

Dans le derme des rats traités au perchlorate de potassium, on constate une diminution significative des mastocytes du groupe I et une augmentation des formes fortement dégranulées (groupe III) (Tableau Ia). Le nombre de mastocytes par mm<sup>2</sup> diminue de façon significative par rapport aux contrôles (Tableau Ib).

Dans la thyroïde, on note une augmentation significative du pourcentage des mastocytes dégranulés sous l'action du perchlorate de potassium (Tableau IIa) ainsi qu'une augmentation significative du nombre de mastocytes par mm<sup>2</sup> (Tableau IIb). La glande des animaux traités présente en outre une hyperplasie correspondant à la description de LAMPÉ<sup>8</sup>.

Dans le foie et le poumon, on constate une légère diminution des mastocytes chez les animaux traités.

L'action des antithyroïdiens (thiouracil, propylthiouracil, etc.) sur le comportement des mastocytes a été étudiée par différents auteurs et revue par SELYE<sup>1</sup>. On peut en déduire que l'hyperplasie de la glande thyroïde s'accompagne d'une augmentation des mastocytes à ce

niveau ainsi que d'une augmentation des formes dégranulées de ces cellules<sup>9</sup>. MÉLANDER et al.<sup>10</sup> observent qu'il existe une relation directe entre le taux de la TSH dans le plasma et le nombre de mastocytes dans la glande thyroïde chez la souris. Nos résultats obtenus dans la thyroïde des animaux traités au perchlorate de potassium sont superposables: il existe en effet une augmentation des formes dégranulées ainsi qu'une augmentation du nombre de mastocytes par mm<sup>2</sup> de surface. On peut donc conclure que le comportement des mastocytes dans la glande thyroïde est lié à son état fonctionnel<sup>9, 11</sup>.

Au niveau des organes et dans le derme en particulier, on observe une diminution du nombre des mastocytes et une augmentation du pourcentage des formes dégranulées chez les animaux traités. Ces faits ne concordent pas avec les résultats obtenus avec d'autres antithyroïdiens et cités dans la littérature. On peut cependant penser que la diminution du nombre de mastocytes par mm<sup>2</sup> chez les animaux traités est due à la disparition de ces cellules par dégranulation. Il est possible que le perchlorate ait un effet direct sur les mastocytes au niveau des organes. Cette action serait cytotoxique et analogue à celle d'autres produits provoquant la dégranulation de ces cellules<sup>1</sup>.

Tableau II. a) Pourcentage de mastocytes dégranulés dans la thyroïde de rats\*

contrôles 7 rats	traités 6 rats
45,41 ± 3,6	57,54 ± 3,4

Pour chaque animal, on compte 1000 mastocytes; les pourcentages sont calculés à partir de cette valeur.

\* 0,05 > p > 0,01.

b) Nombre de mastocytes par mm<sup>2</sup> de surface thyroïdienne<sup>b</sup>

contrôles 7 rats	traités 7 rats
10,9 ± 1,8	15,8 ± 2,7

Pour chaque animal, on compte les mastocytes sur une surface totale de 40,56 mm<sup>2</sup>. Les résultats sont exprimés en nombre de mastocytes par mm<sup>2</sup>.

<sup>b</sup> p < 0,01.

Sont reportées dans ce tableau les valeurs moyennes et les déviations standards.

**Summary.** The authors study the number of mastocytes and the percentage of degranulated types in rats treated with potassium perchlorate. This product has an anti-thyroid activity. An increase in absolute number of mastocytes in the thyroid gland of treated animals is shown. There is also an increase of degranulated types of these cells. In the other organs (skin, liver, lungs) a decrease in absolute number of mastocytes is observed with a concomitant increase of degranulated types. The results are discussed according to the literature.

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<sup>8</sup> L. LAMPÉ, L. MODIS et A. GEHL, Acta med. hung. 23, 223 (1967).

<sup>9</sup> F. SANTINI, Arch. ital. Anat. Embriol. 67, 443 (1962).

<sup>10</sup> A. MELANDER, CH. OWMANN et F. SUNDLER, Endocrinology 89, 528 (1971).

<sup>11</sup> G. ZILLOTTO et N. PELLEGRINI, Riv. Anat. Pat. 11, 903 (1956).

## Structural Changes in the Chromosomes of Malpighian Tubes of *Rhynchosciara americana* (Diptera) During Normal Larval Development and After Temperature and Hormonal Treatment

Cell physiology and differentiation involves in many cases specific morphological modifications in chromosomes. Our present knowledge of this problem is based in great part on several classical studies on the polytene chromosomes of the salivary glands of Diptera<sup>1-5</sup>. The available information is not clear enough to give us a complete idea of the structural changes which occur at chromosome level during development, as well as in the course of different physiological or experimental conditions. A specially favorable organism for this study is *Rhynchosciara* in which polytene chromosomes are found in many tissues of the larva and the adult. The present paper deals with some changes observed in a particular type of chromosomes of the Malpighian tube cells of *Rhynchosciara americana* during some stages of prepupal larval period and after treatment with 2 different agents: temperature and the hormone ecdysone.

**Material and methods.** Larvae of *Rhynchosciara americana* were used in our experiments. Cultures of the flies were maintained in the laboratory according to the method described by LARA et al.<sup>6</sup>. The biological and cytological characteristics of this species of fly were discussed in the papers of DREYFUS et al.<sup>7</sup> and BREUER and PAVAN<sup>2</sup>. All experiments were carried out with larvae on the 3rd,

<sup>1</sup> W. BEERMAN, Chromosoma 5, 139 (1952).

<sup>2</sup> M. C. BREUER and C. PAVAN, Chromosoma 7, 371 (1955).

<sup>3</sup> R. PANITZ, Naturwissenschaften 47, 383 (1960).

<sup>4</sup> H. O. BERENDES, F. M. A. VON BREUGEL and TH. K. H. HOLT, Chromosoma 16, 35 (1965).

<sup>5</sup> U. CLEVER, Chromosoma 12, 607 (1961).

<sup>6</sup> F. J. S. LARA, H. TAMAKI and C. PAVAN, Am. Natur. 99, 189 (1965).

<sup>7</sup> A. DREYFUS, E. NONATO and C. PAVAN, Revta bras. Biol. 11, 439 (1951).

4th, 5th and 6th periods of the 4th instar. This subdivision of this instar in these periods is that proposed by TERRA et al.<sup>8</sup>. Daily analysis of the Malpighian tubes chromosomes was carried out from the 4th day after the beginning of the cocoon formation (3rd period) up to the end of the 4th instar. For the normal cytological observations, usually 3 larvae were killed daily. Temperature treatment was performed by transferring the larvae from 20°C to 32°C. Changes in the chromosomal structure were studied after 24 h of treatment. Changes in the chromosomal pattern of Malpighian tubes occurring after injection in the haemocoel of 0.002 ml of a 0.02% solution of ecdysone, were analysed 24 h after injection.

**Description.** *Rhynchosciara americana* possess 4 Malpighian tubes. They show 3 anatomically and functionally differentiated zones: basal, medial and distal. Each one has a typical cytological pattern. The 4 chromosomes show clear cut structural differences in these 3 areas. The cytological analysis of the chromosomes of basal and median regions, just at the beginning of the 3rd period, shows that they have a small degree of polyteny and their distension is not so complete as in salivary gland chromosomes. However, the band pattern characteristic for each of the chromosomes becomes more evident in successive

days and after 7 days of the beginning of the analysis period, the band pattern is clear enough to permit a careful analysis. The nuclei of the distal zone have a smaller diameter than those of the basal and median zones. The chromosomes in non-squashed nuclei are ordered inside the nuclear membrane in a characteristic and constant fashion. The 4 heterochromatic centromeric regions are placed in the central part of the nucleus and the chromosomal arms are radially oriented toward the nuclear membrane. In squashed nuclei we can easily see that the chromosomes of this region are much shorter and with a much smaller degree of polyteny than those of the basal and median portions. In general they do not show typical bands, although in some cases it is possible

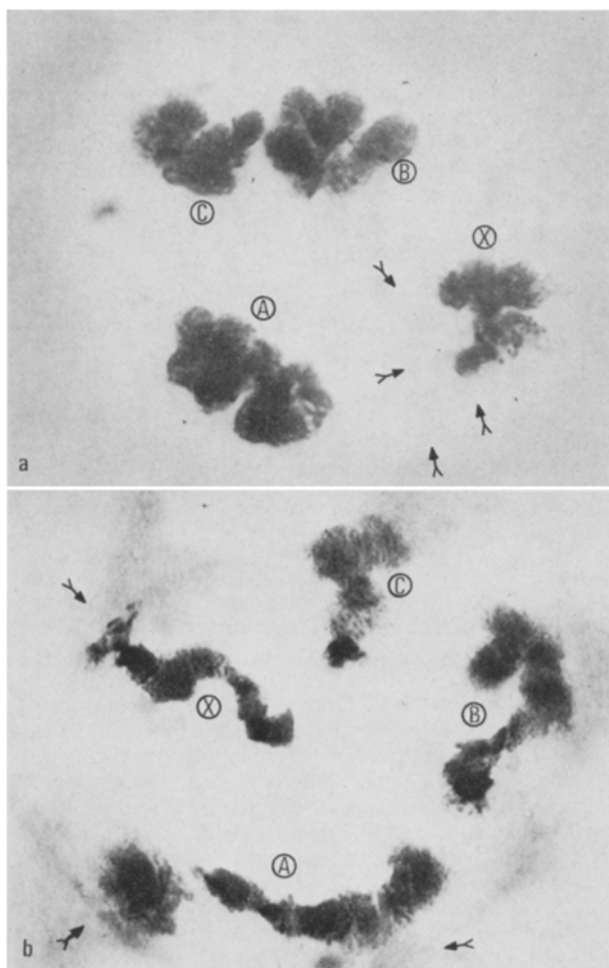


Fig. 1. Chromosomes of the distal part of Malpighian tubes of normal *Rhynchosciara americana* larvae. A) condensed state at the 4th day after cocoon formation. The arrows point out several nucleolar bodies. B) Uncondensed state at the 8th day after cocoon formation. The arrows signal points of contact between the chromosomes and the nuclear membrane. Acetic orcein.  $\times 1000$ .

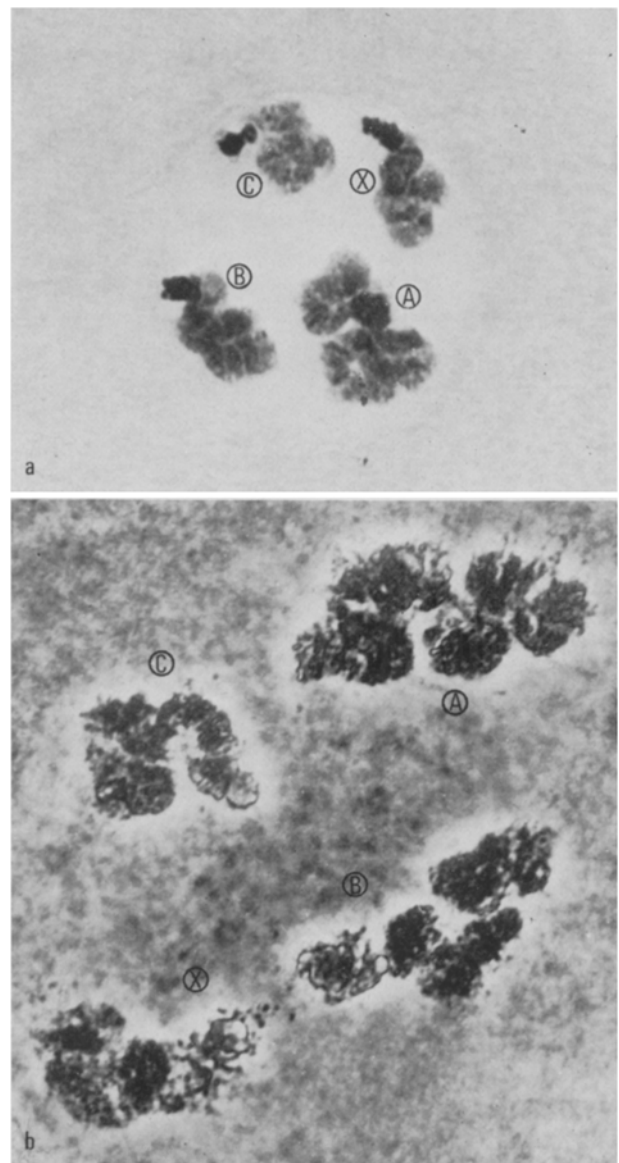


Fig. 2. Chromosomes of the distal part of Malpighian tubes of larvae of *Rhynchosciara americana* at the 4th day after cocoon formation. Note in B) the presence of minute granules adherent to the chromosomal surface (phase contrast), A)  $\times 450$ ; B)  $\times 1000$ .

<sup>8</sup> W. R. TERRA, A. G. DE BIANCHI, A. G. GAMBARINI and F. J. S. LARA, J. Insect. Physiol., in press.

to reveal a banding pattern in some sectors of the chromosome. In preparations made when the larvae were in the first 3 days of the 4th instar, the chromosomes have a condensed appearance (Figure 1a). The chromosomes are almost entirely heterochromatic and they are free into the nuclear sap. Several nucleolar bodies can be observed. In some cases, and using phase contrast optics, it is possible to reveal the existence of minute granules and spines closely adherent to the heterochromatic chromosomes (Figure 2b). As larval development progresses and the animals continue to build up the common cocoon, the cytological aspect of the chromosomes begins to change. They become more diffuse and the amount of heterochromatic areas considerably decreases (Figure 1b). Simultaneously with this progressive process of decondensation, several areas of close contact between the chromosomes and the nuclear membrane begin to appear (Figure 1b). In many cases the C chromosome is to be observed as a roundish slightly stained mass with a little heterochromatic block corresponding to the centromeric heterochromatin. This shape recalls, to a certain extent, that of the so-called 'pompon-like' chromosomes described in salivary gland in some viral and protozoan infections<sup>9</sup>. Important structural changes at chromosomal level were observed in the Malpighian tubes of larvae after temperature and hormonal treatments. In both cases the treatments were made on the 4th day of the 3rd period, when the chromosomes appear in the control animals with a high degree of condensation. The most important fact appearing after 24 h of ecdysone injection was an intense process of decondensation of the chromosomes. They show a loosened aspect and increased areas of contact with the nuclear membrane begin to appear (Figure 4a). Essentially the same chromosomal changes were revealed after 24 h of temperature treatment (Figure 4b), but in this case the chromosomes showed a more diffuse aspect than in hormonally treated animals. At the same time they showed larger areas of puffed aspect, which in some cases involved even the centromeric heterochromatic region (Figure 4b).

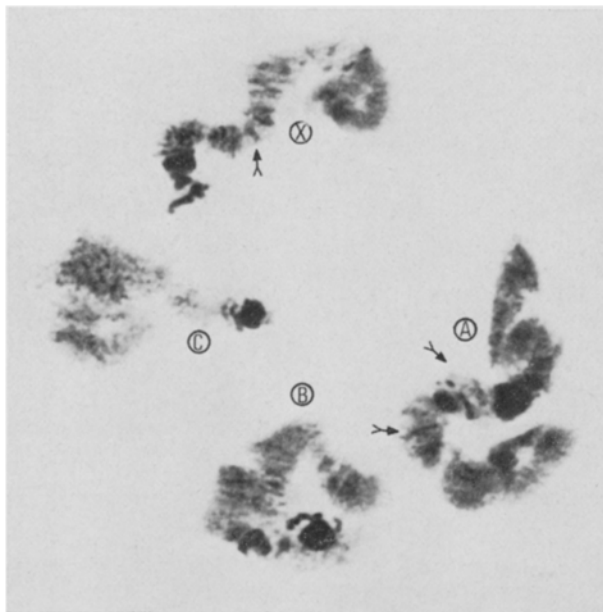


Fig. 3. Chromosomes of the median zone of Malpighian tubes of *Rhynchosciara americana* larvae at the 4th day after cocoon formation. Note the entirely puffed aspect of the C chromosome and the bands in the X and A (arrows). Acetic orcein.  $\times 1000$ .

**Discussion.** It is generally accepted that the formation of puffs and Balbiani rings are morphological manifestations of gene activity in polytene chromosomes. The formation of a puff involves the accumulation of acidic proteins, the despiralization of DNA within the puff region, the synthesis of RNA and the storage of newly synthesized RNA<sup>10</sup>. In non-polytene chromosomes, the possibilities of visualization of morphological changes in the chromosomal structure are of course much more restricted, and there are only few data concerning chromosomal structural modifications in the course of cellular differentiation or during different cellular activities. A good material for the analysis of this problem seems to be constituted by the chromosomes of distal part of Malpighian tubes of *Rhynchosciara*, because

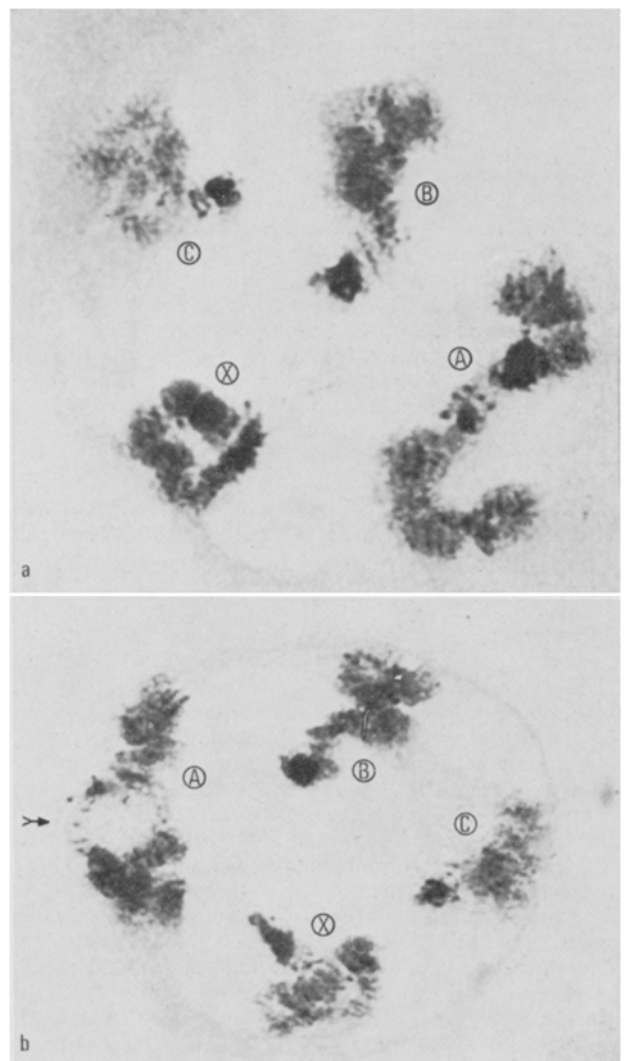


Fig. 4. The chromosomes of the distal part of Malpighian tubes of *Rhynchosciara americana* larvae. A) Treated with 0.02% ecdysone. Note the diffuse aspect of the chromosomes and their close contact with the nuclear membrane. B) Larvae maintained at 35°C for 24 h. The arrow signals a puff in the heterochromatic centromeric region of the A chromosome. Both treatments were performed at the beginning of the 3rd period of the 4th instar. Acetic orcein.  $\times 1000$ .

<sup>9</sup> M. DIAZ, C. PAVAN and R. BASILE, *Revta bras. Biol.* 29, 191 (1969).

<sup>10</sup> W. BEERMAN, *Naturwissenschaften* 52, 365 (1965).

they represent an intermediate stage between typical polytene chromosomes. As follows from the foregoing account, these manifest several clear cut structural modifications on successive days of the prepupal period. The most relevant phenomenon is a gradual process of decondensation of the chromosomes as the maturation of the larvae progresses. In fact during the 3 first days of the period studied, the chromosomes are almost entirely heterochromatic and condensed, and shortly thereafter, on the 7 day of cocoon formation, when the process of building of the common capsule takes place, they become more diffuse and light stained. At the same time they show several areas of close adherence with the nuclear membrane, sometimes with a typical puff-like granular appearance (Figure 1b). Similar changes were induced in the 4th day of cocoon formation by means of hormonal and temperature treatments. Apparently both experimental procedures provoke an acceleration of the decondensation process, as well as a phenomenon of fusion with the nuclear membrane. A number of investigations on the relation of chromosome differentiation to gene activity suggest that euchromatin should represent the stage at which the genetic information carried in the chromosome is being transcribed<sup>11</sup>. Conversely the heterochromatin should be the morphological counterpart of the diminution or suppression of the genetic activity. If we can extend to these chromosomes that theory, then, whatever genetic messages they carry out must be transcribed mostly in the latter days of the stage studied, while the chromatin is in the decondensed state. On the other hand, it has been pointed out that many condensed chromosomes are composed of blocks of material with genetic function, which are not heterochromatic at all stages of the life cycle<sup>11</sup>. The same would be the case for the nucleolus

associated heterochromatin blocks appearing in nerve cells of several avian species which seems to pass through a sort of cycle of condensation-decondensation<sup>12</sup>. These studies and others<sup>13</sup> would suggest that heterochromatin and euchromatin are reversible states, and that chromosomal regions traditionally called heterochromatic are at least in some cases merely stages of transitory chromosomal differentiation.

**Résumé.** Les auteurs décrivent quelques aspects structuraux des chromosomes dans les tubes de Malpighi de *Rhynchosciara americana* (Diptera). L'apparition de modifications dans la structure chromosomique au cours du développement larval normal, et après traitement hormonal et thermique est suivie. La signification fonctionnelle possible de ces changements structuraux est discutée.

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<sup>12</sup> T. P. PESSACQ, *Cytologia* 34, 375 (1969).

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<sup>15</sup> We thank Prof. F. J. S. LARA for valuable discussion and advice and for reading and correcting the manuscript, and Dr. A. G. GAMBARINI for many interesting suggestions made in the course of the research, and STA. A. C. STEFFEN for valuable technical assistance. The Ecdysterone was a kind gift from Prof. J. DE WILDE.

## Degeneration of Noradrenergic Nerve Terminals in Submucous Ganglia of the Rat Duodenum Following Treatment with 6-Hydroxydopamine

Since it was first shown that administration of 6-hydroxydopamine (6-OHDA) results in selective degeneration of noradrenaline-containing nerve terminals<sup>1</sup>, there have been a number of studies in which its effect at neuroeffector sites have been examined<sup>2-8</sup>. Fluorescence histochemical studies have established that noradrenaline-containing nerve terminals are present in mammalian enteric plexuses<sup>9,10</sup>, and ultrastructural studies have demonstrated the presence of axosomatic synapses in the myenteric plexus of the guinea-pig<sup>11,12</sup>. The distribution of noradrenaline-containing nerve terminals in ganglionated plexuses of the rat duodenum, however, has not been determined. In this study the distribution of degenerating nerve terminals found in submucous ganglia of the duodenum of the rat following administration of 6-OHDA has been examined using electronmicroscopy.

Rats were given a single i.v. injection of 100 mg/kg 6-OHDA HCl (25 mg/ml 6-OHDA HCl dissolved in a solution containing 1 mg/ml ascorbic acid). Control rats were injected i.v. with an equivalent volume of ascorbic acid. Subcutaneous heparin (1,000 U) was administered to alternate rats in each of the treated and control series 1 h before the animals were killed. 2, 4, 6, 12 and 19 h after injection of 6-OHDA, the duodenum was fixed by vascular perfusion with a solution containing 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.5 mg/l CaCl<sub>2</sub>. After perfusion the duodenum was distended slightly by intraluminal injection of fresh fixative. A short segment of gut was

removed, cut into thin rings, post-fixed in osmium tetroxide and embedded in Araldite. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in an Hitachi HS-8 electron microscope.

In the submucous ganglia of untreated rats, noradrenergic axons were identified by their content of small vesicles (about 50 nm in diameter) which contained an

<sup>1</sup> J. P. TRANZER and H. THOENEN, *Experientia* 25, 155 (1968).

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<sup>6</sup> J. P. TRANZER and J. G. RICHARDS in *6-Hydroxydopamine and Catecholamine Neurons* (North-Holland Co., Amsterdam, London 1971) p. 15.

<sup>7</sup> J. A. GOSLING and J. S. DIXON, *J. Cell Sci.* 10, 197 (1972).

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<sup>9</sup> G. GABELLA and M. COSTA, *Experientia* 24, 706 (1968).

<sup>10</sup> M. COSTA and G. GABELLA, *Z. Zellforsch.* 122, 357 (1971).

<sup>11</sup> G. GABELLA, *Experientia* 27, 380 (1971).

<sup>12</sup> G. GABELLA, *J. Anat.* 111, 69 (1972).