Butterworth, F.M., H. Dene and D. Wachnicki, Oakland University, Rochester, Michigan. Acid phosphatase in the protein granules of the larval fat body.

Cells and tissues programmed to die during development undergo a preparative and lytic phase. The preparative phase is generally marked by an increase in the number of lysosomes and during the lytic phase the acid hydrolases contained in these bodies are released presumably causing the breakdown of the cells. Although there are many kinds of hydrolases in lysosomes (Barrett, 1969), the usual criterion for identifying lysosomes cytochemically is by the presence of acid phosphatase activity. In the following discussion it will be demonstrated that granules containing acid phosphatase activity are present in the larval fat body, and that the presence of these granules is correlated with the cell death of normal and mutant tissue.

Acid phosphatase was demonstrated cytologically by both the lead nitrate method of Gomori (1950) and the azo dye technique of Seligman and Mannheimer (1949). The lead nitrate method was modified from Gomori (1950, as prescribed by Pearse, 1968, P. 728) which utilized sodium glycerophosphate (Sigma) as the substrate, lead nitrate and acetate buffer at pH 5. The azo dye method of Seligman and Mannheimer (1949) used was modified (Seligman, personal communication). The reaction medium consisted of 50 mg sodium α-naphthol phosphate (Dajac Laboratories), 30 mg fast blue salt B (Matheson, Coleman and Bell), 90 ml of 4.0M sodium chloride, and 10 ml of 0.07M acetate buffer at pH 5.0. Both media were mixed just prior to each experiment, and the control media were identical in every way except the respective substrate was omitted. Although similar results were obtained from both procedures, the azo dye method was used predominantly.

Both methods were used on whole tissues or sections of paraffin embedded tissues. The whole tissues were either unfixed or fixed in acetone for 24 hours at 4°C. Material to be sectioned was fixed in acetone (as above), dehydration completed in several changes of acetone, cleared first in cedarwood oil followed by xylene, infiltrated in paraffin at 50-53°C for not more than 1 hour, embedded in paraffin, and sections were placed on slides without fixative. Incubation in the reaction media was at 37°C for 15-24 hours. Paraffin was removed from the sections just prior to mounting in balsam. Whole tissues following the reaction procedure were rinsed in distilled water and either mounted directly in glycerine jelly or dehydrated and embedded in paraffin for sectioning. Tissues were also prepared for general cytological examination according to Butterworth and Bodenstein (1967). The larvae, pupae and adults studied were reared under standard conditions (see Butterworth 1972).

In the normal development of the larval fat body cell, during the third instar, the cells achieve a 5-fold increase in cross-sectional area. Midway in the instar proteinaceous granules begin to form, increasing in size and number such that by the end of the instar they comprise 20% of the cytoplasm. During metamorphosis the cells separate and begin to histolyze. Most of the cells survive metamorphosis and have the same percentage of protein inclusions. However the cells have become smaller due to a loss of background cytoplasm. During the first two days of adult life the cells rapidly histolyze until, by the third day of adult life, none remain (Butterworth et al., 1965; Butterworth, 1972). In mutant, ap^4, the larval fat body which has protein granules in normal amounts histolyzes in the adult state at a much lower rate than normal (Butterworth and King, 1965; Butterworth, 1972).

Acid phosphatase activity was found in the protein granules of the fat body of wild type animals from the time that the granules were first detected in the mid-third instar larvae and throughout development as long as the cells were present. Acid phosphatase could not be demonstrated in cytoplasm at earlier stages although activity in small granules might not be resolvable in the light microscope. Individuals homozygous for ap^4 which could be detected as early as the second day of pupation also had acid phosphatase in the protein granules of the fat body for the remainder of metamorphosis and through the fifth day of adult life. Flies of this genotype rarely survive longer than five days. Nevertheless large numbers of their larval fat cells are present during that period; and the intensity of the acid phosphatase stain in the granules is similar throughout this period. Furthermore, the level of staining of enzyme in the granules of both ap^4 and wild-type flies is similar.

It is of interest to note that the fat body of Acph-1 null-activity mutants (Bell, et al. 1972), generously supplied by Dr. R.J. MacIntyre, possesses qualitatively normal numbers of protein granules; and that the cells of this tissue break down at the normal rate in the adult stage. Preliminary experiments with several alleles generally indicated no acid phosphatase activity in the granules. Occasionally activity was detected particularly in whole tissues. Presumably other genetically distinct acid phosphatases present are being inactivated by the

cytological processing. Clearly cell death in the fat body does not require acid phosphatase-1.

Acid phosphatase has been demonstrated in the protein granules of the larval fat body in other insect species (beetles: DeLoof, 1972; moths: Locke and Collins, 1968; muscid flies: Stay, 1959) but the function of acid phosphatase in the protein granules is not yet clear. Since the protein granules of the larval fat body of Drosophila contain acid phosphatase, and since Gaudecker (1963) finds the granules to be autophagic, it is reasonable to think of these granules as being lysosomal. Attempts to identify other acid hydrolases in the granules are underway.

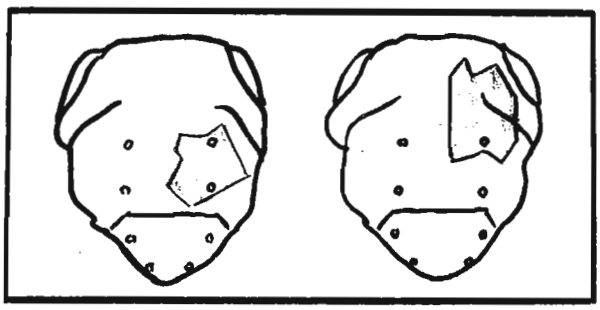
References: Barret, A.J. 1969 In: Lysosomes in Biology and Pathology, Vol. 2 (Ed. by Dingle and Fell) Chapt. 6, American Elsevier Co., N.Y.; Bell, J.B., R.J. MacIntyre and A.P. Olivieri 1972, Biochem. Gen. 6:205-216; Butterworth, F.M. 1972, Devel. Biol. 28:311-325; Butterworth, F.M. and D. Bodenstein 1967, J. Exp. Zool. 164:251-266; Butterworth, F.M. and R.C. King 1965, Genetics 52:1153-1174; Butterworth, F.M., D. Bodenstein and R.C. King 1965, J. Exp. Zool. 158:141-154; DeLoof, A. 1972, J. Insect Physiol. 18:1039-1047; Gaudecker, B.V. 1963, Z.F. Zellforsch. 61:56-95; Gomori, G. 1950, Stain Tech. 25:81; Locke, M. and J.V. Collins 1968, J. Cell Biol. 36:453-483; Pearse, A.G.E. 1961, Histochemistry, Theoretical and Applied, 2nd ed. Little, Brown & Co., Boston; Seligman, A.M. and L.H. Mannheimer 1949, J. Nat. Cancer Inst. 9:427-434; Stay, B. 1959, J. Morph. 105:457-494.

Behnia, A. and G. Koliantz. Teachers Training College, Tehran, Iran. The effect of uric acid on a wild-type strain of D. melanogaster.

In the summer of 1970, from a natural population of Azarbaijan province in Iran, a wild type strain, called Gayaneh, was isolated which showed a high viability, fecundity and also a longevity more than Java wild type (Mostashfi, P. and G. Koliantz, 1970, 1971). The strain was divided into three lines each of which was exposed to different genetical experiments.

Two-day old males of the second line, were let feed for 24 hours on Whatman filter paper which had been saturated with uric acid in distilled water. Then each male was made to mate with two virgin females in fresh Mostashfi medium. After 24 hours the males were discarded a and females remained to lay eggs for 48 hours. To obtain F_2, a mass mating method was used (Spencer 1947a, Tsuno 1970). In this experiment the evidence was saccharose solution with the same concentration as uric acid.

The effect of uric acid in 1, 3, 5 and 10 percent concentrations are summarized in Table 1.



% Concent.	Gener.	l	sl	qn	malform.	mosai.
1	F_1	1	2	7	-	-
	F_2	-	1	6	3	-
3	F_1	-	-	10	1	-
	F_2	-	2	8	3	-
5	F_1	2	-	8	8	-
	F_2	-	1	7	3	9
10	F_1	-	1	9	9	-
	F_2	5	3	2	13	-

The number of malformations in the F_1 increased with uric acid concentrations and reached its highest point in the F_2 of 10% concentration.

In 5% concentration, two gynandromorphs and nine cases of mosaism were observed, two of which are drawn diagramatically. Highest lethality in F_1 and F_2 was observed in 5% and 10% concentrations, respectively.

The results obtained by double bound (with saccharose solution), showed relatively lower malformations and lethal effects.

References: Godbole, N.N., R.M. Kothari and V.G. Vaidya 1971, DIS 46:116; Hochman, B. 1971, Genetics 67; Mostashfi, P. and G. Koliantz 1971, 3rd Iran. Cong. of Genet.