

chromosomes exhibit a clear picture of synapsis, a feature so characteristic of meiotic chromosomes in pachytene, and carry multiple strands¹⁰ whereby they have entered a permanent prophase¹¹. It is in this context that it will be interesting and significant to know and compare the chromosomes of a local coccid, *Icerya aegyptica* Dougl. (Tribe: Iceryini, Monophlebinae) with that of the salivary chromosomes of a dipteran insect.

The embryos fixed in BRADLEY-CARNOY mixture were stained with SCHIFF's reagent as well as with the modified alcoholic carmine of SNOW¹². *Icerya aegyptica* is a haplo-diploid Iceryine coccid and the males are found in very small numbers and live for a very brief period. The karyotype of this species, like other Iceryine coccids, is $2n = 4$ (♀), $n = 2$ (♂). The 2 pairs of chromosomes differ slightly in their length.

In Figures 1 and 2, the coccid chromosome presents the characteristic chromatic and achromatic banding normally noticed in the salivary gland chromosomes. These chromosomes of the coccid, however, are not giant in their size although they exhibit the band structure. As is usual with the salivary chromosomes, many chromatic bands form the bulb here also. Such differences in the individual bands due to the formation of the bulbs necessarily lead to the varied appearance and stainability of the chromosomes, which in itself is a reflection of the functional

activities of the chromosomes in particular and of the metabolism and differentiation of the cell in general. Further, based on the staining reaction (with SCHIFF's reagent) it is possible to state that wherever such bulb formation on the chromosome has taken place, there is an equal increase in the DNA content of the bulb also¹³.

Résumé. Démonstration d'une structure analogue à celle des chromosomes géants des Diptères chez un Coccide.

T. S. S. DIKSHITH¹⁴

Indian Lac Research Institute, Namkum, Ranchi (Bihar, India), 6 November 1967.

¹⁰ P. C. KOLLAR, Proc. R. Soc. [B] 118, 371 (1935).

¹¹ E. J. AMBROSE and A. R. GOPAL AYANGAR, Nature 169, 652 (1952).

¹² R. SNOW, Stain Technol. 38, 9 (1963).

¹³ Grateful acknowledgment is made to Dr. G. S. MISRA, Director of the Institute, for his keen interest and encouragement. Thanks are also due to Mr. P. DAS, for the preparation of photomicrographs.

¹⁴ Present address: Central Food Technological Research Institute, Mysore-2, India.

Development of the Middle Lamella in Rib Meristem Cells

A rib meristem is characterized by a complexus of longitudinal rows or ribs of cells which divide at right angles to the growth axis. This growth pattern is clearly represented in the cortex of the root. The longitudinal walls of the rib are primary longitudinal walls, the transverse walls being formed, basically, by middle lamella. At each division cycle, 2 daughter cells are formed, and the cell plate forms the new middle lamella between them. The fine structure of the cell plate formation has been studied by several authors¹⁻¹⁰. Moreover, FREY-WYSSLING et al.² showed that the middle lamella formed by the coalescence of small Golgi vesicles, grew thicker through the supply of new Golgi vesicles. The aim of this paper is to study the development of the middle lamella in relation to the cell division cycles.

Material and methods. Seeds of *Phalaris canariensis* were germinated at room temperature, using filter paper and tap water. The seedlings were grown for 2 or 3 days. At the end of this period the root tips (2-3 mm) were removed and immediately fixed by the following method: KMnO₄ 2% in distilled water for 2 h at 20-22°C. The fixed material was dehydrated in an acetone series and embedded in Durcupan ACM (Fluka). During the dehydration, the material was stained overnight in lead uranyl acetate¹¹. To obtain the ultrathin sections an Ultratom L.K.B. was employed. The observations were made with a Siemens Elmiskop I, and the pictures taken on Scienza Gevaert plates.

Results and discussion. The study of longitudinal sections of ribs from the cortex gave clear evidence of the existence of a certain range of types among the middle lamellae, which could be characterized by their different thicknesses and differences of contrast on staining. The thinnest middle lamella showed a thickness of from 0.1-0.2 μ , with a markedly sinuous outline (Figure 1a) as well as a fair degree of electronic density. As the thickness

of the middle lamellae increases, their contours become more uniform and their electronic density decreases. The thickest of them to be observed were 0.4-0.5 μ in diameter, and similar to the longitudinal walls of the rib of cells in electronic density.

The daughter cells observed in late telophase showed the peculiarity of being bounded by 3 transverse walls with clearly different characteristics. The middle lamella recently formed between the 2 daughter cells was seen to possess the characteristics of young walls, with a thickness of 0.1-0.2 μ , sinuous outline and relatively high electronic density, probably due to the high proportion of pectins. The 2 other transverse walls differed from it and from each other in thickness.

¹ K. ESAÚ and R. H. GILL, Planta 67, 168 (1965).

² A. FREY-WYSSLING, J. F. LÓPEZ-SÁEZ and K. MÜHLETHALER, J. Ultrastruct. Res. 10, 422 (1964).

³ J. F. LÓPEZ-SÁEZ, M. C. RISUEÑO and G. GIMÉNEZ-MARTÍN, J. Ultrastruct. Res. 14, 85 (1966).

⁴ J. D. PICKETT-HEAPS and D. H. NORTHCOTE, J. exp. Bot. 17, 20 (1966).

⁵ J. D. PICKETT-HEAPS and D. H. NORTHCOTE, J. Cell Sci. 1, 121 (1966).

⁶ J. D. PICKETT-HEAPS and D. H. NORTHCOTE, J. Cell Sci. 1, 109 (1966).

⁷ K. R. PORTER and S. B. CAUFIELD, Proc. 4th Int. Congr. Electron Microscopy, Berlin 1958 (Springer, Berlin 1960), vol. 2, p. 503.

⁸ K. R. PORTER and R. D. MACHADO, J. biophys. Biochem. Cytol. 7, 167 (1960).

⁹ W. G. WHALEY, M. DAUWALDER and J. E. KEPHART, J. Ultrastruct. Res. 15, 169 (1966).

¹⁰ W. G. WHALEY and H. H. MOLLENHAUER, J. Cell Biol. 17, 216 (1963).

¹¹ G. GIMÉNEZ-MARTÍN, M. C. RISUEÑO and J. F. LÓPEZ-SÁEZ, Experientia 23, 316 (1967).

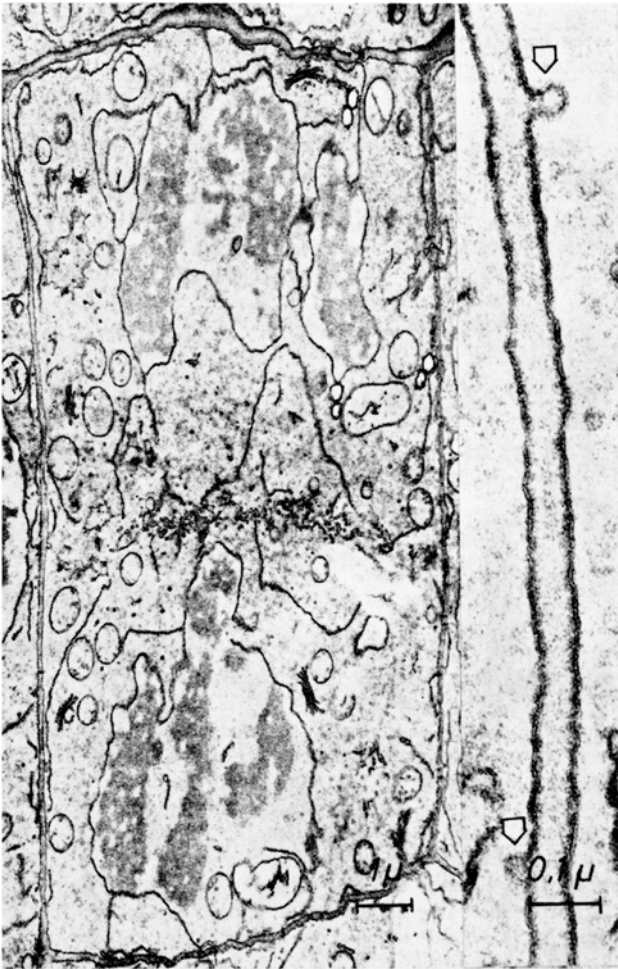


Fig. 1. On the left, early telophase cell showing in the middle the cell plate formation, and bottom and top the transverse walls of different thicknesses ($\times 7300$). On the right, middle lamella showing fusion of Golgi vesicles ($\times 85,000$).

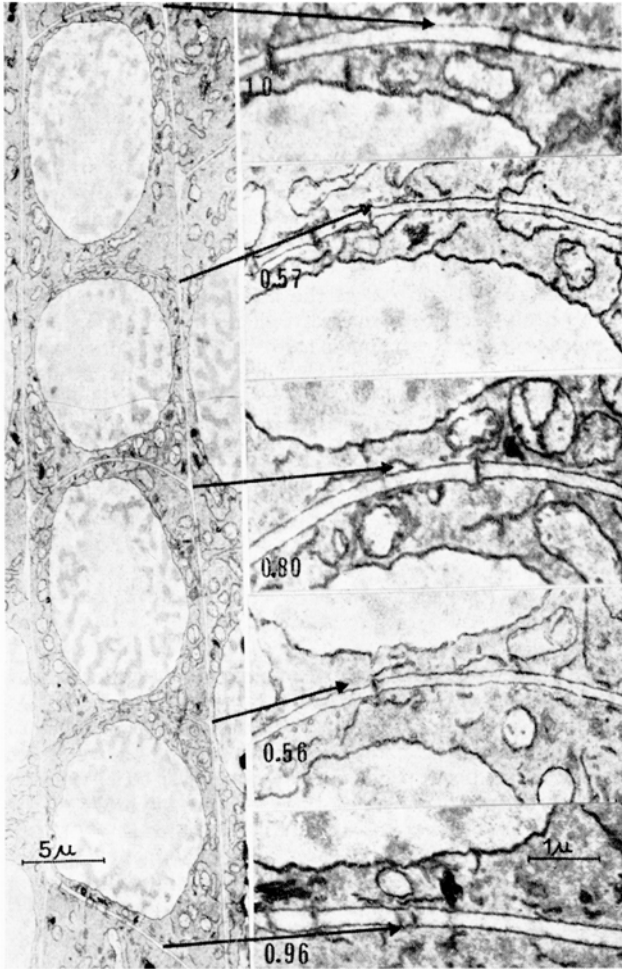


Fig. 2. On the left, rib of 4 cells originated from a mother cell in 2 generation cycles ($\times 2000$). On the right, the transverse walls and their relative mean thickness ($\times 8500$).

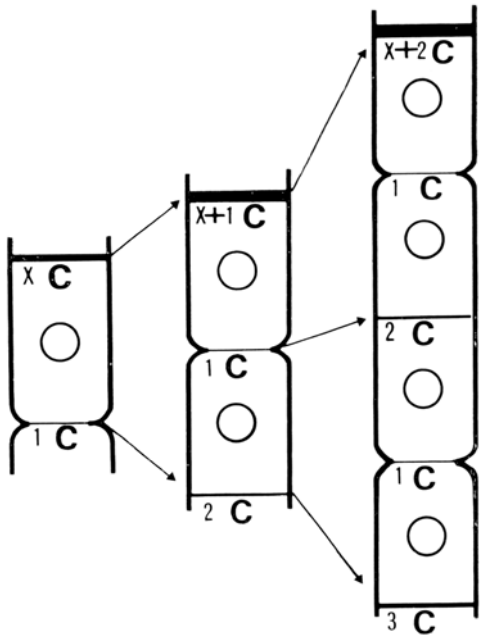


Fig. 3. See text.

The study of numerous longitudinal sections revealed a picture of small vesicles with the characteristics of Golgi vesicles fusing with the middle lamellae and contributing their content to the matrix of the wall and their membrane to the plasmalemma (Figure 1b). A similar process was observed by MOLLENHAUER et al.¹² in the development of the primary cell wall of the outer rootcap cells of *Zea mays*, where the Golgi cisternae produced large vesicles whose contents increased the cell wall volume and whose membranes were incorporated into the plasma membrane. Our observations confirm the findings of FREY-WYSSLING et al.², who concluded that the development of the middle lamella owed its origin, at least in part, to the supply of materials through the Golgi apparatus, and suggested that it depended on the length of time this had existed. As a yardstick for measuring this factor, we used the cell division cycle.

If our hypothesis is correct, a newly formed cell would be bounded by 2 transverse walls, one of which, the thicker of the 2, will have been formed x division cycles ago, while the new one will be initiating the first cycle.

¹² H. H. MOLLENHAUER, W. G. WHALEY and J. H. LEECH, J. Ultrastruct. Res. 5, 193 (1961).

When this cell has come to the end of one division cycle, we should observe 2 daughter cells with a first-cycle middle lamella between them, a second-cycle lamella at one end, and an $(x + 1)$ th-cycle lamella at the other. This hypothesis, further developed, will lead us to a rib or column of 4 cells at the following division cycle (Figure 3).

These expectations were confirmed by the study of 4-cell groups from a rib meristem (Figure 2), which shows that the middle lamella can be distinguished by their morphological characteristics up to an age of 3 or 4 cycles.

These results show that the middle lamella evolve by thickening, decreasing in electronic density, and assuming a more and more rectilinear form. The supply of material for the matrix of the wall is ensured by the contribution made by Golgi vesicles, and the diminishing electronic density of the wall suggests that it gets a proportionate increase in cellulose matter in relation to the original pectic components. The various characteristics to be ob-

served in the transverse walls enable us to distinguish the sister cells with ease, and even the 4 cells which one mother cell must produce in 2 division cycles.

Resumen. La lámina media que separa transversalmente a las células del meristemo radical en columna se desarrolla, en virtud del aporte de pequeñas vesículas del aparato de Golgi, incrementando su espesor desde 0,1–0,2 hasta 0,4–0,5 μ . Las características morfológicas de estas paredes transversales permiten distinguir fácilmente las células hermanas y hasta las 4 células que se originan de 1 célula madre en 2 ciclos de división.

M. C. RISUEÑO, G. GIMÉNEZ-MARTÍN
and J. F. LÓPEZ-SÁEZ

*Instituto de Biología Celular, C.S.I.C., Madrid (Spain),
1 December 1968.*

Content and Synthesis of γ -Aminobutyric Acid in the Larval Brain of *Drosophila melanogaster*

In our previous studies we reported the occurrence of γ -aminobutyric acid in *Drosophila melanogaster*^{1,2}. Its presence has been also recorded in several other insects, including the cornmeal moth (*Ephestia kühniella*)³, the honeybee (*Apis mellifica*)⁴ and the housefly (*Musca domestica*)⁵. From a number of investigations it is further known that this amino acid is characteristic of the central nervous system of various invertebrates and vertebrates⁶. Decarboxylases, which convert glutamic acid to γ -aminobutyric acid, have been demonstrated in the mammalian and chick brain^{7–11}. It is suggested that γ -aminobutyric acid may possibly play a role in the transmission of the action of inhibitory neurons^{12,13}. In order to obtain information about the neurophysiological significance of this amino acid in insects, experiments have been carried out by us to determine its content and synthesis in the larval brain of *Drosophila*.

Larvae of the wild type (Sevelen) of *D. melanogaster* were raised on the standard sugar-corn meal-agar-yeast medium at 25°C. Shortly before pupation about 150 brains (2 hemispheres plus ventral ganglion) were dissected out individually in a drop of Ringer's solution under a binocular microscope. These were collected in 80% methanol and homogenized in a glass microhomogenizer. After centrifuging, the supernatant solution was transferred to a Whatman No. 1 filter paper (24 × 46 cm) for two-dimensional chromatography, using 70% *n*-propanol as the first solvent (ascending) and water-saturated phenol as the second solvent (descending). The optical density of each ninhydrin-positive spot on the chromatogram was determined according to procedures described previously¹⁴. For comparison, extracts of 15 whole larvae of the corresponding age were also prepared and the content of individual substances was analysed by the same method.

The data expressed in percentage of the total ninhydrin-positive components are summarized in the Table. As can be seen, the relative concentration of γ -aminobutyric acid is at least 2 times higher in the brain than in the whole larva. Its content in the brain has been determined to be 1.23 μ M/g wet weight. Similarly the brain extract contains significantly more aspartic acid, glutamic acid and taurine than the entire larva extract. It is of

interest to notice that, with the exception of taurine, all these amino acids may serve as transmitting substances in the central nervous system of both mammals and other invertebrates^{12–13}. The function of taurine is unknown, but according to FLOREY¹² this compound is also characteristic of the nervous tissue. From data presented in the Table, it is further evident that the relative contents of glutamine, tyrosine and tyrosine phosphate are distinctly higher in the whole larva than in the brain. Our previous observations showed that glutamine derives largely from the haemolymph, whereas tyrosine and tyrosine phosphate are involved in the synthesis of cuticular proteins and the tanning reaction¹⁵. Thus, their unequal distribution appears understandable.

In order to test the synthesis activity of γ -aminobutyric acid in brain homogenates, the following in vitro studies were carried out. Brains of 25 fully grown larvae were dissected out in a drop of ice-cold Ringer's solution and homogenized in a small volume of sodium-potassium-phosphate buffer (0.067 M, pH 7.65). The homogenate was then diluted to 200 μ l with the phosphate buffer in a micropipette and transferred to an incubation tube. As

¹ P. S. CHEN and E. HADORN, *Revue suisse Zool.* 61, 437 (1954).

² P. S. CHEN and F. HANIMANN, *Z. Naturf.* 20b, 307 (1965).

³ P. S. CHEN and A. KÜHN, *Z. Naturf.* 11b, 305 (1956).

⁴ N. FRONTALI, *Nature* 191, 178 (1961).

⁵ N. FRONTALI, in *Comparative Neurochemistry* (Proc. 5th Int. Neurochem. Sym. 1962, 185, 1964).

⁶ H. H. TALLAN, in *Amino Acid Pools* (Ed. J. T. HOLDEN; Elsevier, Amsterdam 1962), p. 471.

⁷ E. ROBERTS and S. FRANKEL, *J. biol. Chem.* 187, 55 (1950).

⁸ E. ROBERTS and S. FRANKEL, *J. biol. Chem.* 188, 789 (1951).

⁹ E. ROBERTS, P. J. HARMAN and S. FRANKEL, *Proc. Soc. exp. Biol. Med.* 78, 799 (1951).

¹⁰ W. J. WINGO and J. AWAFARA, *J. biol. Chem.* 187, 267 (1950).

¹¹ B. SISKEN, K. SANO and E. ROBERTS, *J. biol. Chem.* 236, 503 (1961).

¹² E. FLOREY, in *Major Problems in Neuroendocrinology* (Eds. E. BAJUSZ and G. JASMIN; Karger, Basel 1964) p. 17.

¹³ K. KRNEVIC, *Endeavour* 25, 8 (1966).

¹⁴ P. S. CHEN and C. DIEM, *J. Insect Physiol.* 7, 289 (1961).

¹⁵ P. S. CHEN, *Adv. Insect Physiol.* 3, 53 (1966).