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Incorporation of the tritiated acetamide into DNA in Drosophila melanogaster.

It was found that acid amides have a strong action in accelerating the facet-formation of the mutant Bar eye. After acetamide treatment, the Bar eye became larger than that of the wild type in extreme cases (Kaji, 1954, 1960). The tritiated acetamide was mainly incorporated into the nuclei in the facet-forming region of the Bar eye discs by means of autoradiographic analysis. The mode of incorporation of tritiated acetamide resembled that of tritiated thymidine. These results suggested that the action of acetamide has a relation to the metabolism of DNA synthesis in the development of the eye discs (Hirose and Kaji, 1968, 1969; Hirose, 1968).

In the present work attempts were made to study the incorporation of the tritiated acetamide into DNA of the Bar larvae. The 70 hour larvae after hatching were exposed to ^{3}H-acetamide (348 μC/ml) or ^{3}H-thymidine (333 μC/ml) for one hour, and then transferred to the condition medium (yeast 1.5%, molasses 17%, agar 1.5%) for growth until they reached the end of the larval stage. Thereafter, the materials were kept in 85% ethyl alcohol at -20°C. They were then transferred to a cold glass homogenizer and broken with cold 85% ethyl alcohol. The Schmidt-Thannhauser method (1945) was used to extract DNA from homogenized tissue.

In the process of extraction of DNA, homogenized extracts can be fractionated into the following fractions: alcohol soluble, hot alcohol·ether soluble, cold PCA soluble, RNA and DNA fractions. The radioactivity of these fractions was assayed by the liquid scintillation counter (Ten Nucleonics, GSL-163). C.P.M. of the fractions are given in Table 1.

Table 1. The radioactivity of the various fractions in the process of DNA extraction.

Fractions	^{3}H-acetamide		^{3}H-thymidine	
	C.P.M.	%	C.P.M.	%
Alcohol soluble	963,588	95.28	408.922	74.62
Hot alcohol·ether soluble	19,520	1.93	20,640	3.77
Cold PCA soluble	3,124	0.31	13,520	2.47
RNA	6,960	0.69	6,880	1.26
DNA	18,105	1.73	98,072	17.90

The ^{3}H-acetamide was apparently incorporated into DNA fraction. However, both of the ^{3}H-acetamide and ^{3}H-thymidine were marked incorporated into the alcohol soluble fraction. Incorporation into the other fractions was much less than that of the alcohol soluble fraction. Incorporation into DNA fraction was varied between the ^{3}H-acetamide and the ^{3}H-thymidine. In this respect, the ^{3}H-thymidine incorporation was 17.90%, while the ^{3}H-acetamide incorporation was only 1.73% of the total value of incorporated isotopes.

After purification of DNA, it was destroyed by 12 N PCA and was neutralized by KOH. Decomposed DNA was separated into base compositions by thin layer chromatography. The radioactivity of the base compositions, that is, thymine, adenine, cytosine and guanine, was determined by the liquid scintillation counter. The results are shown by C.P.M. in Table 2.

Table 2. The radioactivity of the base components of DNA.

Bases	^{3}H-acetamide		^{3}H-thymidine	
	C.P.M.	%	C.P.M.	%
Thymine	526	73.6	7196	96.2
Adenine	58	8.1	103	1.4
Cytosine	62	8.7	119	1.7
Guanine	68	9.5	49	0.6
(Background)	50		50	

As is apparent in the Table, the ^{3}H-acetamide was incorporated mainly into thymine among the base components of DNA. The mode of incorporation of ^{3}H-thymidine showed a similar tendency. These facts indicate that the acetamide has a close connection with the metabolism of DNA during larval development.

References: Kaji, S. 1954, Annot. Zool. Japon. 27:194-200; ____ 1960, Mem. Konan Univ., Sci. Ser. 4:1-17; DeMarinis, F. 1966 DIS 41:149-150; Hirose, Y. and S. Kaji 1968, Proc. Japan. Acad. 44:363-368; ____ 1969, Experientia 25:199-200; Hirose, Y. 1968, Mem. Konan Univ., Sci. Ser. 11:29-41; Schmidt, S. and S.J. Thannhauser 1945, J. Biol. Chem. 161:83.