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 observed 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.

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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 vertebrates[6]. 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 previously[14]. 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 μM/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 FLOREY[12] 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 reaction[15]. 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 μl with the phosphate buffer in a micropipette and transferred to an incubation tube. As

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Relative concentration (% total) of free ninhydrin-positive components in the brain and the whole larva of *Drosophila* (M = mean, S = standard error, No = number of determinations).

Ninhydrin-positive components	Brain		Whole larva	
	No.	M ± S	No.	M ± S
α-Alanine	11	25.3 ± 0.73	8	20.7 ± 1.57
β-Alanine	–	–	8	1.9 ± 0.14
γ-Aminobutyric acid	11	2.6 ± 0.37	6	1.2 ± 0.16
Arginine	–	–	8	2.2 ± 0.30
Aspartic acid[a]	11	5.2 ± 0.54	8	1.5 ± 0.11
Asparagine	–	–	8	1.4 ± 0.32
Glutamic acid[a]	11	28.9 ± 0.67	8	11.8 ± 0.69
Glutamine[a]	11	11.1 ± 0.52	8	17.8 ± 0.77
Glycine	11	2.7 ± 0.25	8	3.3 ± 0.12
Histidine	–	–	8	1.6 ± 0.24
Leucine/isoleucine	8	2.1 ± 0.36	8	3.1 ± 0.19
Lysine	11	3.7 ± 0.39	8	5.4 ± 1.29
Phosphoethanolamine	11	2.7 ± 0.46	8	1.6 ± 0.20
Proline	2	0.7 ± 0.10	8	0.7 ± 0.06
Serine	11	3.3 ± 0.34	8	2.8 ± 0.11
Taurine[a]	10	1.8 ± 0.21	8	0.3 ± 0.04
Threonine	11	2.7 ± 0.20	8	3.6 ± 0.56
Tyrosine[a]	11	1.4 ± 0.22	8	4.2 ± 0.29
Tyrosine phosphate[a]	11	2.2 ± 0.27	8	9.4 ± 0.78
Valine/Methionine	10	2.0 ± 0.27	8	3.2 ± 0.31
Unknown	11	4.0 ± 0.49	8	2.8 ± 0.25

[a] Differences in the relative distribution of these compounds have been shown to be statistically significant at 1% level.

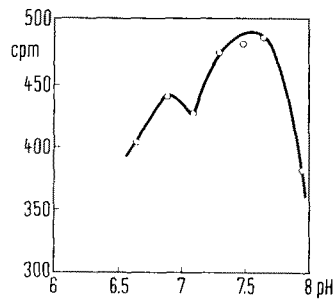

Fig. 1. Effect of pH on the synthesis of γ-aminobutyric acid. Ordinate: radioactivity in counts per minute (cpm) of γ-aminobutyric acid. Abscissa: pH of Na-K-phosphate buffer.

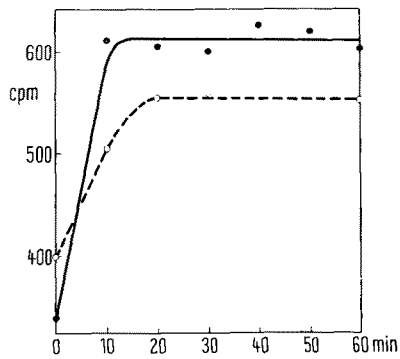

Fig. 2. Formation of γ-aminobutyric acid from C^{14}(U)-glutamic acid. Ordinate: increase in the radioactivity (cpm) of γ-aminobutyric acid. Abscissa: incubation time in minutes. The in vitro experiments were carried out at either pH 7.5 and 25°C (–o–) or pH 7.65 and 38°C (–•–).

coenzyme 10 µl of pyridoxal phosphate ($0.14 \times 10^{-3} M$) were added. After a few min of temperature equilibrium in the water bath, 10 µl of cold glutamic acid ($0.05 M$) and 2 µl of C^{14}(U)-glutamic acid (2.5 µc/µl, specific activity 130 mc/mM, the Radiochemical Centre, Amersham, England) were again added. The reacting mixture was kept at 25° or 38°C with constant shaking. At intervals of 10–30 min samples of 10 µl were drawn for the determination of γ-aminobutyric acid production. Each sample was deproteinized by adding 20 µl of absolute ethanol. Subsequent to centrifugation, 25 µl of the supernatant was mixed with 5 µl of authentic γ-aminobutyric acid ($0.004 M$) as carrier, and then applied to a Whatman No. 3 MM filter paper (37 × 41 cm).

The separation of the amino acids was done by either one-dimensional high voltage electrophoresis with 8% formic acid as the buffer (2300–2700 V), or a combination of electrophoresis and paper chromatography with 70% *n*-propanol as the running solvent. After developing the ninhydrin color at 60°C, the γ-aminobutyric acid spot on the paper was located, cut out and the radioactivity measured in a windowless gas flow counter (Nuclear Chicago).

For establishing the pH optimum of the enzyme activity, experiments were performed by incubating the brain homogenate in buffer solutions ranging from pH 6.65 to 7.95. The values of radioactivity in γ-aminobutyric acid after an incubation period of 10 min are plotted in Figure 1. It is clear that there are 2 activity maxima, one at about pH 6.9 and another one at about pH 7.65. The picture bears a remarkable similarity to that found for the brain of the honeybee by FRONTALI[5].

The average progressive curves for γ-aminobutyric acid synthesis at pH 7.5 and pH 7.65 from 4 parallel experiments are depicted in Figure 2. The increase in radioactivity of the reaction product is apparently very rapid and reaches a plateau already at 10–20 min after the beginning of incubation. The initial rate of synthesis at 38°C and pH 7.65 has been estimated to be 22.1 µM/g (wet weight)/h.

Since the only substrate used in our in vitro experiments was glutamic acid, it seems that, similar to that already reported for the mammalian brain[7–10], glutamic acid decarboxylase is involved in the enzyme reaction described here. However, based on her manometric measurements of CO_2 production FRONTALI[5] postulated the possible existence of an alternative mechanism of γ-aminobutyric acid formation. A more detailed analysis of purified enzyme preparations would be desirable to give a clear-cut answer. In any case, the present results demonstrated clearly that active synthesis of γ-aminobutyric acid takes place in the larval nervous system of *Drosophila*[16].

Zusammenfassung. Papierchromatographische Untersuchungen an verpuppungsreifen Larven von *Drosophila melanogaster* zeigten, dass der relative Gehalt an γ-Aminobuttersäure im Gehirn rund zweimal höher ist als in der ganzen Larve. Mittels Isotopentechnik wurde eine aktive Synthese der γ-Aminobuttersäure im Hirnhomogenat nachgewiesen.

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