Bakula, M. Saint Louis University, Missouri. Beta-galactosidase activity in axenic and nonaxenic adults of D. melanogaster.

Beta-galactosidase activity of adult flies was measured by a method modified from Sellinger et al (1) using 5mM o-Nitrophenyl-β-D-galactoside (Sigma) as the substrate. In preliminary experiments (Figure I) the optimal buffer and pH were determined. Citrate-phosphate buffer (ionic strength 0.05) at pH 5.6 was chosen for all subsequent experiments on the basis of these results. An adult homogenate prepared as follows was used as the enzyme source. A number of flies sufficient to give a final concentration of 5 flies/0.5ml (minimum number necessary for a detectable reaction) were hand homogenized in cold 0.25M sucrose with added Triton X-100 (0.01%)(Rohm and Haas). Since beta-galactosidase is typically a lysosomal

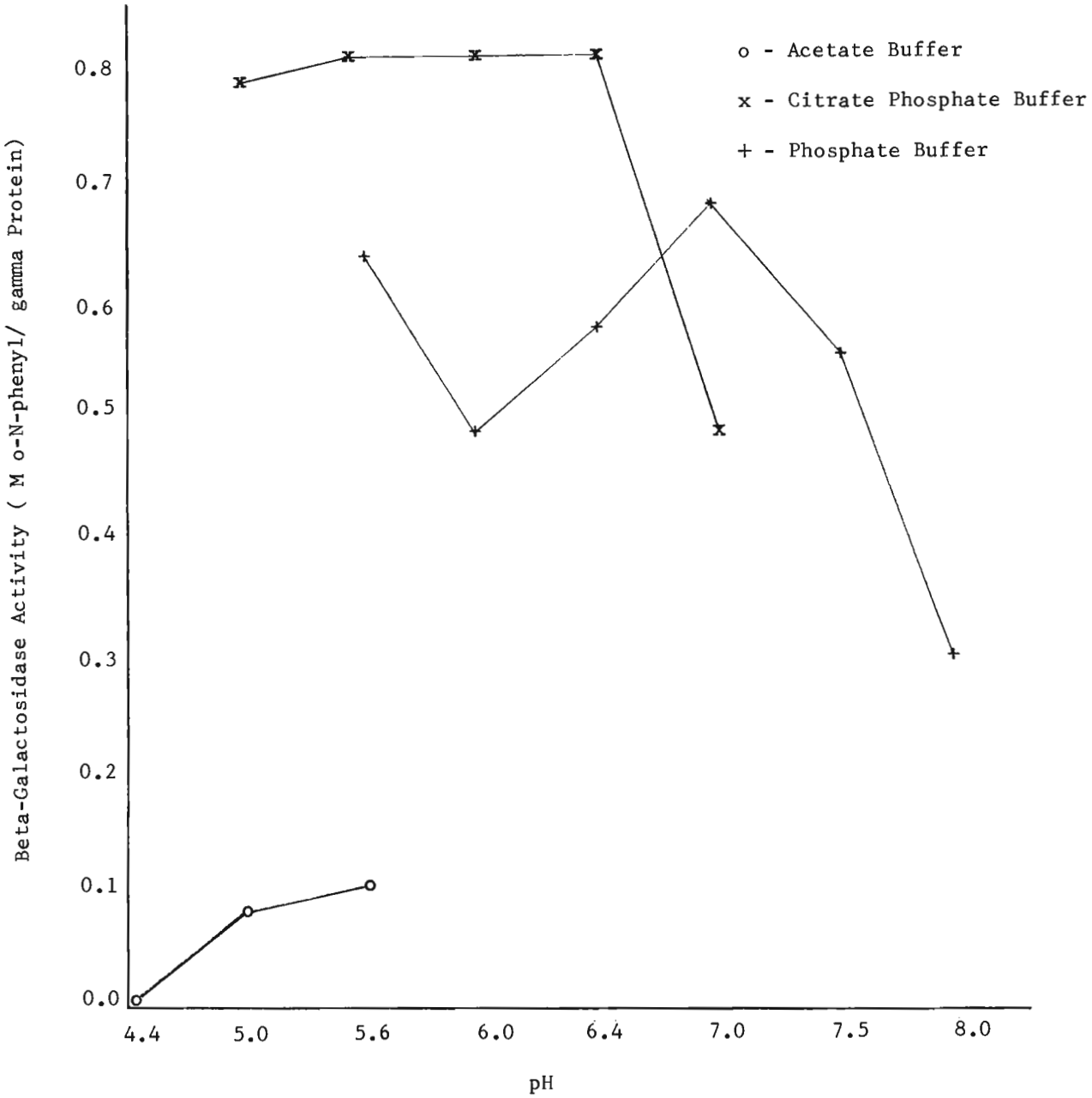

Figure I. Beta-galactosidase activity per gamma protein in the pH range 4.4-8.0. Three buffers (ionic strength 0.05) were employed as indicated. All reactions were carried out at 25°C for 2 hours.

enzyme in other animals the Triton X-100 was used to rupture the lysosomal membranes. The homogenate was centrifuged at high speed in a clinical centrifuge, the precipitate rehomogenized and centrifuged as before. After the final centrifugation the supernatants were combined, their volumes adjusted, and 0.5ml aliquots were pipetted into tubes containing 1.25ml buffer and 0.5ml substrate. The reaction was allowed to proceed for 2 hours at 25°C, and was stopped by plunging the tubes into an ice bath. The amount of o-nitrophenol liberated by the enzyme was measured colorimetrically at 420mu immediately after adding 0.5ml 1M NaOH to each tube. Protein determinations were performed according to the method of Lowry (2). The results of the assay were expressed as uM o-nitrophenol per gamma protein.

The enzyme determinations were run on non-axenic live yeast fed adults (P_1) and on 2 successive axenic generations of adults (P_2 and P_3) raised on sterile medium containing 0.5% Brewer's yeast, 1.5% agar and either 0.8% sucrose or 0.8% lactose. All tests were made on flies 2 to 5 days of age. In Table I the beta-galactosidase activities of the axenic lactose

Table I. Beta-galactosidase Activity of Non-Axenic and Axenic D. melanogaster Adults.

	Non-Axenic		Axenic				
			Lactose Fed		Sucrose Fed		
Generation	No. of Tests	Mean uM o-N-phenyl/ gamma protein	No. of Tests	Mean uM o-N-phenyl/ gamma protein	No. of Tests	Mean uM o-N-phenyl/ gamma protein	t
P_1	3	0.336					
P_2			4	0.14	4	0.12	o.490
P_3			5	0.73	5	0.47	4.19*

* Significant at 5% level

and sucrose raised are compared to each other. These results are not compared to the P_1 generation since it is probable that bacteris may be biasing the result by contributing to the total enzyme activity.

Beta-galactosidase levels were highest in the P_3 adults after an initial, though non-significant decrease. It appears that the lactose fed flies have the greatest enzyme activity.

References: Sellinger, O. Z., Beaufay, H., Jacques, P., Deyen, A. and deDuve, C. 1960. Biochem. J. 74: 450-456. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. J. Biol. Chem. 193: 265-275.

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Schalet, A., G. Lefevre and K. Singer. University of Connecticut, Storrs, Connecticut; San Fernando Valley State College, Northridge, California. Preliminary cytogenetic observations on the proximal euchromatic region of the X chromosome of D. melanogaster.

We have undertaken a cytogenetic investigation of deficiencies located in the proximal region of the X chromosome covered by the $y^+Y\ mal^+$ chromosome (Schalet and Finnerty, DIS 43: 128). Salivary analysis based upon at least 12 deficiencies of independent origin permits the following preliminary observations.

1) Cytological extent of the proximal X covered by $y^+Y\ mal^+$: From a left breakpoint in 18F through sections 19 and 20.

2) Location of visible loci: ot, 19A3-6; sw and mel, 19B3-19C2; mal, 19C4-19D3; lf, 19E5-6; unc, 19F1-2; su(f), to the right of 20A2 (probably to the right of 20A). The "mal" locus of Lifschytz and Falk, (see note of Schalet and Finnerty in this issue), defined by the overlapping deletions A118/Q539, 19E7 or immediately next to it.

3) Lethal loci in section 20: Lethal A7 has been localized to 20A1-2. Complementation tests have demonstrated at least 7 lethal loci between lethal A7 and su(f). Consequently, these 7 lethals and su(f) are located within bands generally considered to be truly chromocentral. Since su(f) is to the left of the proximal breakpoint of the sc^4 inversion, these results are in conflict with Cooper's assignment of 19F for that breakpoint.