ception of a line at 5876Å which was probably due to HeI.

The partial pressure of H_2 in the atmosphere is given as 0.5×10^{-6} atm (*3*). This is probably equilibrated by diffusion through the glass. Since a density as low as this probably could not be detected by our methods, it is likely that the hydrogen is present as a compound, possibly water vapor, which is decomposed by our discharge.

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11 December 1963

Xanthine Dehydrogenase: Differences in Activity among Drosophila Strains

Abstract. *In* Drosophila melanogaster, *mutants at two loci are known to lack detectable amounts of xanthine dehydrogenase activity. These are the maroon-like eye-color locus on the X chromosome and the rosy-eye-color locus on the third chromosome (52±). A survey was made of the xanthine dehydrogenase content of 98 wild-type strains of* D. melanogaster. *One strain with 25 percent of the xanthine dehydrogenase activity found in normal flies is described. Strains with high xanthine dehydrogenase activity have also been obtained by selection.*

Screening strains for differences in amounts of protein or enzyme activity, such as reported by Lewis (*1*), might result in a class of mutants involved in the regulation of protein synthesis in higher organisms. Xanthine dehydrogenase in the fruit fly, *Drosophila melanogaster,* has been of special interest because of the existence of two separate gene loci, maroon-like (*ma-1*) and rosy (*ry*), which mutate independently to produce eye-color mutants that lack detectable amounts of this enzyme. In order to obtain other mutants controlling the level of xanthine dehydrogenase activity, wild-type strains of diverse geographic origins were surveyed. In addition, some of these strains were crossed, and the progeny were selected for many generations for high or low xanthine dehydrogenase activity. This paper is a preliminary account of two strains obtained by these methods, one with low enzyme activity, and one with high activity.

Xanthine dehydrogenase was assayed by a modification of the fluorometric technique used for single flies (*2*). All preparative procedures were carried out at temperatures of 5°C or less. A single fly (or a pair of flies, as in the selection experiments) was homogenized in 4 ml of 0.1*M* tris buffer, *p*H 7.5, containing 5 mg of crystalline bovine serum albumin (Armour) per milliliter. Approximately 40 mg of Norite-A (Fisher) was added and the mixture was allowed to stand with occasional stirring for 1 hour. The solution was then centrifuged for 30 minutes at 30,000*g*. The supernatant was removed and filtered through a coarse sintered glass filter to remove the last remnants of the charcoal. One milliliter of the supernatant was placed into a fluorometer cuvette and 0.02 ml of 1×10^{-3} *M* methylene blue was added. The solution was allowed to stand for 4 minutes in a Thermolyne dry bath adjusted to 30°C, after which 0.01 ml of $6.67 \times 10^{-4}M$ 2-amino-4-hydroxypteridine was added. The solution was then mixed and the readings were taken at 2-minute intervals for 10 minutes in a No 540 Photovolt fluorometer equipped with a 347-mμ primary filter (Photovolt) and a 405-mμ secondary filter (Turner, No. 110-812). The fluorometer was initially adjusted so that a standard solution of $1.6 \times 10^{-6}M$ quinine in 0.1*M* sulfuric acid read 100 on the photometer scale. The high blank of the enzyme reaction mixture was suppressed by appropriate adjustment of the photometer. In all cases the enzyme assays were performed on randomly coded extracts. During the assay, the cuvettes were returned between readings to the dry bath. One unit of enzyme activity is defined as that amount of enzyme which converts 1 $\mu\mu$mole of 2-amino-4-hydroxypteridine to isoxanthopterin per minute. Under the conditions of the assay the rate of the reaction was linear for at least 30 minutes, and was proportional to enzyme concentration.

Figure 1 shows the distribution of the mean ($\bar{X}$) (three to four individual females assayed per strain) xanthine dehydrogenase activity in 98 wild-type strains of *D. melanogaster.*

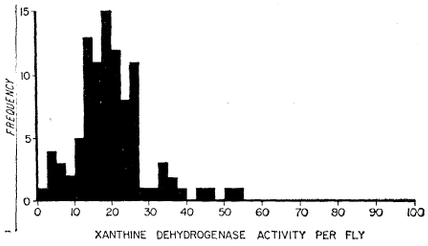

Fig. 1. Distribution of the mean activity of xanthine dehydrogenase in 98 wild type strains of *Drosophila melanogaster*. Three to four flies of each strain were assayed individually for xanthine dehydrogenase activity. The mean activity (units) per fly is plotted against the frequency of occurrence.

The mean enzyme activities of the 98 wild-type strains of *D. melanogaster* and their source of origin are as follows:

Amherst College: (1) Oregon-R^{368}, 19.1.
Cold Spring Harbor Laboratory: (2) Oregon-R, 16.0.
Institute of Animal Genetics: (3) Oregon-S, 16.3; (4) Pacific, 24.0.
Cancer Research Institute: (5) Amherst-3, 30.7; (6) Canton-SA, 18.2; (7) Crimea, 13.6; (8) Florida, 19.5; (9) Lansanne, 20.2; (10) Oregon-R, 18.9; (11) Oregon-RS, 11.6; (12) Samarkand, 26.0; (13) Seto, 34.4; (14) Swedish-b6, 20.5; (15) Urbana, 22.7; (16) Wageningen-A, 25.1.
Johns Hopkins University: (17) Oregon-RJ, 11.3.
Johns Innes Institute: (18) Hampton Hi U-2, 15.2; (19) Teddington-4, 23.1; (20) Samarkand-3, 14.9; (21) Bayfordbury-5, 13.3; (22) Oregon^{355}, 19.5; (23) Samarkand-8, 12.2; (24) Bayfordbury-1, 25.3; (25) Bayfordbury-B6, 28.2.
Oak Ridge National Laboratory: (26) Canton-S, 44.8; (27) Oregon-R, 13.6; (28) Oregon-RC, 20.6; (29) Samarkand, 26.2; (30) Swedish-C, 25.2.

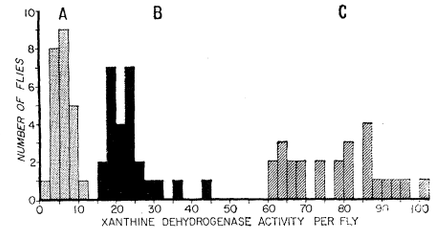

Fig. 2. The distribution of xanthine dehydrogenase activity in 25 flies from three strains of *Drosophila melanogaster*. (*A*) Strain with low enzyme activity (*lxd*). (*B*) Standard strain (Oregon-R wild-type). (*C*) Selected line with high enzyme activity. Twenty-five flies from each stock were assayed singly for xanthine dehydrogenase activity. The mean activity (per fly) is plotted against frequency.

Pennsylvania State University: (31) A2I[90]-18, 10.9; (32) A3I[81]-18, 8.8; (33) A3IV[160]-26, 6.1; (34) A3V[165]-26, 19.0; (35) A3IX[89]-18, 2.5; (36) A3X[89]-18, 15.1; (37) A6IV[134]-26, 5.3; (38) A6V[84]-18, 14.9; (39) A6V[133]-22, 3.7; (40) A6IX[88]-18, 18.4; (41) A6X[137]-22, 5.6; (42) A6X[106]-26, 3.8; (43) A17VI[156]-26, 9.9; (44) A17VIII[163]-26, 2.6; (45) A18X[123]-22, 23.4; (46) A21IV[161]-26, 3.1; (47) A-1, 19.4; (48) A-3, 22.0; (49) A-15, 22.4; (50) 115602, 20.1; (51) 115612, 16.1; (52) 933602, 19.4; (53) 933612, 21.0.

Radiation Research Unit, Harwell, England: (54) Nettlebed[307], 34.0; (55) Wild Edinburgh[327], 45.8; (56) Oregon-K, 21.3.

Rutgers University: (57) Z72, 26.1; (58) Z79, 37.6; (59) Z76, 22.2.

Stockholm University: (60) Oribron, 14.9; (61) Skafto, 15.1; (62) Tunnelgatan, 14.1; (63) Stäket, 16.5; (64) Karnäs, 17.9; (65) Djursholm[55], 15.1; (66) Algeria, 14.6.

University of Iowa: (67) Ames, 28.0; (68) Oregon, 19.8; (69) Florida, 21.5.

University of North Carolina: (70) Oregon-RNJ, 27.0.

University of Texas: (71) Austin, 13.4; (72) Canton-S, 26.4; (73) Espanola, 18.4.

University of Uppsala: (74) HiKone-R, 20.2; (75) Karnäs, 14.7; (76) San Mignel, 19.5; (77) Formosa, 17.4; (78) Samarkand, 14.7; (79) Gruta, 20.1; (80) Boa Esperanca, 19.9; (81) Curitibia, 23.4; (82) Stäket, 14.7; (83) Tunnelgatan, 25.1; (84) Oregon-R, 25.6.

University of the Witwatersand: (85) Limpopo River, 36.4; (86) Bloemfontein, 22.6; (87) Bethylie, 54.7; (88) Nelsprint, 50.9; (89) Cape Town, 17.9; (90) West Rand, 38.6; (91) Inhaca Island, 12.7; (92) Graff Reinet, 32.8; (93) Cedara, 14.0; (94) Stanfordlake, 25.4; (95) Stellenbosch, 15.7; (96) Zoutpansberg, 17.1.

Yonsei University: (97) Y•8, 17.0; (98) Y•D, 16.2.

The mean activities of these strains ranged from 2.5 to 54.7 enzyme units. Since environmental variation was at a minimum, it seems likely that most of these differences were due to genetic effects. Indeed, statistical analyses of the data indicated that over 80 percent of the total observed variation was due to genetic differences (*3*). In order to check this, two of the strains included in the distribution in Fig. 1 (one with low enzyme activity and one with modal activity) were analyzed further (Fig. 2, *A* and *B*). The strain with the lowest activity in Fig. 2*A* is an inbred strain derived at the Pennsylvania State University ($\bar{X} = 5.8$; $S_{\bar{x}} = 0.48$); the central distribution (Fig. 2*B*) represents the enzyme activity found in a strain of Oregon-R flies ($\bar{X} = 23.4$; $S_{\bar{x}} = 1.17$). Thus, the differences between the mean enzyme activity of these two strains (and their variances) are highly significant. Genetic analysis (*4*) has demonstrated that this difference is due to a recessive gene, called *lxd* (low xanthine dehydrogenase), located on the left end of the third chromosome near locus 33[±] and is therefore allelic neither to *ry* nor *ma-l* (*4*). This gene, when homozygous, limits the enzyme activity to about 25 percent of the average in the Oregon-R strain. Experiments are now in progress to ascertain the mechanism of action of this possible regulator locus.

The flies having very high enzyme activity (Fig. 2*C*) ($\bar{X}$=77.7; $S_{\bar{x}}$=2.39) are the result of a complex breeding and selection experiment (*3*). The parents of each generation were those progeny of the previous generation whose parents exhibited the highest xanthine dehydrogenase activity. The selection intensity was one pair in ten during the entire experiment (20 generations). The amount of inbreeding was changed in three distinct phases of the experiment. In the first six generations there was no inbreeding; from the 7th to the 14th generation inbreeding was gradually increased; at the 15th generation inbreeding was intensified by full sibling mating and continued to the 20th generation. An increase in enzyme activity occurred at two specific points: at the beginning of the experiment, presumably as a result of heterozygosis of the background genes (*5*), and at the 16th and 17th generations, where the genes responsible for this added increase were presumably fixed by intense inbreeding.

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Acceptance or Rejection of Male Skin by Isologous Female Mice: Effect of Injection of Sperm

Abstract. *Female C57B1/6J mice given one intraperitoneal injection of 1 to 8 million isologous epididymal sperm cells may exhibit either delayed or accelerated rejection of isologous male skin, depending on both the number of sperm cells injected and the time of application of the graft. A long time interval or a large dose of sperm cells results in maintenance of the male graft or delayed rejection, while a small dose and short time interval produces an accelerated rejection phenomenon.*

In inbred strains of mice, isografts of skin are accepted permanently with the sole exception that male skin grafts are rejected by the female (*1*). Furthermore, a female previously grafted with skin from an isologous male exhibits accelerated rejection of a second graft. This accelerated rejection is evidence of an immune response initiated by the first transplant. Rejection of male transplants by the female is attributed to a histocompatibility gene located on the Y chormosome (*2*).

Billingham (*3*) has induced 100 per-

Table 1. Skin isografts in male (*M*) and female (*F*) C57B1/6J mice.

Group	Graft	Treatment	Dose ($\times 10^6$ cells)	Time of grafting (days)	No. of animals	Rejection (%)	Median rejection time (days) and range	Termination (days)
1	M–M	None			16	0		60
2	F–F	None			15	0		60
3	F–M	None			15	0		60
4	M–F	None			15	100	28 ± 3	
5	M–F	Sperm	1	5 or 14	12	100	15 ± 3	
6	M–F	Sperm	2.5	5	16	100	16 ± 2	
7	M–F	Sperm	1.25	14	10	100	14 ± 2	
8	M–F	Sperm	4	5	8	100	27 ± 3	
9	M–F	Sperm	8	5 or 14	16	0		100
10	M–F	Sperm	1	21	4	0		80
11	M–F	Spleen cells	5.5	5	10	100	15 ± 3	