Michinomae**M. and S. Kaji. Kyoto Prefectural University of Medicine, Kyoto, and Konan University, Kobe, Japan. The various lysosomal frequencies during the development of the Bar eye disc.

Fristrom (1969, 1972) has demonstrated that degenerating cells appear in the presumptive eye cells during the initial stage of development of the Bar eye disc. Previously, we reported that this cell death is associated with the presence of lysosomes (Kaji and Michinomae 1973; Michinomae and Kaji 1973). During the development of the Bar eye disc, different types of specific structures were observed by electron microscopy. These are simple vesicular figures, myelin figures, fragments of cell organelles each bound with a single membrane and lipid droplets. The presence of these specific structures are observed by acid phosphatase reactions by heavy deposition of osmium black. These acid phosphatase-positive and membrane-bound structures may be defined as lysosomes.

In this paper will be examined various lysosomal frequencies during the development of the Bar eye disc, asetamide-treated Bar eye disc and wild type disc. The lysosomal frequencies and their types are differenciated during the development of the eye disc. The developmental process of the eye disc was correlated with the changes in specific fine structures. In the Bar eye disc, the first visible signs of the specific structures appeared already in the 48 hour larvae after hatching as simple vesicular figures. In the 60 hour disc, there were found simple vesicular figures and myelin figures. Later in this stage, the simple vesicular figures were gradually decreased, accompanied by progress of the eye development. In the 70 hour disc, myelin figures and cell fragments were mainly detected. In the 85 hour disc lipid droplets were observed in addition to myelin figures and cell fragments. After puparium formation, most of the degenerating bodies were changed to lipid droplets. Acid phosphatase activity could be detected in the specific structures of the eye discs except in the case of simple vesicular figures. From this sequence of changes it is interpreted that these fine structures are lysosomes which are undergoing change from the primary (simple vesicular figure) to secondary (myelin figure and cell fragment) and derivative types (lipid droplet). The results of these observations are summarized in Table 1.

Table 1. Relative value of the various lysosomal frequencies of Bar, acetamide-treated Bar and wild type during the development of the eye discs.

Time after hatching	Strain	Primary lysosomes	Secondary lysosomes	Derivative lysosomes
	B	+	-	-
48 hours	BA*	+	-	-
	W	+	-	-
	B	+ +	+ +	-
60 hours	BA	+ +	+ +	-
	W	+ +	+ +	-
	B	+	+ + +	±
70 hours	BA	±	+	-
	W	-	+	-
	B	±	+ + + +	+
85 hours	BA	-	+	-
	W	-	+	-
	B	-	+ + + +	+ + + +
95 hours	BA	-	+	±
	W	-	+	±
	B	±	+	+ + + +
100 hours	BA	+	±	+
(pre-pupae)	W	+	+ +	+

B: Bar eye disc. BA: acetamide-treated Bar eye disc. W: wild type (Oregon-R) eye disc.

*The Bar larvae for the acetamide treatment, were reared in the normal medium from hatching to 42 hours and then transferred to 1.5% acetamide mixed medium for growth until they reached the specific stage.

As apparent in the Table, lysosomal precursor has appeared already in the 48 hour discs. However, in the case of the acetamide-treated Bar eye discs, relative values of various lysosomal frequencies were highly decreased during the development of 70 hour to pre-pupal stage. Moreover, in the case of the wild type discs, these frequencies were also less than that of the untreated Bar eye discs.

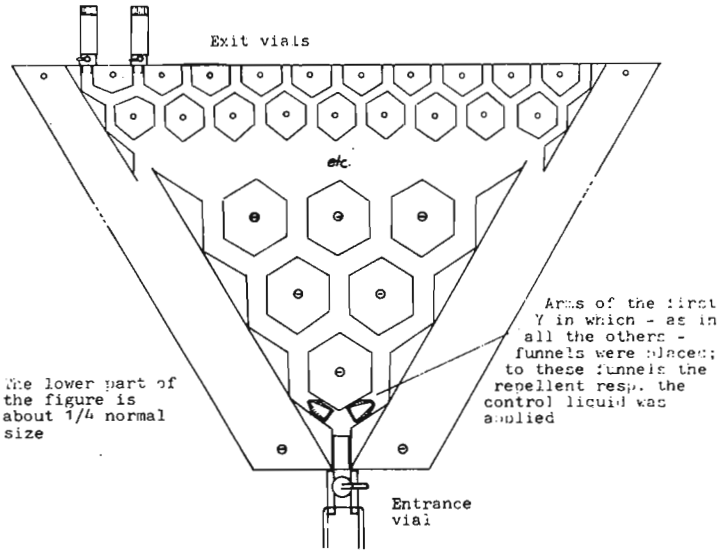
These results suggested that acetamide acts to inhibit the appearance of lysosomes on the metabolic process of the mutant Bar eye disc during development.

References: Fristrom, D. 1969, Molec. Gen. Genet. 103:488-491; _____ 1972, Molec. Gen. Genet. 115:10-18; Kaji, S. and M. Michinomae 1973, Genetics 74:Suppl. 22,2:130; Michinomae, M. and S. Kaji 1973, Japan. J. Genetics 48:297-300.

** will be on leave from Kyoto Prof. Univ. of Medicine to Konan Univ., April 1974.

Gerresheim, F. Universität München, Germany. An attempt to induce and select mutants with abnormal chemotactic behavior.

A search was made for mutants which do not show the avoidance reaction of normal flies toward an insect repellent, i.e. for mutants which behave indifferently or at least show a significantly lower sensitivity. Wild-type Berlin males were fed with EMS and crossed to attached-X females. F_1 males with abnormal behavior were selected and then, in order to determine whether an X-chromosomal mutation had actually been induced, their male progeny in the F_2 were tested.



In order to distinguish the abnormal flies from the normals, a Y-maze, described by Becker (M.G.G. 107:194-200, 1970), was used (Figure). In each of ten consecutive Y's the flies choose between, on one side, the repellent (primarily N,N-Diethyl-meta-toluamide) and, on the other side, a neutral control liquid. After ten choices, the exit vial a fly arrives at tells how often the repellent containing arms of the Y's have been avoided. And the mean of the distribution of a group of animals in the exit vials reflects the average orientation behavior of the group.

Large groups of F_1 males showed in general a strong asymmetric distribution. In 35 test series, out of 13500 F_1 males 60 deviating ones were singled out, i.e. ones that ended up in one of the central exit vials. From each of them an F_2 was raised. None of these showed a significant deviation from the controls. In 3 doubtful cases the test was repeated in the F_3. They, also, did not differ from the controls.

Of all the possible explanations for the lack of success two seem to be most prominent. (1) The success depends on the degree to which the distribution is asymmetric. Among my test series there were too many in which normal flies arrived at one of the central exit vials. Under such conditions the selection for possible mutants was not sufficiently effective. More important is probably (2) that the chemical sense in insects is generally based on a number of different sensory receptors, some being excitable by only specific substances, others being less specific. Correspondingly, some chemical compounds excite only a single receptor type, others excite several types or all of them. The former, specific type of compound is probably suitable for a study of the kind described; the repellent, however, probably belongs to the latter, non-specific type of compound. Although mutants insensitive to such non-specific compounds are conceivable, mutants insensitive to specific compounds seem more probable. - Investigations which take these considerations into account are under way.