

extensively to the phenomenon of increased neurosecretory substance. The question remains to be answered in how far subelectronmicroscopic particles are packaged within the distended neurotubules.

The current concept of the mechanism of protein synthesis<sup>18</sup> undoubtedly favors the concept of the existence of a biologically inactive precursor molecule of vasopressin<sup>8,9</sup> which is likely to be activated in the disconnected neurohypophysis by local enzymes which have been shown to increase considerably in the distal stump of transected axons<sup>19</sup>.

Incidentally, the high uptake of labeled substance in the intermediate lobe beautifully demonstrates the increased activity of this lobe following withdrawal of the inhibitory hypothalamic influence<sup>20</sup>.

**Zusammenfassung.** Nach Durchschneidung der proximalen Hypophyse wird im proximalen und distalen Stumpf der Neurohypophyse ein erhöhter Gehalt an neurosekretorischen Granula festgestellt. Die Tatsache, dass nur

proximal S<sup>35</sup>-L-Cystein-Hydrochlorid nachgewiesen werden kann, schliesst die immer wieder vorgebrachte Hypothese einer distalen Neurosekretsynthese endgültig aus.

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<sup>18</sup> E. BRESNICK and A. SCHWARTZ, *Functional Dynamics of the Cell* (Academic Press, New York and London 1968), p. 230.

<sup>19</sup> L. LUBINSKA, in *Progress in Brain Research* (Ed. M. SINGER and J. P. SCHADE; Elsevier, Amsterdam-London-New York 1964), vol. 12, p. 1.

<sup>20</sup> W. ETKIN, in *Neuroendocrinology* (Ed. L. MARTINI and W. F. GARNONG; Academic Press, New York 1966), p. 261.

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## Effect of Mitomycin-C on the Development of the Eye-Disks in *Drosophila melanogaster*

It is well known that mitomycin-C has a intensive effect, as well as a specific inhibition, on DNA formation in the bacteria, *Escherichia coli*<sup>1-4</sup>. Previously it has been found that mitomycin-C also inhibits the development of *Drosophila* larvae. Above all, the facet-formation of the compound eyes was markedly inhibited by the treatment of this chemical during their larval stage<sup>5</sup>.

Nevertheless, in our previous studies of the Bar eye in *Drosophila*, it was pointed out that acid amides have a strong action in accelerating the facet-formation of the mutant Bar eye. When these chemicals were administered, the Bar eye became larger than that of the wild type in extreme cases<sup>6-8</sup>.

In the present work, the autoradiographic analysis of the mechanism of the development in the Bar eye was carried out in respect to the nucleic acid metabolism.

The 60-h larvae were treated after hatching with the tracer (<sup>3</sup>H-acetamide 5.64 µC/ml or <sup>3</sup>H-thymidine 100 µC/ml) for 6-8 h with or without mitomycin-C (60 µg/g)

and then transferred to normal medium until the end of larval stage. The larvae were fixed in Carnoy, embedded in paraffin, sectioned at 3 µ, and then finally dipped in nuclear emulsion. Autoradiographic exposure for <sup>3</sup>H-acetamide was 7 days and that for <sup>3</sup>H-thymidine, 3 days. Number of grains were the counts/25 µ<sup>2</sup>, taken as a unit area of the tissue.

The results of <sup>3</sup>H-acetamide incorporation treated with or without mitomycin-C are summarized in Table I.

<sup>3</sup>H-acetamide incorporation was marked in those of the eye disks, of the fat bodies and of the salivary glands.

Table I. Results of autoradiographic grain counts for <sup>3</sup>H-acetamide incorporation treated with or without mitomycin-C

Treatment	Average grain No. <sup>a</sup> per 25 µ <sup>2</sup>		
	Eye disk	Fat body	Salivary gland
<sup>3</sup> H-acetamide (8 h)	30.3	27.3	24.0
<sup>3</sup> H-acetamide + mitomycin-C (8 h)	18.8	23.4	19.5
<sup>3</sup> H-acetamide → mitomycin-C (6 h)	17.1	18.9	17.1
Mitomycin-C → <sup>3</sup> H-acetamide (6 h)	15.4	16.3	15.3

<sup>a</sup> Mean of 15 samples.

<sup>1</sup> H. KERSTEN, *Biochim. biophys. Acta* 55, 558 (1962).

<sup>2</sup> I. MATSUMOTO, M. KOZAKA and Y. TAKAGI, *J. Biochem.* 60, 653 (1966).

<sup>3</sup> I. MATSUMOTO, *Kagaku-to-Seibutsu* 4, 368 (1966).

<sup>4</sup> V. N. IYER and W. SZYBALSKI, *Proc. natn. Acad. Sci. US* 45, 355 (1963).

<sup>5</sup> S. KAJI and Y. HIROSE, *Proc. Jap. Acad.* 44, 369 (1968).

<sup>6</sup> S. KAJI, *Annotnes zool. jap.* 27, 194 (1954).

<sup>7</sup> S. KAJI, *Annotnes zool. jap.* 28, 152 (1955).

<sup>8</sup> S. KAJI, *Mem. Kōnan Univ. Sci. Ser.* 4, 1 (1960).

Table II. Results of autoradiographic grain counts for <sup>3</sup>H-thymidine incorporation treated with or without mitomycin-C

Treatment	Average grain No. <sup>a</sup> per 25 µ <sup>2</sup>		
	Eye disk	Fat body	Salivary gland
<sup>3</sup> H-thymidine (8 h)	29.5	29.1	25.7
<sup>3</sup> H-thymidine + mitomycin-C (8 h)	18.7	16.8	15.4

<sup>a</sup> Mean of 15 samples.

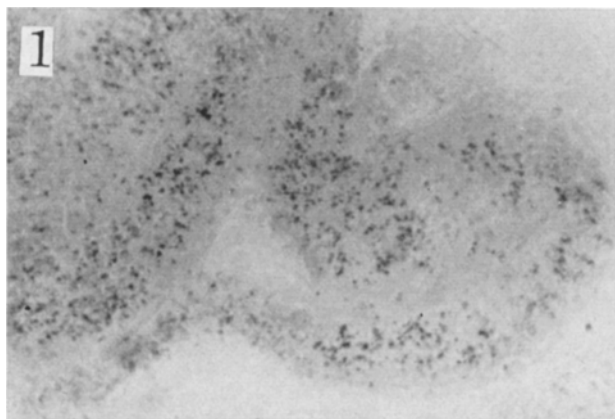


Fig. 1.  $^3\text{H}$ -acetamide incorporation into the Bar eye disk. The incorporation was distinct at the facet-forming region, located on the basal part of the disk.

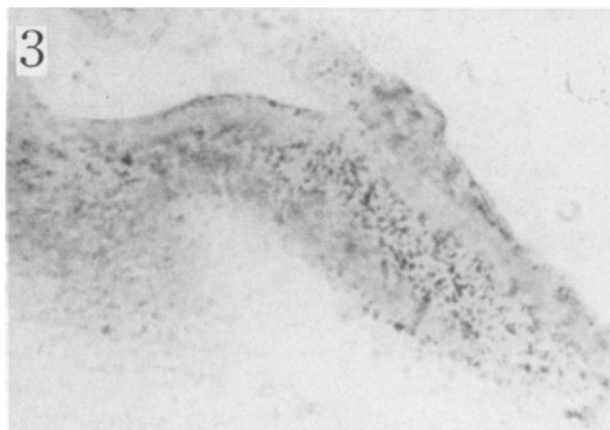


Fig. 3.  $^3\text{H}$ -thymidine incorporation into the Bar eye disk. The incorporation was marked at the facet-forming region.

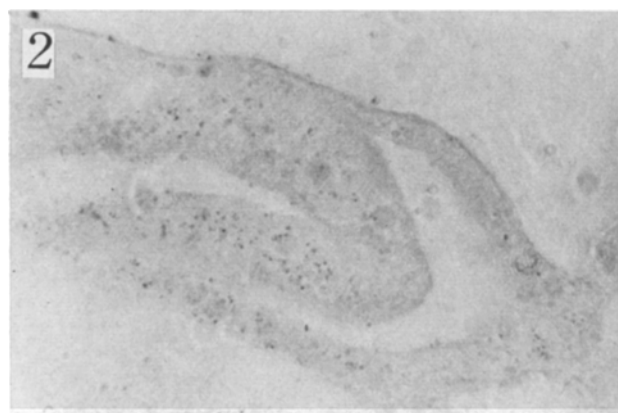


Fig. 2.  $^3\text{H}$ -acetamide incorporation into the Bar eye disk treated with mitomycin-C. The incorporation was markedly inhibited by the pre-treatment with mitomycin-C before labelling.

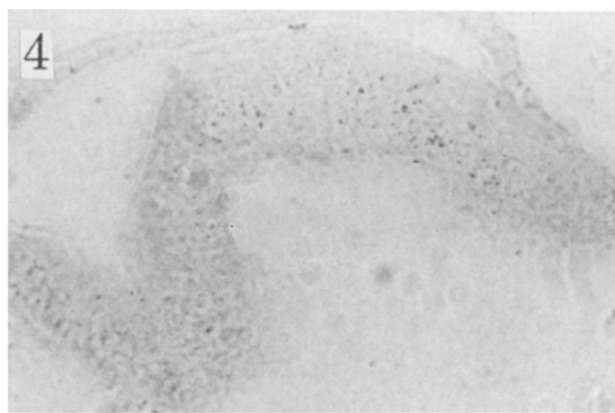


Fig. 4.  $^3\text{H}$ -thymidine incorporation into the Bar eye disk treated with mitomycin-C. The number of grains were decreased in those treated with  $^3\text{H}$ -thymidine-mitomycin-C mixture.

However, grains were decreased when they were treated with mitomycin-C. In the extreme cases, incorporation was markedly inhibited by the pre pretreatment with mitomycin-C before labelling with  $^3\text{H}$ -acetamide. Especially, in the eye disks, incorporation decreased to half the amount because of the inhibitory action of mitomycin-C. The labelling was observed in nuclei of all the tissues examined. In the eye disks, incorporation was distinct at the facet-forming region, which are located on the basal part of the disks. It was decreased, however, by the treatment of mitomycin-C (Figures 1 and 2). Similar results were observable in the  $^3\text{H}$ -thymidine incorporation (Table II).

The incorporation of  $^3\text{H}$ -thymidine into the tissues resembled that of  $^3\text{H}$ -acetamide. As observable in the eye disks, number of grains were marked at nuclei in the facet-forming region. However, grain number decreased in those treated with  $^3\text{H}$ -thymidine-mitomycin-C mixture (Figures 3 and 4).

Apparently,  $^3\text{H}$ -acetamide and  $^3\text{H}$ -thymidine were mainly incorporated into the nuclei of the tissues examined. Among the tissues, incorporation into the eye disks was distinct. It may be considered that acetamide acts on the cluster cells of the eye disks, which are the pre-

cursors of the facets. It could also be assumed that this effect promotes the differentiation of the cells in the Bar eye disks. However, such effect was inhibited when it was treated with mitomycin-C. Also, the consequences of incorporation of  $^3\text{H}$ -acetamide and  $^3\text{H}$ -thymidine into eye disks resemble each other under the influence of the mitomycin-C, which is an inhibitor of DNA formation. These results suggest that the action of acetamide has a connection with the nucleic acid metabolism in the development of the eye disks.

**Résumé.** L'incorporation de l'acétamide- $^3\text{H}$  et de la thymidine- $^3\text{H}$  aux disques de l'œil a été expérimentée chez *Drosophila melanogaster*. Ces 2 substances radioactives ont été nettement incorporées dans les disques, mais le traitement avec la mitomycine-C a fait décroître le nombre des grains.

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