairs of minute, yet quite distinct barbels.

Nichols [4] describes a new species, *Varicorhinus robustus*, from Yenping, Fukien, recording from the same locality also the very close *Scaphestes tamusuiensis*, a species previously known only from Taiwan island. According to him [4], [5], *robustus* differs from *tamusuiensis* only in its deeper body : depth about 27.0% of st. length, as against about 23.2%. Rendahl [7] and Lin [3] consider *robustus* a synonym of *tamusuiensis*.

In order to clarify this problem, I made measurements of most of the available specimens mentioned above. The most variable characters are the body depth, the least depth and the caudal peduncle length. The results are presented in table 1 (the values of body depth being given in % of both standard length and caudal peduncle length). One remarks that in most series determined *robustus* by Nichols (and especially in the Yenping specimens, including the holotype and paratypes), the values of body depth and even of least depth are slightly higher than in "typical" *tamusuiensis*, including the Taiwan specimens and the Yenping specimens (A.M.N.H. 10684 & 11637) determined *tamusuiensis* by Nichols. Yet the overlap of extreme values is too wide to suggest two distinct species. The variation of many body proportions, including depth, being to a certain degree allometric, I represented graphically (Figs 1 and 2) the variation of body depth in % of st. length and caudal peduncle length, each individual value being represented by a point. Regression lines would have been more suggestive, but the number of specimens in each series was too small to allow the calculation of these lines.

Both graphs suggest that, in spite of the rather wide intrapopulation variability, a trend of the values to group around a central line (regression line) is noticeable in each population. The values of body depth are somewhat higher in the population from Yenping than in that from Foochow, lower in that from Chuang-Hsien and in the series A.M.N.H. 10696. It seems that in all populations the body depth shows a positive allometry till the specimens reach 100—120 mm st. length, then a negative one. The changing of the allometry trend can be recognized in the Chuang-Hsien population and in the series A.M.N.H. 12170; the allometry is apparently positive in the series A.M.N.H. 10696, from which only smaller specimens were available, and apparently negative in Foochow population, from which large-sized specimens were available. Important

Table 1

Body proportions (body greatest depth, least depth, caudal peduncle length) in Onychostoma (Scaphestes) tamusuiensis

Locality, Series	St. length mm	n	in % of standard length			Body depth in % of caud. ped.
			b. depth	least d.	caud. ped.	
Taiwan island	55—186	4	22.9—25.1	9.9—11.2	22.5—23.2	100—107
Foochow (U.S.N.M. 87981 & 130561)	121—167	7	23.1—25.4	8.8—9.6	21.4—24.0	100—118
Chungan Hsien (A.M.N.H., 3 series)	39—183	23	20.0—25.1	8.7—10.8	21.1—25.1	81—119
No local. (A.M.N.H. 12181)	51—166	12	21.2—25.9	9.1—11.4	20.8—25.0	86—114
"Fukien"(A.M.N.H. 10691)	61—110	6	21.8—24.5	9.2—11.4	20.1—24.4	96—108
Yenping (robustus including holo- a. paratypes)	52—105	24	23.4—28.2	—	→	—
	88—105	7	—	9.7—10.7	21.7—23.6	110—125
Yenping (tamusuiensis according to Nichols)	47—124	14	21.4—27.8	9.1—10.4	21.2—26.2	100—127
"Fukien" (A.M.N.H. 10696)	52—69	8	19.0—23.6	8.6—10.1	20.2—24.7	84—104
Fuching (A.M.N.H. 10690)	47—117	10	21.4—25.4	—	—	—
	98—117	4	—	10.0—10.4	21.6—23.0	98.7—117
No local. (A.M.N.H. 12170)	44—141	8	23.4—27.0	9.0—9.8	20.4—25.0	91—126

(vertical label at left of the Minkiang rows: *Minkiang drainage (Fukien province)*)

is the fact that the values of all Yenping specimens, considered by Nichols some as *tamusuiensis* (A.M.N.H. 10684 & 10693), the others as *robustus* (holotype, paratypes and A.M.N.H. 11634 & 10693) are evidently grouped around a single regression line, representing a single population. Nichols selected the elongate specimens as *tamusuiensis*, the deeper-bodied ones as *robustus*; yet the graph demonstrates that they all belong to a single species and population.

These data demonstrate that *Varicorhinus robustus* is a synonym of *Onychostoma tamusuiensis*.

The range of *O. tamusuiensis* includes Taiwan island, Minkiang drainage in Fukien and Tientai in Chekiang [10].
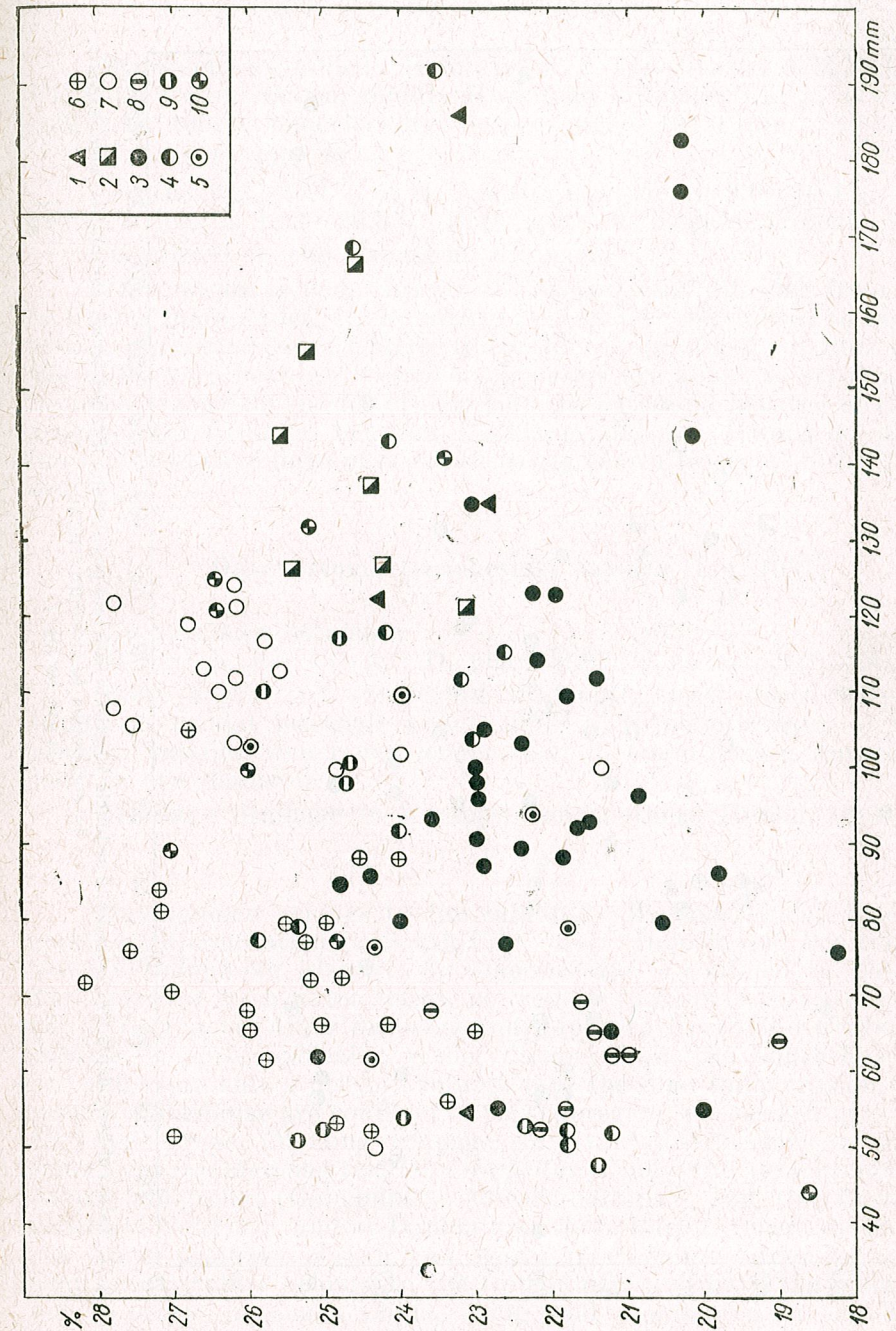

Fig. 1. Values of body depth (in % of standard length) in different populations of Onychostoma (Scaphestes) (tamusuiensis), in correlation to standard length.

1. Taiwan Isl. ; 2. Foochow, mouth of Minkiang r. ; 3. Chungan Hsien ; 4. no locality (A. M. N. H. 12181) ; 5 "Fukien" (A. M. N. H. 10691) ; 6. Yenping, middle Minkiang r. (robustus) ; 7. Yenping, middle Minkiang r. (tamusuiensis according to Nichols) ; 8. "Fukien" (A. M. N. H. 10690) ; 9. Fuching, Minkiang drainage ; 10. no locality (A. M. N. H. 12170).
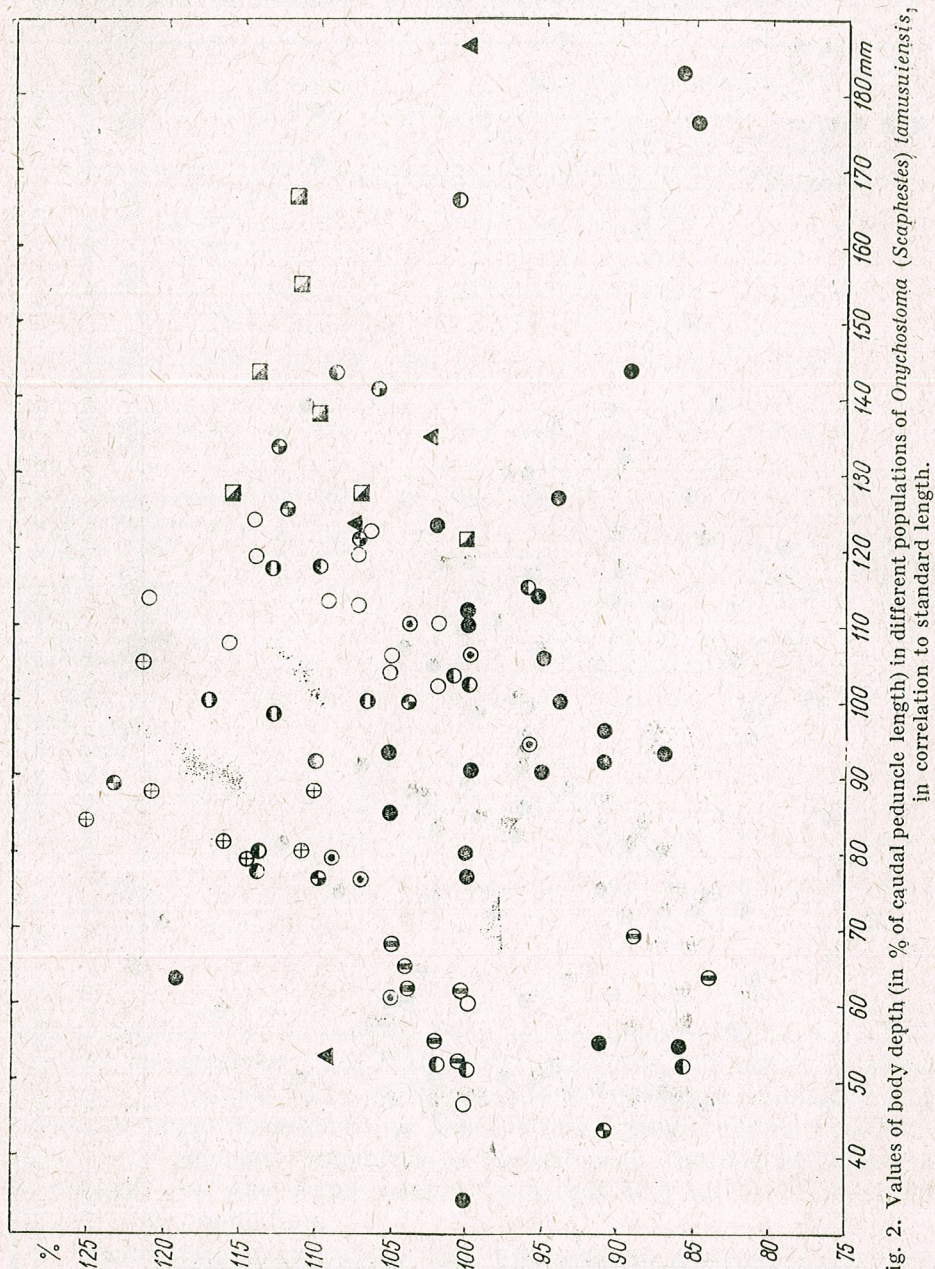
Fig. 2. Values of body depth (in % of caudal peduncle length) in different populations of *Onychostoma* (*Scaphestes*) *tamusuiensis*, in correlation to standard length. 1–10: see Fig. 1.

Onychostoma (Scaphestes) shansiensis (Nichols, 1925)

Specimens examined : Holotype of *V. shansiensis*, A.M.N.H. 8425, Niang-tze-kwan, Shansi, middle Hwang-ho drainage, China, 174.0 mm ; A.M.N.H. 10680, same locality, 5 spec., 91.0—194.0 mm.

D 3/8 ; L. lat. 49—53.

Depth 19.2—25.4% of st. length ; least depth 9.6—10.4% ; caudal peduncle length 18.2—20.8%. Both pairs of barbels present, minute.

Restricted to the Hwang-ho drainage.

This species is very close to *O. tamusuiensis*, differing from it only in having more scales. H. Rendahl [7] considers *shansiensis* to be a subspecies of *tamusuiensis* ; I prefer to consider it specifically distinct because the differences between it and *tamusuiensis* are about as great as those between sympatric species of *Onychostoma* s.str. and because of the wide geographical distance between Chekiang and the Hwang-ho drainage (no representative form of *tamusuiensis* is known to occur in the Yangtze drainage).

Onychostoma (Scaphestes) barbata (Lin, 1931)

No specimen available.

D 3/8 ; L. lat. 48—50 ; D. phar. 5.3.2—2.3.5 or 5.3.2—2.4.5.

Body depth 21.2—22.2% of st. length ; least depth 7.6—8.6% ; length of caudal peduncle about 13.6—15.4%. Both pairs of barbels present, maxillar one much longer than in *tamusuiensis*, sometimes as long as eye diameter.

Hsikiang drainage in Kwangsi and southern Hunan, China.

★

Onychostoma belongs to the subfamily Barbinae which reaches its greatest differentiation in South-East Asia (Indochinese Peninsula and western Indonesian islands) ; yet *Onychostoma* is confined to East Asia. The fauna of fresh-water fishes, as well as that of fresh-water molluscs [8] of East Asia (Amur drainage and Japan to North and Central Vietnam) is, zoogeographically, rather distinct from the South-East Asian one and represents a distinct subregion of the Sino-Indian region. Several genera of Barbinae are confined to this subregion, not occurring in South-East Asia proper (Mekong drainage, etc.) ; the most specious among these genera are *Onychostoma* and *Acrossocheilus*. Unlike the autochtonous East Asian subfamilies (Gobioninae, Acheilognathinae) and the East Asian genera of Cultrinae and of Danioninae, these Barbin genera do not reach the Amur drainage, Japan and Korea (*Hemibarbus* occurring in these countries, actually belongs to the Gobioninae) and have the main distribution center in South China, being well represented in the Yangtze drainage too ; only two such genera reach northern East Asia, e.g. the Hwang-ho and the Pai-ho drainages (*Onychostoma* and *Acrossocheilus*). One specimen of an *Onychostoma* species, *macracantha*, was recorded from

the Mekong drainage in South Vietnam, but I have doubts whether it actually occurs there; the specimen was taken from the market of Kon-Tun and I think it was probably caught from some river flowing into the Tonkin Bay, the fish fauna of these rivers being close to that of the Song-Koi, while the fish fauna of the Mekong is quite different.

The distribution of the 16 species of *Onychostoma* — 11 of *Onychostoma* s. str. reviewed previously [1] and 5 of *Scaphestes* — is the following :

Hwang-ho drainage : two species, one occurring also in the Yangtze (*sima*), one endemic (*shansiensis*).

Yangtze (except its southern tributaries in Kweichow) : three species, two endemic (*angustistomata* and *macrolepis*), one also in the Hwang-ho.

Southern tributaries of the Yangtze in Kweichow province : three species, two of them also in the Hsikiang drainage (*gerlachi* [1] and *rara*) and one endemic (*rhomboides*).

Chekiang province : one species, *tamusuiensis*, occurring also in the Minkiang drainage and Taiwan.

Minkiang drainage : two species, *roulei* endemic, *tamusuiensis* also in Taiwan and Chekiang.

Taiwan island : only *O. tamusuiensis*.

Hsikiang drainage : five species, three endemic (*elongata*, *barbata*, *lini*), two also in the southern part of Yangtze drainage (*gerlachi* and *rara*).

Song-Koi drainage in North Vietnam : four species, two endemic (*ovalis* and *vietnamensis*), one also in the coastwise rivers from Central Vietnam (*macracantha*), one also in Hainan island (*leptura*).

Hainan island : only *O. leptura*, occurring also in the Sonk-Koi drainage.

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[1] Tang [9] records from a southern tributary of the Yangtze near Kweiyang, Kweichow, also *O. laticeps* (=*sima*), but the values he indicates for it — L. lat. 49 — are characteristic of *gerlachi*.

L'APPAREIL GÉNITAL FEMELLE CHEZ QUELQUES ESPÈCES D'ARANEAE «HAPLOGYNAE» (ARACHNIDA)

PAR

ELENA TRACIUC

The paper deals with the microscopic anatomy of the female genital apparatus in six species of spiders lacking epigynum and copulatory duct. The author describes both the types of gonads and the structure of the copulatory organ.

L'anatomie microscopique de l'appareil génital femelle chez les espèces abordées dans le mémoire ci-présent n'a pas été décrite jusqu'ici, exception faite pour l'espèce *Dysdera crocata*, chez laquelle Cooke (1966) a décrit la région vulvaire seulement.

Liste des Araneae étudiées :

Dysdera crocata
Harpactes rubicundus } fam. Dysderidae
Scythodes thoracica —— fam. Scythodidae
Pholcus opilionoides —— fam. Pholcidae
Tetragnatha extensa
Pachynatha degeeri } fam. Tetragnathidae

MATÉRIEL ET MÉTHODE

Les espèces ont été capturées dans plusieurs régions : Hotarele (Bucarest), Cozia (Vîlcea) et Pătrăuți (Suceava).

Les Dysderidae ont été capturées dans les pièges au formol 4% dans lesquels a eu lieu aussi la fixation. Pour les autres espèces, le fixateur a été le mélange Hollande. Après la déshydratation le matériel a été inclus en paraffine. Les coupes sériées de 7 μ d'épaisseur ont été colorées à l'hémalun-éosine.

Dysdera crocata C. L. Koch, 1839

Chez cette espèce, la glande sexuelle a la forme d'un fer à cheval, sa position dans l'abdomen étant médiane. Les deux bras du sac ovarien sont joints l'un à l'autre, et dans leur partie antérieure ils se prolongent par deux oviductes courts, courbés de façon latéro-ventrale. Au-delà de cette courbure, ils se réunissent en une poche sous-ovarienne qui s'ouvre dans la paroi postérieure de l'atrium. En ce qui concerne cette partie interne de l'appareil génital, elle est semblable à celle de *Segestria seno-culata*. Les différences apparaissent seulement dans la région vulvaire (Pl. I, fig. 1).

La vulve manque d'épigyne. Dans l'atrium débouche un diverticule postérieur court et très large, entouré dans sa portion d'ouverture par un épithélium glandulaire. Du côté antéro-dorsal de l'atrium débouche un deuxième diverticule, petit, ressemblant à une enflure de l'atrium. Depuis la partie médiane de la paroi antérieure de l'atrium part un canal large, aplati de façon dorso-ventrale, fort chitineux sur ses côtés latéraux et qui se prolonge au milieu de la vulve par une spermathèque trilobée (Pl. II, fig. 3). L'un des lobes a une position ventrale et médiane par rapport aux autres qui sont latéraux. On doit noter que l'on a trouvé des spermatozoïdes dans la spermathèque, aussi bien que dans les diverticules de l'atrium.

Harpactes rubicundus (C.L. Koch, 1839)

L'appareil génital est tout à fait semblable à celui décrit pour *D. crocata* en ce qui concerne sa partie interne. L'espèce manque d'épigyne. Dans la vulve il y a une spermathèque qui part de la paroi antérieure de l'atrium, notamment de la partie médiane de celle-ci, et qui a une forme tubulaire non ramifiée tout en remplissant l'espace vulvaire tout entier (Pl. I, fig. 2).

Scythodes thoracica (Latreille, 1804)

On doit noter dès le début la présence remarquable chez cette espèce d'une glande sexuelle en forme de fer à cheval. La forme et la position de celle-ci, aussi bien que les annexes (oviductes, poche sous-ovarienne) sont semblables à celles décrites pour la fam. Dysderidae (Pl. I, fig. 3). La vulve chez *S. thoracica* manque d'épigyne, tout en étant plus simple que chez les Dysderidae. L'atrium a des parois lisses formées par une couche de cellules cubiques. Depuis les parties latérales de l'atrium partent deux canaux longs et contorsionnés, tapissés de chitine et débouchant par deux spermathèques ovoïdes à parois lisses.
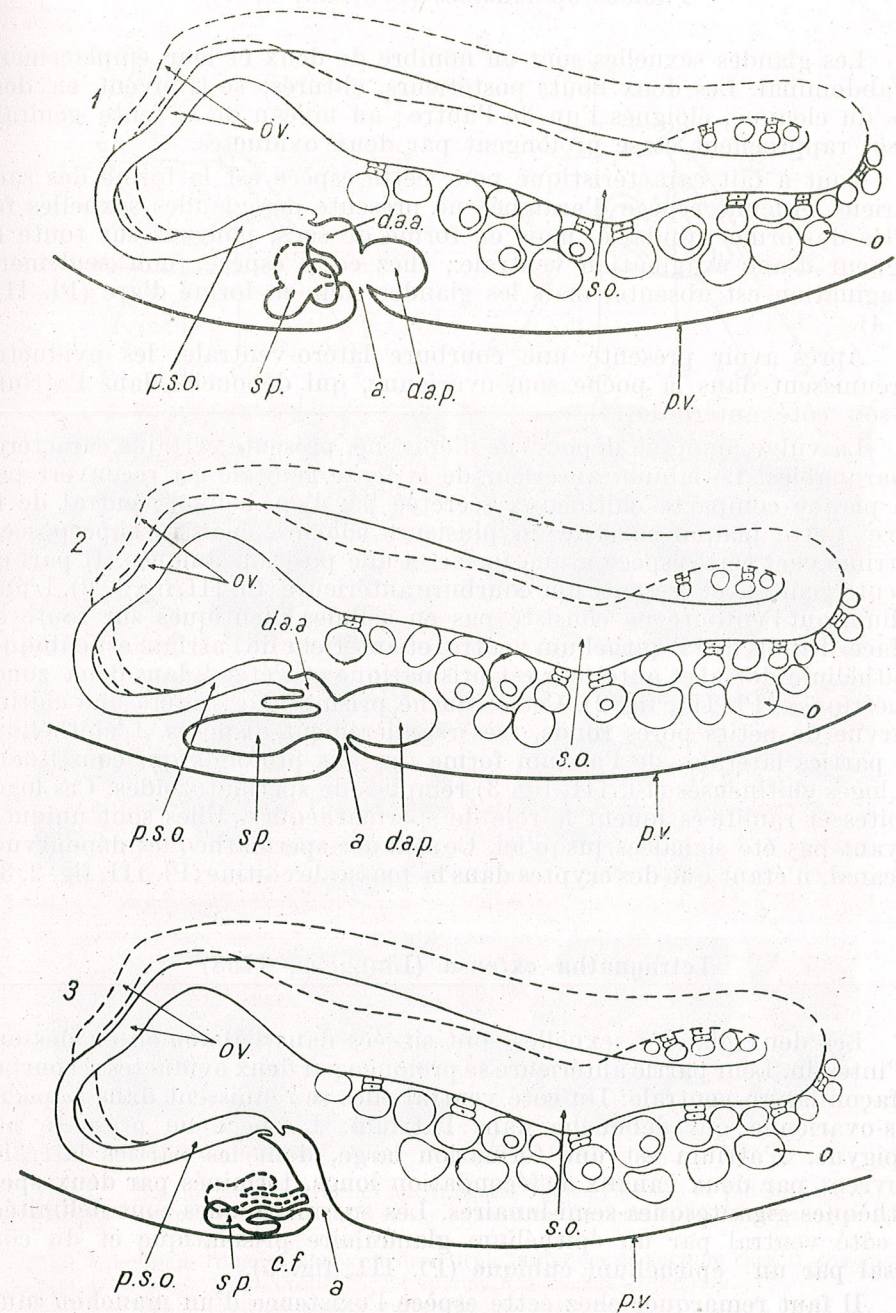
PLANCHE I — Représentation schématique de l'appareil génital femelle.
Fig. 1. — *Dysdera crocata*
Fig. 2. — *Harpactes rubicundus*
Fig. 3. — *Scythodes thoracica*

Pholcus opilionoides (Schrank, 1781)

Les glandes sexuelles sont en nombre de deux et leur emplacement est abdominal. Les deux bouts postérieurs, obturés, se trouvent en dessous du cloaque, éloignés l'un de l'autre; au niveau de la fente génitale ils se rapprochent et se prolongent par deux oviductes.

Tout à fait caractéristique pour cette espèce est la forme des sacs ovariens. Aucune espèce d'araignée ne présente des glandes sexuelles femelles de forme tubulaire, mais en forme de sacs, pourvus sur toute la longueur d'une évagination ventrale; chez cette espèce, non seulement l'évagination est absente, mais les glandes sont en forme d'arc (Pl. III, fig. 4).

Après avoir présenté une courbure latéro-ventrale, les oviductes se réunissent dans la poche sous-ovarienne, qui débouche dans l'atrium de son côté antéro-dorsal.

La vulve, quoique dépourvue d'épigyne, présente certains caractères remarquables. Le labium antérieur de la fente génitale est recouvert par une plaque compacte chitineuse, sécrétée par l'épithélium ventral de la vulve. Cette plaque consiste en plusieurs couches épaisses superposées. L'atrium chez cette espèce a une forme et une position uniques. Il part de la fente génitale et présente une courbure antérieure (Pl. III, fig. 1, 2). L'épithélium qui l'entoure ne consiste pas en cellules identiques sur toute sa surface. Tandis que l'épithélium ventral et antérieur de l'atrium est cubique, l'épithélium dorsal et antérieur est prismatique et s'étend dans deux zones symétriques (Pl. III, fig. 2). Cette couche prismatique sécrète une chitine pourvue de petits pores ronds, très régulièrement disposés. L'épithélium des parties latérales de l'atrium forme des plis profonds qui constituent des loges chitineuses (Pl. III, fig. 3) remplies de spermatozoïdes. Ces loges étroites et ramifiées jouent le rôle de spermathèques. Elles sont uniques, n'ayant pas été signalées jusqu'ici. Ce sont des spermathèques dépourvues de canal, n'étant que des cryptes dans la masse de chitine (Pl. III, fig. 2, 3).

Tetragnatha extensa (Linnaeus, 1758)

Les deux glandes sexuelles sont situées dans l'abdomen en dessous de l'intestin. Leur partie antérieure se prolonge par deux oviductes recourbés de façon latéro-ventrale. Du côté ventral elles se réunissent dans la poche sous-ovarienne qui débouche dans l'atrium. L'espèce ne présente pas d'épigyne. L'atrium est une formation large, dont les parties latérales s'ouvrent par deux canaux de fécondation longs, terminés par deux spermathèques gigantesques semi-lunaires. Les spermathèques sont délimitées du côté ventral par un épithélium glandulaire prismatique et du côté dorsal par un épithélium cubique (Pl. III, fig. 5).

Il faut remarquer chez cette espèce l'existence d'un manchon musculeux qui entoure la jointure entre la poche sous-ovarienne et l'atrium. Nous pensons que celui-ci joue le rôle de sphincter au moment de l'élimination des ovules (Pl. III, fig. 6).
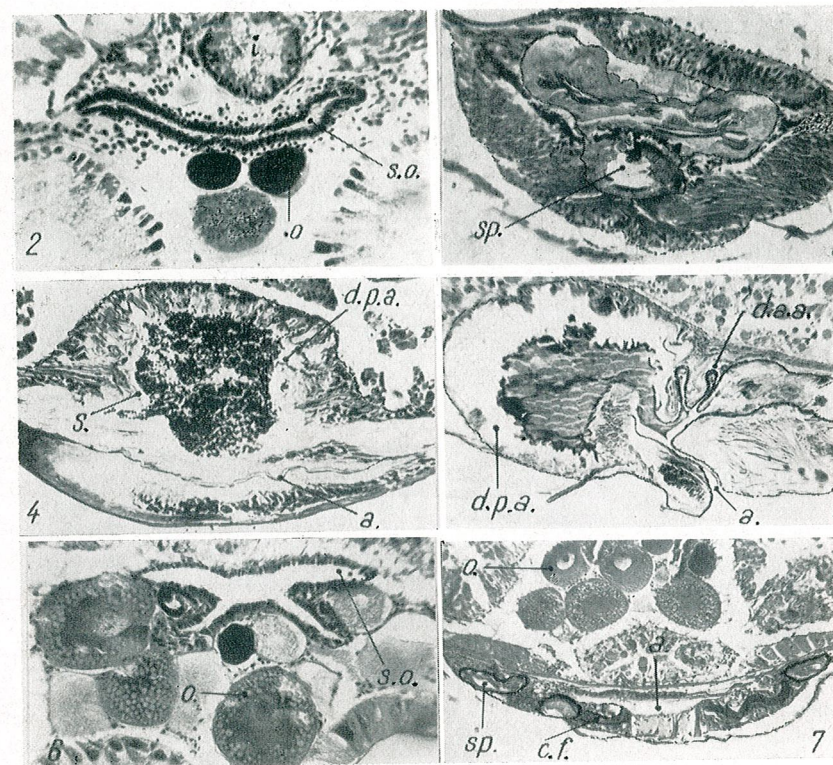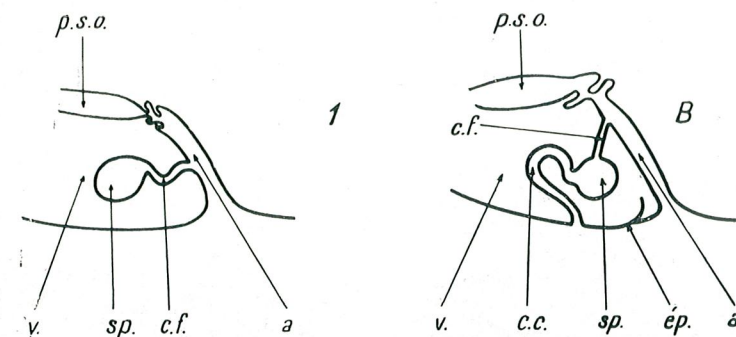

Fig. 1. — Schéma de la vulve chez les Araignées Haplogynae (A) et Entelegynae (B).
Fig. 2. — Coupe transversale par le sac ovarien au niveau de sa courbure postérieure chez *D. crocata*.
Fig. 3. — Coupe transversale par le diverticule postérieur de l'atrium chez *D. crocata*.
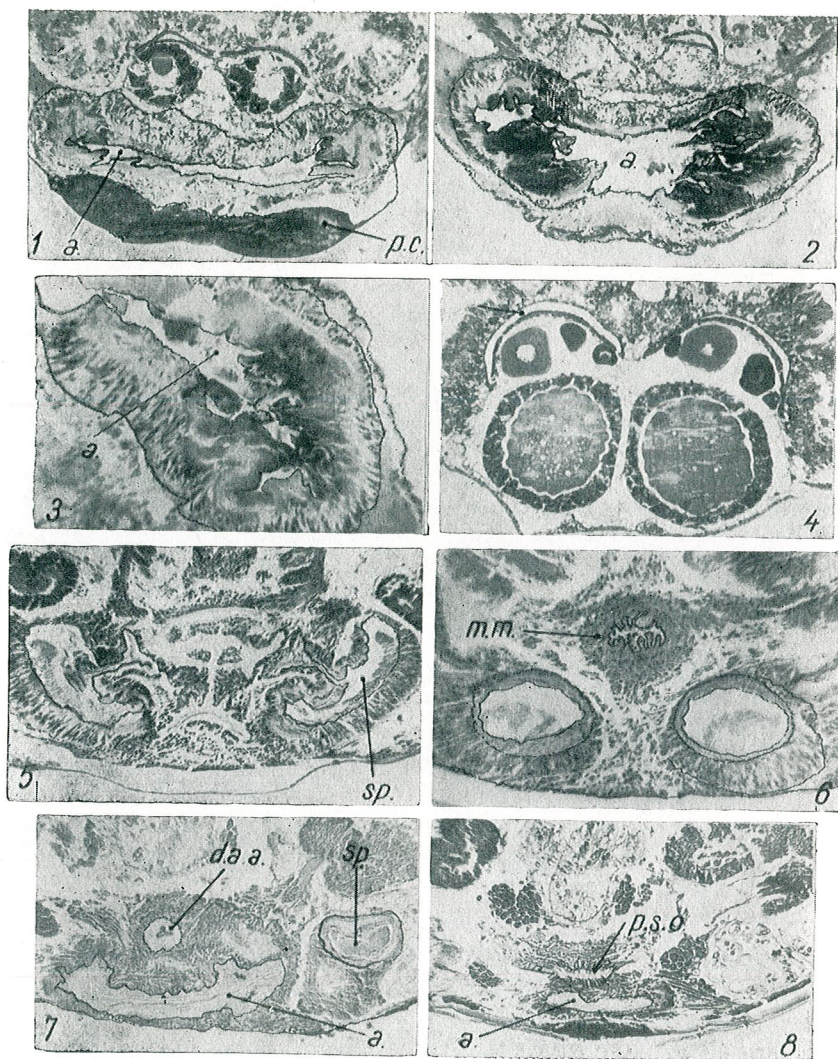Fig. 4. — Coupe transversale par la spermathèque chez *D. crocata*.
Fig. 5. — Coupe sagittale par la vulve chez *H. rubicundus*.
Fig. 6. — Coupe transversale par la courbure postérieure du sac ovarien chez *S. thoracica*.
Fig. 7. — Coupe transversale par la vulve chez *S. thoracica*.

Abréviations: a., atrium; c.c., conduit copulateur; c.f., conduit de fécondation; c.g., cavité générale; d.a.a., diverticule antérieur de l'atrium; d.p.a., diverticule postérieur de l'atrium; é.g., épithélium glandulaire; ép., épigyne; g.s. glandes séricigènes; i., intestin; m.m., manchon musculeux; o., ovocytes; ov., oviductes; p.c., plaque chitineuse; p.s.o., poche sous-ovarienne; p.v., paroiventrale de l'abdomen; s.o., sac ovarien; sp., spermathèque.

Pachynatha degeeri Sundevall, 1830

Les glandes sexuelles aussi bien que les annexes ont la même forme et la même position que chez l'espèce précédente. La vulve manque d'épigyne. L'atrium se remarque par l'existence d'un diverticule tubulaire antérieur, situé sous la partie ventrale de la poche sous-ovarienne (Pl. III, fig. 7). Ce diverticule est obturé à son côté antérieur. Depuis les parties latérales de l'atrium partent deux canaux courts qui ont aux extrémités deux spermathèques à parois ondulées. Chez cette espèce on rencontre aussi un manchon musculeux au niveau de la jointure entre l'atrium et la poche sous-ovarienne (Pl. III, fig. 8).

CONCLUSIONS

— L'appareil génital femelle a une glande sexuelle en forme de fer à cheval chez les fam. Atypidae (3), Dysderidae et Scythodidae.

— L'appareil génital femelle a deux glandes sexuelles chez les fam. Pholcidae et Tetragnathidae.

— L'épigyne est absente chez toutes les espèces étudiées.

— L'atrium chez la fam. Dysderidae présente certaines particularités par rapport aux autres familles, notamment la présence du diverticule atrial postérieur et d'une spermathèque antérieure.

— A l'exception de *Pholcus opilionoides*, toutes les espèces étudiées ont des canaux de fécondation qui partent de l'atrium et se terminent par des spermathèques.

— L'accouplement a lieu dans l'atrium chez toutes les espèces.

— Nous considérons les espèces étudiées comme des « Haplogynae », puisque, par tous ses caractères, l'appareil génital diffère de celui des araignées *Entelegynae*.

REMERCIEMENTS

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TRACIUC E., Bulletin du Muséum National d'Histoire Naturelle, 1969 (1970), 2e Série, **41**, Suppl. 1, 18—23.
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Coupes histologiques par la vulve chez les Pholcidae et les Tetragnathidae.

Fig. 1. — La plaque chitineuse antérieure à la fente génitale chez *Pholcus opilionoides*.

Fig. 2. — Atrium chitineux chez *P. opilionoides*.

Fig. 3. — Epithélium glandulaire entourant les cryptes de l'atrium chez *P. opilionoides*.

Fig. 4. — Sacs ovariens en forme d'arc chez *P. opilionoides*.

Fig. 5. — Coupe transversale par l'atrium et les spermathèques chez *Tetragnatha extensa*.

Fig. 6. — Manchon musculeux jouant le rôle d'un sphincter de l'atrium chez *T. extensa*.

Fig. 7. — Coupe transversale par la vulve chez *Pachynatha degeeri*.

Fig. 8. — Coupe transversale par l'atrium et la poche sous-ovarienne chez *P. degeeri*.

A SPECIFIC FORMATION OCCURRING IN THE PERIPHERAL CYTOPLASM OF YOUNG STURGEON OOCYTES

BY

MARIA CALOIANU-IORDĂCHEL

The study of sturgeon oocytes during the period of previtellogenic development evinces important changes of cytoplasmic organelles. Special emphasis is laid upon the progressive modifications of Golgi complexes disposed in the cortical and subcortical zones of oocyte cytoplasm.

A general phenomenon met during oocyte development in most animal groups is the cytoplasmic organelles enrichment and the migration of these organelles towards the peripheral zone of the cytoplasm. This process was also described in the sturgeon oocytes [1]. A minute study bearing upon the stages of the previtellogenic development of sturgeon oocytes led to the demonstration of a specially-looking formation which represents the purpose of this paper.

MATERIAL AND METHOD

The *Acipenser güldestädti* Brandt females were caught in the Romanian Black Sea coast. For electron microscopy, ovary fragments were initially fixed with 2% glutaraldehyde in caccodylate buffer. From the ovarian stroma the oocytes were detached and postfixed with osmium tetroxid 2% in caccodylate buffer. Following rapid dehydration the oocytes were infiltrated and embedded in Araldyte or Epon. The coloured fine sections (Reynolds, 1963) were examined in a JEM-7 microscope. For light microscopy, the ovaries of young females were fixed in the Bouin, Carnoy, 10%

neutral formol mixtures. Parallelly with the general Azan, picro-indigo-carmine stainings, some specific reactions at PAS, toluidine blue (at different pH) and alcian blue were also used.

RESULTS

During the development, the young sturgeon oocytes show a series of characteristic qualitative changes in the nucleus and cytoplasm, as well as in the oocyte follicular cells ratio [1].

Concomitantly with follicle formation and microvilli growth in the space between the oocytes surface and that of the follicular cells, the mitochondria and the Golgi complex are abundant in the peripheral zone of the young oocytes cytoplasm. The small, round or elongated mitochondria (Fig. 1) are distributed in compact groups near the oolemma. Often they come in close contact with the circular or oval vesicles (Fig. 2). The Golgi complexes, abundant in the peripheral zone of the young oocytes cytoplasm, stand out by their various aspect and their distribution near the cortical cytoplasm. In a more advanced oocyte development stage, characterized by the formation of the unpaired zona pellucida and the appearance of lipochondria, important changes are noticed in the peripheral zone of the cytoplasm (Fig. 2). Within the Golgi saccules, a fine granular material accumulates (Figs 2 and 3) and increases in quantity. The few tubular elements left, as well as the Golgi vesicles, are disposed in a single row delimiting the granular material zone (Figs 3 and 4). The granular content of this special formation gives an intensely positive PAS reaction pointing to the presence of polysaccharides, namely of a principal mucopolysaccharidic component. While the dictyosomes occurring in the immediate proximity of the plasma membrane practically lose their typical structure under the changes described, in the subcortical zone of the cytoplasm numerous Golgi complexes are observed, which maintain their typical structure and actively participate to the elaboration of specific substances and formations (Fig. 4).

DISCUSSION

The increase in volume of the female sexual cells during the development is accompanied by important changes of the cytoplasmic organelles.

In the young oocytes, mitochondria occur under two types : some are filamentous and the others small, round, with a few transverse cristae.

Wartenberg and Stegner [10] suggest that the presence of the two interconnected types represents the formation of the mitochondria from precursors as a response to the growth of the oocyte. The mitochondria of the sturgeon young oocyte often appear closely related to membranous vesicles.

The changes occurring in the Golgi complex are more difficultly interpreted. Dictyosomes distribution in the peripheral cytoplasm of the oocyte under the oolemma is a generally known phenomenon in the
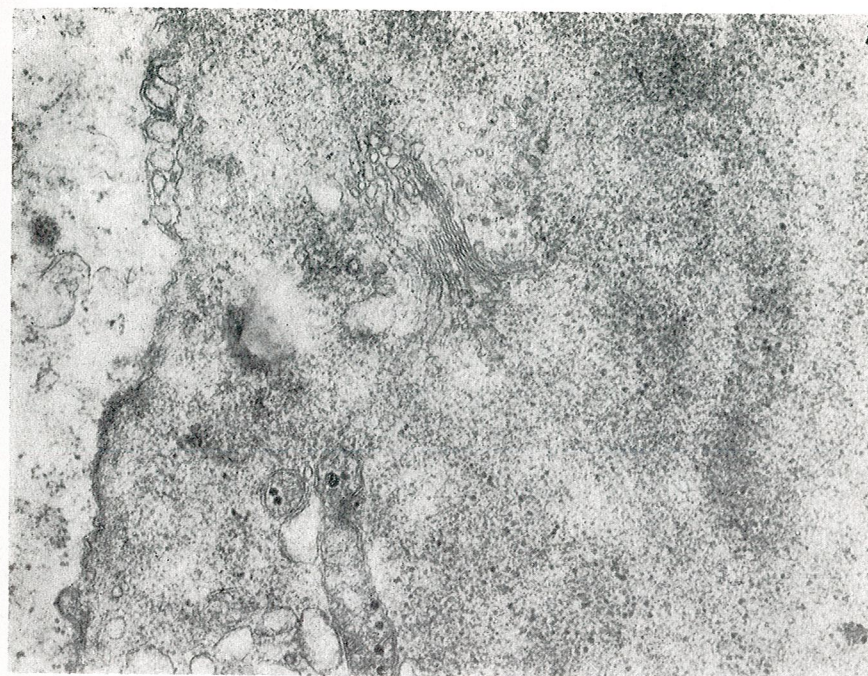
Fig. 1. — Electron micrograph of the peripheral region of a young oocyte. The two types of mitochondria and the Golgi complex are disposed in the cortical oocyte cytoplasm in the proximity of the plasma membrane. The surface of the oocyte is in close junction with the plasma membrane of the follicle cells × 28,000.
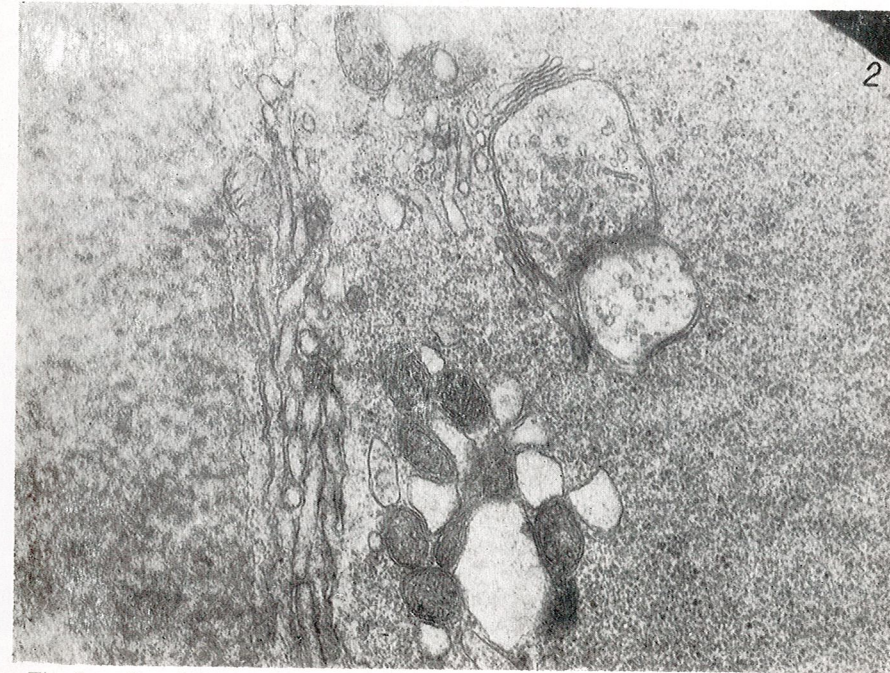
Fig. 2. — In a later stage of the oocyte development mitochondria are grouped in clusters and associated with the vesicles. In the interior of Golgi saccules appears a specific material. × 28,000.

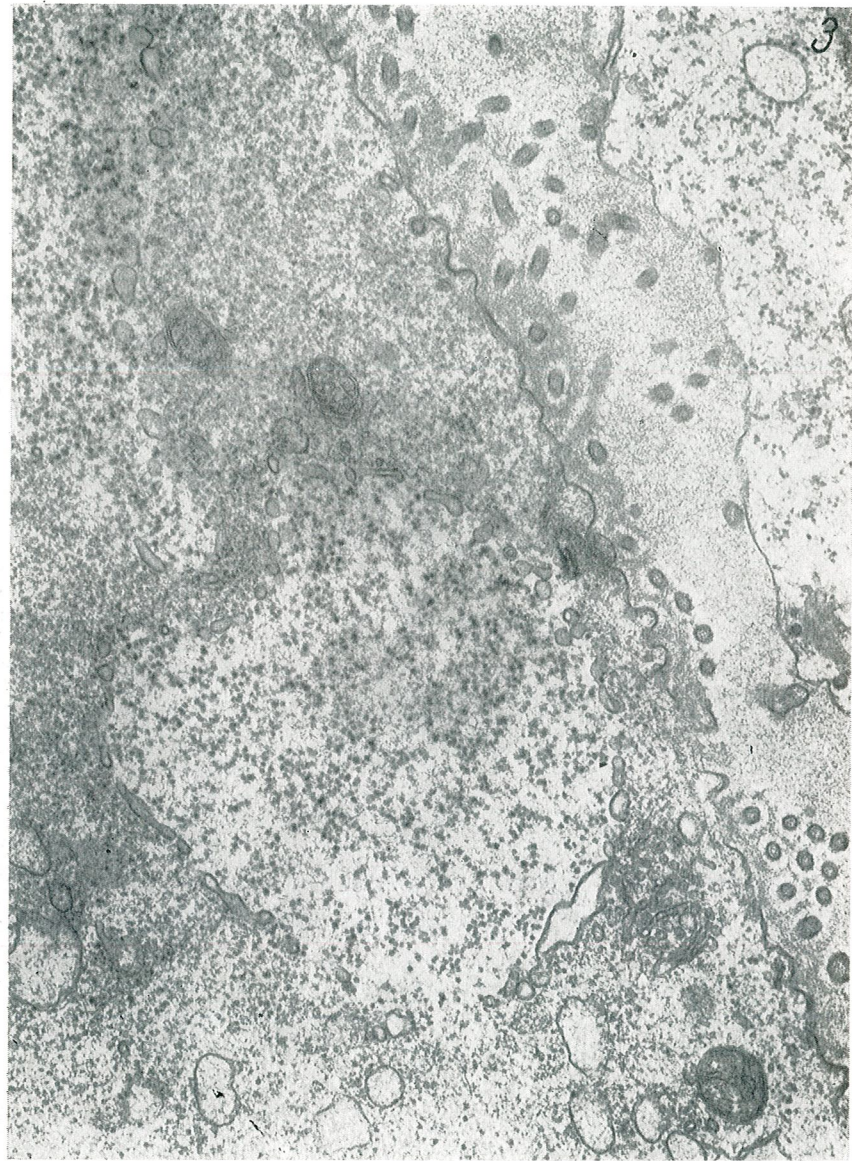Fig. 3. — Portion of an oocyte in the formation stage of the
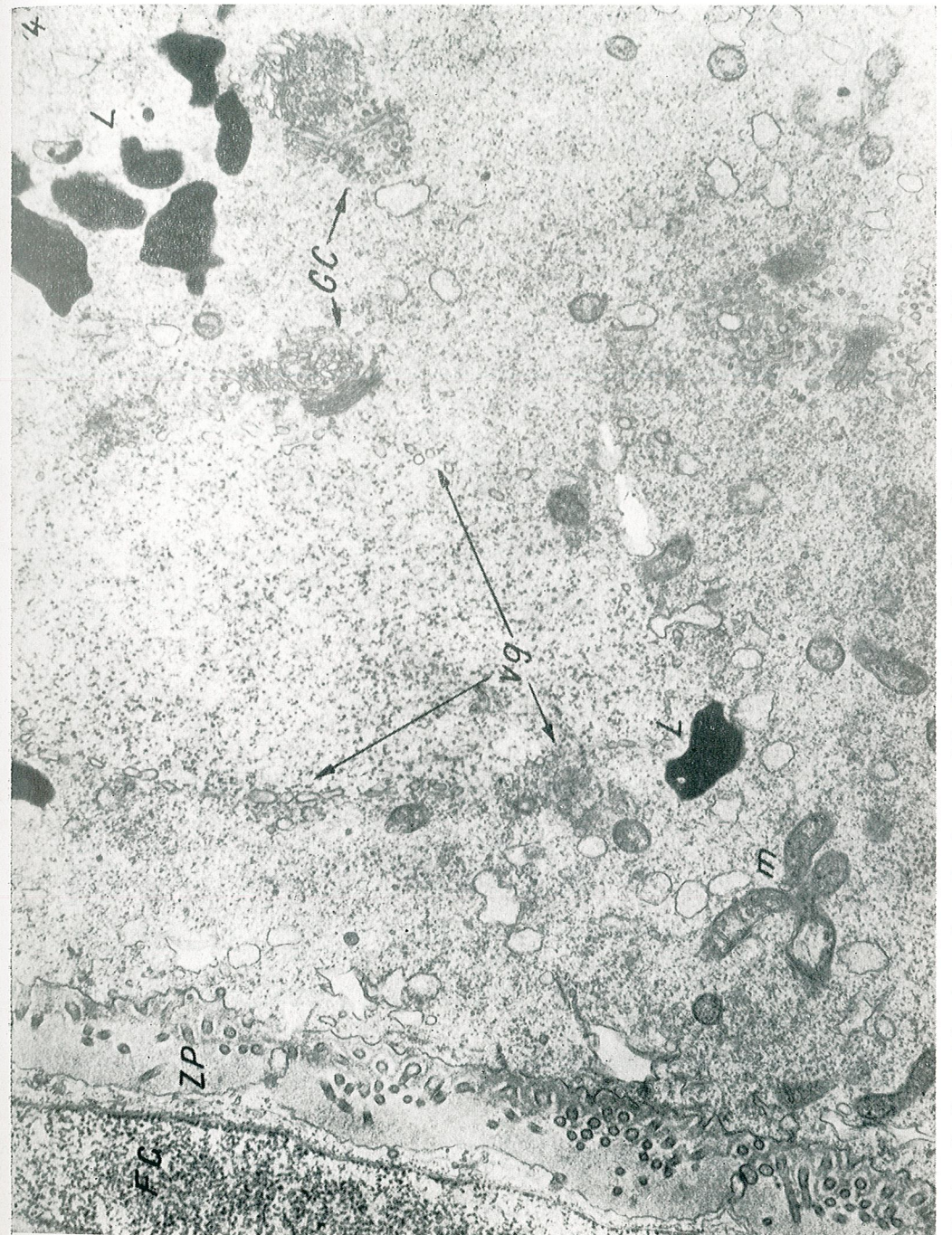zona pellucida. The fine granular material grows. ×27,000.

Fig. 4. — In the peripheral cytoplasm the changes of the Golgi complex disposed in the proximity of the oolemma are illustrated. The
granular material is surrounded by small Golgi vesicles (Vg arrows). The unpaired zona pellucida (ZP) is differentiated. The subcor-
tical zone of the oocyte marks an intense accumulation of Golgi complexes (GC) which shows an intense secretory activity. ×30,000.

animal groups. Odor [6] and Hope [3] suggest that the Golgi complex has certain functions connected to the zona pellucida formation. Of a special importance is the presence in the interior of some Golgi saccules of a granular material which progressively grows and remains surrounded only by small vesicles and vacuoles. It seems that the formation of these zones is the result of dictyosomes secretion and transformation. The granular material delimited demonstrates — following the results of the reactions applied — a rich carbohydrate content. By using ^{3}H-glucose and ^{3}H-galactose, Neutra and Leblond showed [5] that in various cell types the Golgi complex is actively involved in both carbohydrate and protein synthesis [2] [3]. In our case, the dictyosomes seem to participate in a first stage to the carbohydrate elaboration. These formations appear as a fine material agglomerating within the Golgi vacuoles and participating to the pellucida substance elaboration, or as granular masses delimited by small vesicles and distributed in the cortical zone. Concomitantly the Golgi complexes of the subcortical zone go on multiplying and accumulate within the parallel saccules an electronic opaque material, showing an intense secretory activity.

Transformations of the Golgi complexes were observed by Robert and Ward [8] [9] in *Rana pipiens* oocytes appearing however at the end of the vitellogenesis period. The changes described are related to the final processes of deposition of the reserve nutritive substances in the mature oocyte cytoplasm. Consequently, they cannot be identified with the formation met by us in the peripheral cytoplasm of the sturgeon oocytes during previtellogenesis.

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CHANGES IN THE RATIO OF INTERRENAL AND CHROMAFFIN TISSUE VOLUMES IN THE ADRENAL GLAND DURING THE DIFFERENT STAGES OF THE SEXUAL CYCLE IN *PHALACROCORAX CARBO* L.

BY

ANCA PETRESCU-RAIANU

Volume measurements of the interrenal and chromaffin tissues were carried out on adrenal glands removed from male and female individuals of *Phalacrocorax carbo* L. In both sexes, the adrenal gland shows a quantitative preponderance of interrenal tissue in each of the three periods examined (mating, egg laying-hatching and sexual repose). Its highest values were found in the mating period, while its lowest ones during the sexual repose. The increase in the interrenal tissue volume during the mating period is, at least partly, a result of an increase in size of its component cells.

The relationships between adrenal glands and the reproductive activity were approached until now in many reports. As far as the changes of the adrenal glands during the annual cycle in birds are concerned, the results are partly in disagreement with one another. Hartman and Brownell [6] did not find any significant difference between the adrenal weight values for the glands examined during the egg laying period and during the sexual repose. However, Fromme-Bouman [3], Bhattacharyya and Ghosh [1] and Hall [5] revealed significant changes in the fractional cortical volume as well as histological and histochemical alterations. An examination of their data reveals a coincidence between the maximal activity of the adrenals and that of the testes. On the other hand, Lorenzen and Farner [11] found in *Zonotrichia leucophrys gambelii* the maximal activity of the testes to be coincident with the lowest values of the fractional cortical volume, i.e. with the minimal activity of the interrenal tissue.

As most of these works are dealing with males only, and their results are greatly contradictory, the present paper reports the volume changes of the two tissues (interrenal and chromaffin) in the adrenal gland during the different stages of the sexual cycle in *Phalacrocorax carbo* L., in males as well as in females.

MATERIAL AND METHODS

Volume measurements of the interrenal and chromaffin tissues were carried out on adrenal glands removed from male and female individuals of *Phalacrocorax carbo* L., captured on the Black Sea shore at the Sulina channel mouth. The captures were scheduled three times a year, corresponding to the mating period, the egg laying-hatching period and the sexual repose period respectively, as shown in table 1.

Table 1

Number of individuals killed in different stages of sexual cycle

Stage	Date	Number of males	Number of females
Mating	15—25 March	7	9
Egg laying-hatching	15—30 April	15	18
Sexual repose	20—30 September	11	10

The glands were fixed in the Bouin-Hollande-mixture and included in paraffin. Sections of 5µ thick were stained with hemalum and eosine. The volume measurements of the interrenal tissue, chromaffin tissue and of the capillaries were performed in two ways :

1. by means of an ocular eyepiece with a network, the results being expressed in per cent values.

2. by means of microphotographs, using a planimeter, the volumes being expressed in per cent values, too.

Every figure from table 2 is a mean of 120 measurements.

RESULTS

Our data concerning the volumes of the interrenal and chromaffin tissues and of the vascular system of the adrenal gland in *Phalacrocorax carbo* are listed in table 2 and represented in figure 1.

An examination of these data shows that in both sexes, the interrenal tissue occupies the major part of the gland in each of the three periods examined (mating, egg laying-hatching, sexual repose). Its highest values were found in the mating period (March), while its lowest ones during

Table 2

Volumes of the different components of the adrenal gland in *Phalacrocorax carbo*, as a function of the stages of the sexual cycle

Stage		Male		Female	
		Eyepiece network measurements p.c.	Planimeter measurements p.c.	Eyepiece network measurements p.c.	Planimeter measurements p.c.
Mating	Interrenal tissue	85.68±0.9	84.65+0.7	83.82±0.7	82.55±1.2
	Chromaffin tissue	9.35±0.7	9.73±0.5	9.47±0.5	11.15±1.6
	Blood vessels	4.97±0.6	5.62±0.5	6.71±0.6	6.30±0.4
Egg laying-hatching	Interrenal tissue	72.60±0.9	72.35±0.7	75.40±1.6	75.15±1.2
	Chromaffin tissue	19.40±1.1	19.20±0.5	16.78±1.0	17.12±0.5
	Blood vessels	8.00±0.7	8.45±0.5	7.82±0.8	7.73±0.7
Sexual repose	Interrenal tissue	65.35±0.8	64.56±1.0	69.90±0.9	67.66±0.9
	Chromaffin tissue	25.81±0.9	25.70±1.1	21.67±0.7	23.59±0.7
	Blood vessels	8.84±0.7	9.74±0.5	8.43±0.5	8.75±0.4

the sexual repose (September-October). After the mating period, a marked decrease in the interrenal tissue volume is to be noted, mainly when the short time in which it takes place is considered (30—45 days). The decrease in the interrenal tissue volume lasts for the whole summer, so that in autumn, the values recorded are lower than those recorded during the egg laying-hatching period. The decrease in interrenal tissue volume after the egg laying-hatching period is less marked as compared to the decrease occurring between the mating and the egg laying-hatching period, and extends over a longer period (approximately 5 months).

The volume alterations of the interrenal tissue in *Phalacrocorax carbo* are parallel in both sexes. The values obtained for the mating period are very close, while in the egg laying-hatching and the sexual repose periods they seem to be different, the males displaying somewhat lower values than the females.

An examination of the histological appearance of the interrenal cords in the three stages of the sexual cycle shows that during the mating period these cords undergo a hypertrophy, being larger in diameter than during the repose, and with higher interrenal cells. Therefore, the conclusion might be drawn that the increase in the interrenal tissue volume during the mating period is, at least partly, a result of the increase in size of its component cells (Plate I A,B).
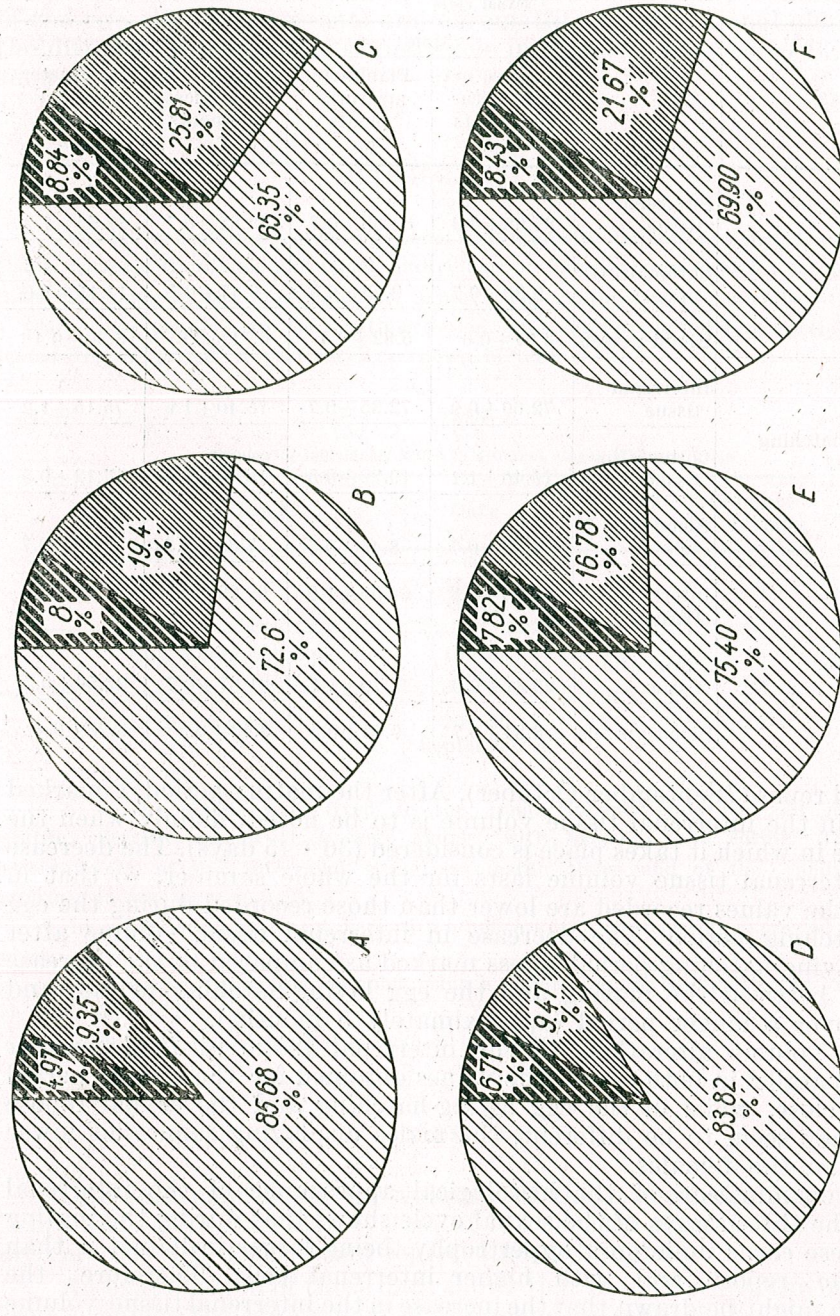
Fig. 1. — Diagram showing the volume changes of the interrenal and chromaffin tissue and of the vascular system as a function of the stages of the sexual cycle. A,B,C, male; D,E,F, female; A,D, mating; B,E, egg laying-hatching; C,F, sexual repose.
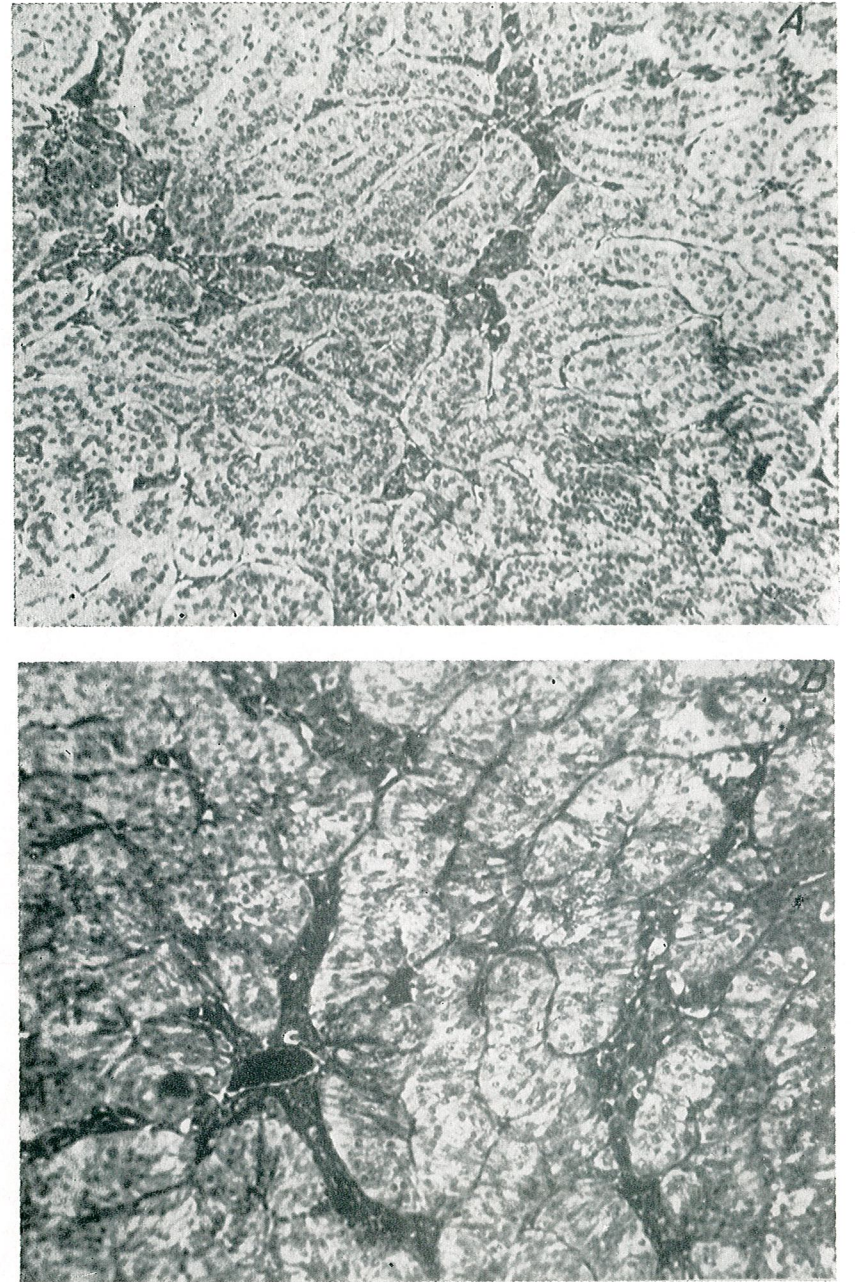
Volume of the interrenal cords   *Volume of the chromaffin tissue*   *Volume of the blood vessels*

Plate I. — A. Interrenal cords appearance during the sexual repose. B. Interrenal cords appearance during the mating period.

As to the volume changes of the vascular system, the higher values recorded by us in *Phalacrocorax carbo* belong to the repose period, while the lowest ones to the mating period. The per cent values of the blood vessels volumes are increasing from the mating period to the repose one in both sexes.

DISCUSSION

Several reports were dealing with the relative proportions of the interrenal (cortical) and chromaffin (medullary) tissues in the adrenal gland. The studies were performed in species of all the classes of vertebrates in which these two glands occur in association. The results are, however, different according to the species. In most species, an obvious preponderance of the interrenal tissue upon the chromaffin one was observed. Thus, according to Lakshman [10] in 4 Anura the interrenal component is preponderant, the chromaffin tissue being preponderant in volume only in *Rana hexadactyla*. Also, Gabe and Martoja [4] showed that in the adrenal glands of Squamata, the interrenal tissue occurs in greater amount than the chromaffin one.

In birds, the studies were concerned with several species : *Anas plathyrhyncos* [7], *Passer domesticus* [1], [8], *Zonotrichia leucophrys gambelii* [11], *Turdus merula* [3], *Platycercus eximius* [5], *Pelecanus occidentalis* [9], *Columba livia* [12] and *Gallus domesticus* [13]. In the adrenal glands of all the species listed above, but *Gallus domesticus*, the interrenal tissue occupies a greater volume than the chromaffin one. Our data ascribe *Phalacrocorax carbo* to the species displaying a preponderance of the interrenal component. Even in the periods in which the interrenal cords volume shows the lowest values, the preponderance of the interrenal tissue upon the chromaffin one is still maintained.

Another aspect to be discussed is the fact that the relative proportions of the interrenal and chromaffin tissues are affected by certain factors. In most species so far examined (in birds, as well as in mammals), seasonal alterations of this ratio, depending upon the sexual cycle, were described. In these species, variations in the interrenal tissue volume were found between minimal values of 60—70 p.c. and maximal ones of 74—90 p.c. from the gland. These changes in the interrenal tissue volume may be or not associated with changes in the gland weight, depending upon the species studied. In *Turdus merula* [3], *Passer domesticus* [1] and *Platycercus eximius* [5] the period in which the interrenal tissue shows the maximal volume is coincident with that of the testes. However, Lorenzen and Farner [11] found in *Zonotrichia leucophrys gambelii* just the opposite situation, i.e. the lowest values of the interrenal tissue volume during the period of maximal activity of the testes. Our data, obtained for *Phalacrocorax carbo*, revealing that the highest values of the interrenal cords volume occur during the mating period, are in agreement with the results of Hall [5], Fromme-Bouman [3] and Bhattacharyya and Ghosh [1]. To account for the reverse situation found in *Zonotrichia leucophrys gambelii*, some authors suggested the fact that certain factors related to the migra-

tion (*Zonotrichia* being a migratory species) might affect in a different way the changes of the interrenal tissue volume. However, the species examined by us, *Phalacrocorax carbo*, is migratory too, but all the same the volume changes of the interrenal tissue do not agree with those found in *Zonotrichia*; thus the factors related to migration do not seem to be involved in the inversion of the rhythm of the interrenal tissue activity.

A comparison between the volume of the interrenal tissue in the adrenal glands of the species so far studied shows that the minimal values range, for all the species, between 60 and 67 p.c. from the total volume of the gland. However, our data indicate in *Phalacrocorax carbo* a minimal volume of the interrenal tissue of 67—70 p.c. from the total volume of the gland. This discrepancy might be due either to the different methods used by various authors, in measuring the volumes, or to the existence of differences between species, differences noted by Knouff and Hartman too [9]. These authors showed that the relative proportion of interrenal and chromaffin tissues is dependent upon the species, pointing out that in *Pelecanus occidentalis* the interrenal tissue occupies a much greater volume than in other species.

Taking into account the phylogenetic closeness between the genera *Pelecanus* and *Phalacrocorax*, the higher value obtained by us for the minimal volume of the interrenal tissue might be explained on this basis too.

As to changes in the vascular system of the gland, our results seem, at first sight, to be in disagreement with what is known about the vascularization of the organs during the intense activity periods. Indeed, the values of the blood vessels should have been greater during sexual repose. However, it could be supposed that the vascular system increases during the mating period too, though in a lower extent than the interrenal tissue, which results in the apparent lower values of the blood vessels volume during the mating period in spite of their actual increase.

CONCLUSIONS

1. The adrenal gland in *Phalacrocorax carbo* shows a quantitative preponderance of the interrenal tissue over the chromaffin one, in females as well as in males.

2. The relative proportion of the interrenal and chromaffin tissues undergoes sexual cycle-induced alterations, pointing to the occurrence of some relationships between the adrenal gland and the reproductive activity. In both sexes the maximal volume of the interrenal tissue occurs during the mating period. The lowest values of the interrenal cords volume were recorded during the sexual repose.

3. The ratio between the amounts of interrenal and chromaffin tissues could be considered as a morphological index for the activity of adrenal glands.

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INSULIN ANOREXIA IN CHICKENS

BY

CONSTANŢA MATEI-VLĂDESCU

The mechanisms of insulin anorexia induction in chickens were studied. It was established that the minimal insulin dose capable of inducing anorexia in chickens is of 3 I.U./kg b.w. The effect of pretreatment with nicotinic acid, a known inhibiting agent of FFA mobilization, and with reserpine, which depletes adrenal catecholamines, was investigated on the anorexigenic action of this dose of insulin. The prior treatment with nicotinic acid administered separately or in combination with reserpine shortened, sometimes to disappearance, the anorexia period. This demonstrates that the mobilization of fat from the adipose tissues, as well as the release of an increased quantity of anorexigenic catecholamines play an important role in setting off insulin anorexia in chickens.

In attempting to induce hyperphagia and weight increase in chickens by injecting insulin, as it happens in rat, Lepkovsky and coll. [5] obtained a decrease of food intake and of body weight.

It was suggested that one of the mechanisms of insulin anorexia in chickens might be the mobilization of fat from adipose tissues [6], as insulin raises their plasma FFA levels [3] [6] in contrast to those of rat, dog and human.

Proceeding from this hypothesis we investigated the anorexigenic effect of insulin in chickens after the inhibition of free fatty acids mobilization by means of nicotinic acid.

In our researches we likewise investigated the possible interference with the anorexigenic action of adrenaline [7] [8], considering that insulin determines in chickens an increased release of hyperglycemiant glycogenolytic catecholamines, responsible to a great extent for their considerable resistance to insulin [9]. To this purpose we studied the effect of reserpinization, respectively of adrenal catecholamine depletion, on insulin-induced anorexia in chickens.
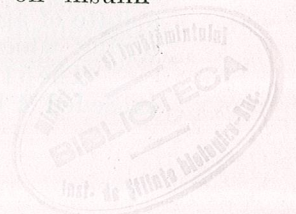
MATERIAL AND METHOD

Experiments were performed on 4 cocks and 28 hens of the Leghorn race weighing between 1.200 and 1.800 kg.

Chickens were kept in separate cages to enable the measuring of the food intake. Before testing they were submitted to a 20—24 hours fast. The food was a mixture of fodder, according to the formula given in a previous work [7], or the concentrated fodder used in poultry farms.

The minimal insulin dose capable of inducing anorexia was first established. We therefore studied the effect of some insulin doses ranging between 0.1 and 10 I.U./kg b.w. namely : 0.1, 0.5, 1, 2, 3, 5, 7 and 10 I.U./kg b.w.

Biofarm insulin with 40 I.U./ml was employed. Insulin was injected intraperitoneally, after the corresponding dilution with distilled water.

Food intake was investigated before injection and 30 minutes, 1,2,3, and 5 hours after it, in the following manner : chickens received food and if they started to eat, consuming in a few minutes 5—10 g fodder, the access to food was stopped and it was considered that their appetite was unaltered ; if they did not eat, even if the food was left for several hours in the cage, it was considered that the state of anorexia had set in.

In the case of the 0.1 and 1 I.U. insulin/kg b.w. dose, food intake after daily repeated injections was likewise measured for 5 days. Insulin was injected 30 minutes before food administration, while food intake was measured for a two hours' interval. The same procedure was applied to chickens intraperitoneally injected with a 9⁰/₀₀ physiological saline solution.

The (Merck) nicotinic acid used for lipolysis inhibition was administered per os, under the form of aqueous solution (1 g/60 ml), 30—50 ml that is 0.5—0.8 g/hen, daily for 4 days before testing the effect of insulin, and 60 minutes prior to insulin injecting, on testing day.

Adrenal catecholamine depletion was performed by means of reserpine (hyposerpil) administered per os under the form of tablets, which contained each 0.25 mg of Rauwolfia serpentina pure crystallized alkaloid. Each hen received 5 tablets daily, for 4 days before testing, and 5 tablets one hour before insulin injection. The total dose was of 6.25 mg reserpine/hen.

Both the effect of separate nicotinic acid or reserpine administration, and that of their combined injection was studied, according to the above shown procedure and doses.

RESULTS

In the graph of figure 1 the variation of the insulin anorexigenic effect according to dose is presented. Doses of 0.1, 0.5 and 1 I.U./kg b.w. did not induce anorexia. Administered daily, for 5 days, the doses of 0.1 and 1 I.U./kg b.w. led to the gradual decrease of food intake, fully evidenced after the 5th injection (Fig. 2).

At about 3 hours from the administration of the 2 I.U./kg b.w. dose, in two of the four treated hens a state of anorexia appeared which lasted $1\frac{1}{2}$—2 hours. With a single dose of 3 I.U./kg b.w. we succeeded to induce anorexia in all the 8 hens under experimenting. Anorexia occurred 30 minutes to 1 hour after insulin administration, when the hens presented a state of agitation and marked polypnea, and could last for 2—4 hours, during which the hens became passive and sleepy.

The 5 I.U./kg b.w. dose had a clear anorexigenic effect which sometimes exceeded 4 hours. Doses higher than 5 I.U./kg b.w. induced lasting ano-

rexia, on the background of which signs of insulin shock began to appear, with loss of movement co-ordination and of the possibility to maintain body position, lethargic condition and sometimes even slight convulsions.
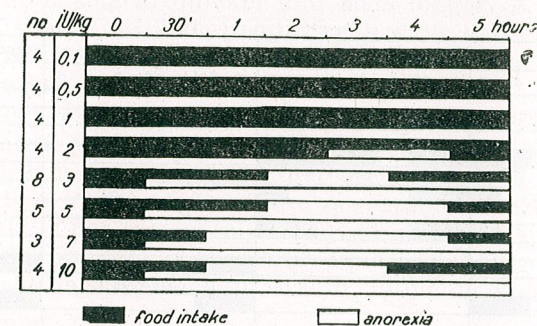
Fig. 1. — Dose dependent variation of insulin anorexia in chickens.
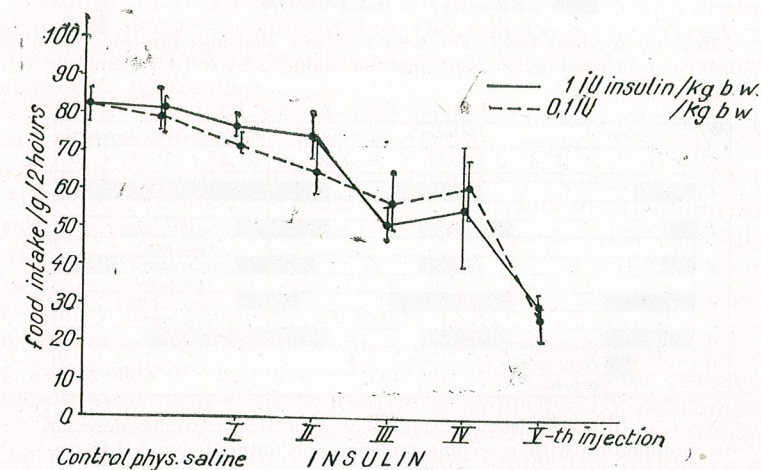
Fig. 2. — Decrease of food intake in repeated daily administration of small insulin doses (0.1 or 1 I.U./kg b.w.) in hens.

For further experimentation we chose the 3 I.U./kg b.w. dose. We performed however also some tests with the 5 I.U./kg b.w. dose.

The response of the hens treated with nicotinic acid and reserpine, administered separately or in combination, to the 3 I.U./kg b.w. dose is represented in the graph of figure 3.

The nicotinic acid pretreatment shortened evidently the duration of anorexia. As regards reserpinized hens, they began by refusing food after the first hour from insulin injection, and to show signs of insulin shock, lethargic condition and sometimes convulsions, necessitating glucose administration.

In two of the four hens submitted to the combined pretreatment with nicotinic acid and reserpine, a clear blocking of the insulin anorexigenic effect was recorded. As seen from the graph, hens 6 and 7 consumed food in all the investigated time intervals and did not undergo any insulin
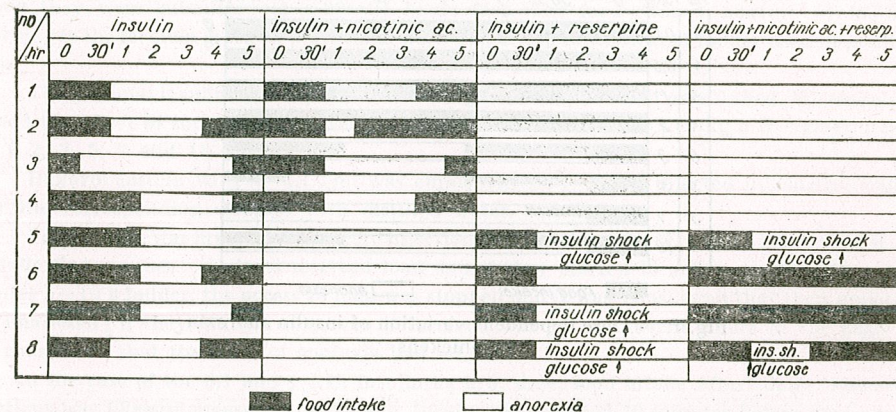
Fig. 3. — Effect of previous treatment with nicotinic acid and reserpine, administered separately or in combination, on anorexia induced by 3 I.U. insulin/kg b.w.
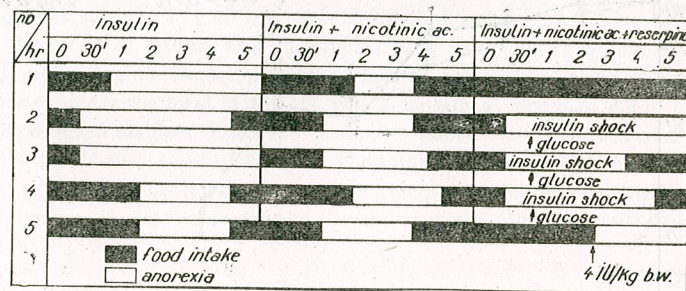
Fig. 4. — Effect of previous treatment with nicotinic acid alone or in combination with reserpine on anorexia induced by 5 I.U. insulin/kg b.w.

shock. The other two hens, 5 and 8, showed strong anorexia accompanied by insulin shock, which required glucose administration.

Similar results were obtained in the case of the anorexigenic effect of the 5 I.U./kg b.w. dose in hens treated with nicotinic acid or with nicotinic acid and reserpine (Fig. 4).

DISCUSSIONS

The insulin anorexigenic effect in chickens is dependent on the dose of insulin. Small insulin doses (0.1—1 I.U./kg b.w.) do not induce anorexia. Nevertheless, their prolonged administration (5 days) diminishes food

intake. This diminution may be correlated with a certain blocking of digestion under the influence of insulin, a phenomenon already recorded by us in a previous paper [1].

As the insulin dose increases, its anorexigenic effect becomes all the more evident, it sets in quicker and lasts longer. A single intraperitoneal injection with 2—3 I.U. insulin/kg b.w. is sufficient for the hen, though after a 22—24 hours' starvation, to refuse food even this is left for hours in the cage.

The tendency to a more rapid restoring of appetite in hens submitted to the treatment with nicotinic acid, a known inhibiting agent of FFA mobilization [2], is a proof that the increased release of fat from adipose tissues induces to a certain extent the occurrence of this anorexia. The fact that anorexia is not completely removed may be accounted for either by an insufficient inhibition of insulin lipid-mobilizing effect or by the co-operation of different mechanisms in inducing insulin anorexia in chickens.

The correlation of this anorexia with the rise of energy metabolism, recorded after insulin injection, seems very probable, a certain parallelism existing between the two phenomena [1].

The inhibition of FFA mobilization on the background of the adrenal catecholamines depletion permitting the blocking, in certain cases, of the anorexigenic effect of insulin suggests an interference of the latter with adrenalin anorexia.

The mere depletion of adrenal catecholamines obtained by reserpinization is not efficient in blocking insulin anorexia, on the one hand because insulin continues to induce an increase of plasma FFA level [3], and on the other, because the organism of hens, lacking an important means of resistance to insulin, namely the increased release of the hyperglycemiant glycogenolytic adrenal medullary hormone [9], is submitted to insulin shock, with corresponding alterations of the activity of the central nervous system, which keeps up anorexia [5].

In case of a sufficient inhibition of FFA mobilization and by removing the possibility of an increased release of anorexigenic catecholamines, the hen maintains and even increases its appetite. By consuming food it enhances its resistance to insulin, while insulin shock no longer occurs.

The importance of lipid mobilization under the influence of insulin in setting off insulin anorexia is also shown by the fact that other fowls, i.e. geese, in which insulin does not induce a rise in plasma FFA [6], increase their food intake and grow in weight after the administration of this hormone [4].

The direct measurement of plasma FFA and adrenaline concentration in all the four test conditions devised by us, which we intend to perform, will show whether we gave a correct interpretation to the behavioural modifications observed by us in these conditions.

CONCLUSIONS

1. The minimal insulin dose capable of inducing anorexia in chickens is of 3 I.U./kg b.w. Smaller insulin doses (0.1—1 I.U./kg b.w.) induce a decrease of food intake if repeatedly administered.

2. Pretreatment with nicotinic acid, an inhibiting agent of FFA mobilization, administered alone or in combination with reserpine, which depletes adrenal catecholamines, decreases, sometimes to disappearance, the period of insulin anorexia. This proves that the lipid mobilization as well as the release of an increased quantity of anorexigenic catecholamines play a significant role in setting off insulin anorexia in chickens.

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L'ACTION PHOTODYNAMIQUE DE LA PROFLAVINE SUR L'ACIDE DÉSOXYRIBONUCLÉIQUE DE DIFFÉRENTS POIDS MOLÉCULAIRES *

PAR

FLORENTINA MOSORA

The photodynamic action of proflavine upon deoxyribonucleic acid remains unchanged when the molecular weight of DNA decreases. This shows that the coupling site of DNA with proflavine is not influenced by the molecular weight of the acid.

Le mécanisme de l'effet photomutagène, de découverte récente [10], a été exploré après cette date par des recherches systématiques entreprises par Duchesne et collab. [2], [4], [8] qui ont mis en évidence, pour la première fois, l'apparition d'un transfert d'énergie lors de l'irradiation, dans la région visible, de toute une série d'acridines en présence d'ADN ou de certains de ses constituants. Les expériences ont été réalisées en solution dans l'eau et à basse température. Dans ces conditions, on a observé, par résonance paramagnétique électronique, la formation de radicaux libres associés aux biomolécules. D'autre part, le rendement en radicaux libres d'un ADN est fonction de son état de dégradation [5], [11]; on trouve une diminution incontestable du rendement en radicaux libres lorsque la masse s'accroît, de telle sorte que pour une variation de masse de l'ordre de 10 on obtient une variation de G_R de l'ordre de 50 pour cent [12].

L'objet de la présente Note est précisément d'analyser l'influence du poids moléculaire de l'acide désoxyribonucléique sur le transfert

d'énergie entre la proflavine et l'ADN (on a choisi la proflavine parce que de toutes les acridines celle-ci a la plus grande activité photodynamique [3]).

On a étudié deux ADN de types différents, extraits du sperme de saumon (CALBIOCHEM) et de l'érythrocyte de poulet. A partir d'échantillons de poids moléculaires égaux, pour l'un à 2.10^6 et pour l'autre à $5,9.10^6$ (coefficients d'extinction rapportés à 1 atome gramme de phosphore, mesurés à 260 mμ de 6600 et 6000), on a procédé à des dégradations au moyen d'ultrasons (solution d'ADN 0,5 M en NaCl) qui ont conduit à des poids moléculaires respectifs de $3,9.10^5$ et $2,5.10^5$. On a vérifié que les produits de scission n'avaient subi au cours de leur formation aucune dénaturation significative. Notons, en outre, qu'après la dégradation, l'ADN était précipité par l'alcool éthylique, puis séché sur l'acétone. La concentration de la proflavine était 5.10^{-4} M. Les différents systèmes ADN-proflavine ont été irradiés pendant environ 2 h à la température de 77° K, dans des tubes en quartz de 4 mm de diamètre scellés sous air, au moyen d'une lampe à vapeur de mercure à haute pression (Osram H.B.O. 500), dont le rayonnement était filtré de telle sorte que seule la région de longueur d'onde supérieure à 3000 Å était utilisée. On a fait usage d'un spectromètre Varian de type 4502—06; la température pendant l'observation était fixée à 150° K.

Dans les conditions de concentrations utilisées, le spectre paramagnétique de la proflavine ne s'est pas manifesté mais est apparu le spectre de l'ADN. L'identification a été faite par comparaison aux spectres obtenus par irradiation directe de ces substances par le rayonnement X [7], [9].

On s'attendait, après les recherches faites sur la structure des complexes aminoacridines-ADN [1], [6], à ce que les caractéristiques de transfert de l'ADN changent en fonction du poids moléculaire. Par contre on a eu la surprise de voir apparaître presque les mêmes signaux : des singulets caractérisés par une largeur aux points d'inflexion égale à 20 œrsteds environ, ainsi que le montrent les figures 1 et 2. Ce résultat si-
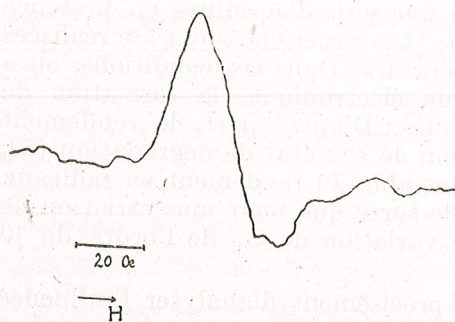
Fig. 1. — Spectre paramagnétique de transfert de l'ADN (poids moléculaire : $5,9.10^6$) irradié dans la région visible 2 h à 77°K ; observé à 150° K.

gnifie incontestablement que le lieu de couplage entre l'ADN et la proflavine n'est pas influencé par le poids moléculaire.

En conclusion, on peut affirmer qu'on est ici en présence d'un fait particulièrement simple dont on doit tenir compte pour l'identification

des centres paramagnétiques produits par irradiation dans l'ADN et aussi pour comprendre le mécanisme de transfert d'énergie ADN-colorant.

Fig. 2. — Spectre paramagnétique de transfert de l'ADN (poids moléculaire : $2,5.10^5$) irradié dans la région visible 2 h à 77°K ; observé à 150°K.
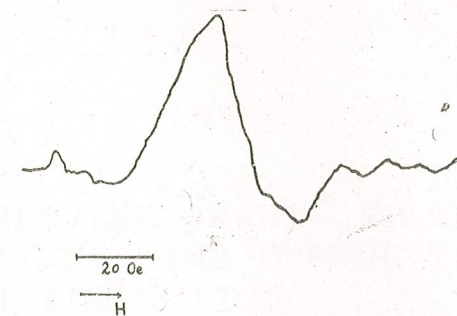
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A HYPOTHESIS CONCERNING THE ORIGIN AND THE MECHANISMS UNITING IN THE SAME OPERON THE MAIN PARTICIPATING FACTORS

BY

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In the present work the author presents a hypothesis concerning the origin of the factors participating in an operon system. The transcriptase, an old enzyme, develops by gene duplication and mutation phenomena, becoming a repressor. The structure with an enzymatic degradation action on the substrate is originating from the mutant transcriptase.

Transcriptase was one of the earliest enzymes on the evolution scale of the organisms. Its presence was compulsory as soon as the DNA became the hereditary bearer of information. Had RNA been originally the genetic information bearer, then it could have been replaced by DNA (a double helicoidal structure which conferred it a greater steadiness) only when transcriptase would have been able to ensure the conveying of information. Provided that DNA was originally the genetical information bearer, then from this very early stage the enzyme had to be functional.

According to Ohno's theory [7], [8], the later transcriptase evolution took place by gene duplication. A similar interpretation of the molecular evolution of cytochromes, hemoglobins, fibrinopeptides, immunoglobulins, hypophyseal hormones and digestive hydrolases was presented by Prof. Ion Moraru, M. Dumitrescu and co-workers, at the First National Conference of Animal Genetics hold in Romania [1—6].

The hypothesis concerning the origin of the factors participating in an operon system is based on the duplication gene theory of evolution. By elucidating the origin of the component factors of the operon system,

one tries to find out an explanation of the mechanisms uniting them in this system, as well as an explanation of the phenomena which assigned to each factor a special action.

Transcriptase was the enzyme representing the means by which genetic information was turned into account. The more important this information became, the more obsolete its dependence on a single factor. If there was a single information-bearer gene for transcriptase at the DNA level, a mutation occurring at this DNA level induced the disappearance of the organism in its earliest stage. The mutants presenting a gene duplication as a consequence of an unequal crossing-over or polyploidization, achieving more than a single locus for transcriptase, represented ensured forms for a possible occurrence of a mutation at the level of this enzyme information bearer. These ensured mutants removed the unensured forms by means of selective pressure.

The occurrence of a mutation inside one of these transcriptase loci at the level of one of the transcriptase subunits was not lethal for the organism. Enzyme synthesis was ensured by other loci which did not undergo mutations.

Let us assume that this transcriptase mutant that we shall denote by "R", is no more able to determine the RNA conveyance depending on DNA, but, stopping at a certain punctation level, i.e. that which delimits its own gene by the factor we call the "operating gene", it no more determines the substrate conveyance on account of the mutation it underwent and thus it fastens on the operon end, blocking it.

When the respective operon was not the bearer of permanent useful and vital information, the organism succeeded in keeping alive in spite of the blocked operon, and the "R" mutant transcriptase biosynthesis did not occur.

The organism is pervaded by substances, one of which has a structure determining the detachment of the mutant transcriptase from the DNA level and its fixing on the new substrate by means of a mechanism which, in case of a repressor, Monod explained as an allosteric transition phenomenon. Therefore, under the influence of a substance penetrating the organism, the mutant transcriptase molecule undergoes a spatial transconformation $R \rightarrow R'$; this influence does not extend on the other transcriptase molecules, which have not underwent mutations and which do not exert a blocking action on the operons, ensuring the information conveyance [9].

If the transconformation of the mutant transcriptase molecule is reversible, $R' \rightarrow R$, when the substance pervading the cells is exhausted they are coming back to their original spatial form and fix themselves at the DNA level; but if it is irreversible, the operon remains functional and the synthesis of a new mutant transcriptase molecule occurs and blocks the operon.

This "R", either newly synthesized or resulted from R', again fastens at the operating gene level, blocking the operon.

The "R" gene bearer segment undergoes an isolocus generating mutation. This isolocus, according to the mutational mechanism it resulted

from, may be situated on the same operon as a continuation of the locus it derived from, on different operons or even on different chromosomes.

One of the two isoloci presents mutational phenomena : segment addition or segment loss, deletions or punctiform mutations, thus becoming the X gene.
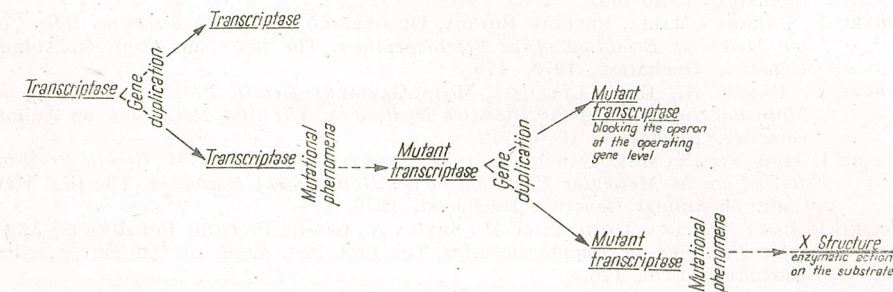
Fig. 1

X structure keeps the situs-bearer segment for the substrate, however presenting also other segments, either modified, lost or newly added ; those mutational phenomena which confer to the X structure a lysis action on the substrate, have the selective advantage to allow the use of the substrate to the benefit of the respective organism which, being thus favoured in food competition, reaches the ecological niche and becomes the form that maintains itself, replacing those forms that lack this supplementary advantage.

X gene which resulted from the "R" gene presents the same factor at the punctuation level known as operating gene and thus "R" keeps its capacity of blocking also the respective operon (namely the X-gene-bearer operon) and from a mutant transcriptase becomes a repressor.

Upon the appearance of the substrate, the repressor releases the operon and fastens on the substrate ; as the operon is being unblocked, X gene is conveyed, while the polypeptide X is now synthesized and exerts an enzymatic activity on the substrate. The substrate is abolished and the repressor "R", which resulted from the mutant transcriptase, blocks again the operon.

The main idea of the present hypothesis is to search an explanation for the striking coincidence that determines the fastening of the repressor at the level both of the operating gene and of the substrate and that at this gene level it blocks just the operon that holds the information for the enzyme acting on the substrate on which the respective repressor is fixed, too.

It is very unlikely that these circumstances had taken place and all the less that they repeated for each operon system.

The purpose of the present hypothesis is to find a single explanation which, by avoiding extraordinary coincidences, could contain the circumstances uniting the factors participating in an operon system. This hypothesis does not claim to be exhaustive, but it is looking for a way to elucidate the events that must be accounted for.

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