Courtright, James B. The Johns Hopkins University, Baltimore, Maryland. Alcohol dehydrogenases (ADH) and octanol dehydrogenases (ODH) in D. melanogaster: Immunochemical differences.

Two clusters of alcohol dehydrogenases, ADH and ODH, with different substrate specificities, have been separated by electrophoresis on agar gels (Ursprung and Leone, J. Exp. Zool. 160:147, 1965). Each group was found to be controlled by a single genetic locus. ADH maps at

2-50.1 (Grell et al., Science 149:80, 1964), ODH at 3-49.2 (Courtright et al., Genetics 54: 1966). It should be noted that both loci are within four Morgan units of the centromere on the respective chromosomes. It seemed possible therefore that the two gene-enzyme systems represented a case in which a locus became duplicated and subsequently translocated to a different chromosome. It was decided to investigate whether these two enzymes were immunochemically related.

Crude extracts of Drosophila melanogaster in 0.1 M Tris-HC1, pH 8.0, were adjusted to a protein concentration of 1 mg/ml, and a total of 2.5 mg were injected suprascapularly with 2.5 ml of Freund's complete adjuvant into each of two adult New Zealand white rabbits. Subsequent injections were carried out every 6-7 days with 2.5 mg protein and 2.5 ml of Freund's incomplete adjuvant. The rabbits were bled from the external marginal ear vein one week after the fourth injection. The serum collected was dialyzed against 18% and then 15% sodium sulfate and the final precipitate re-dissolved in  $\rm H_{2}O$  and dialyzed against 0.1 M NaAc pH 5.8. The resulting gamma globulin was dialyzed against 0.1 M Tris HC1 pH 8.0.

ADH and ODH were separated from one another as previously described (Courtright et al., 1.c.). Agarose (Nutritional Biochem.) was used for electrophoretic separation; on agarose gels, ADH migrates to the cathode, ODH to the anode.

Aliquots containing equal activities of ADH, ODH, or of a 1:1 mixture of ODH and ADH were applied to a circular well with a diameter of 4 mm in an agarose gel and electrophoresed for 10 minutes at 250 volts. Slits parallel to the direction of migration were then cut and filled with gamma globulin prepared from rabbits injected with the Drosophila extract. Double diffusion was allowed to proceed for 48 hours in the cold, at which time the gels were washed with several changes of Tris HCl, pH 9.0 for two days. The precipitin bands were stained directly for ADH and ODH activity with a solution of 5 mg Nitro Blue Tetrazolium (Dajac), 0.4 mg phenazine methosulfate, 50 mg NAD, 20 ml 0.5 M Tris-HCl, pH 9.0, and 0.5 ml of either ethanol or octanol. Bands of formazan deposition which coincided with the precipitin lines were found on the cathode side of the well but never on the anode side. No precipitin lines were detected when the well contained only ODH. The precipitin line patterns of both the ADH and the ADH-ODH mixture were identical. It is concluded that anti ADH antibodies do not precipitate ODH molecules under these conditions. Supported by NIH training grant GM-5708 and NSF grant GB-4451.

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In a study of total genetic damage induced in entire genomes of mature spermatozoa by 4000r of X-rays (Abstr. Eb-5, p. 85, Rad. Res. Soc., May 1967), translocations were collected in both X-bearing and Y-bearing genomes. Since the genomes

were collected in the daughters of the irradiated males (regular daughters for X-bearing genomes and exceptional daughters for Y-bearing genomes), and both X and Y were kept in the female until fertility could be determined, no translocations were lost due to male lethality or sterility. In 181 X-bearing genomes there were 6 T(X;2)s, 18 T(2;3)s and 1 T(2;4). In 129 Y-bearing genomes there were 6 T(Y;2)s, 9 T(Y;3)s, 1 T(Y;4), 11 T(2;3)s, 1 T(2;4) and 1 T(3;4). The frequency of T(A;A)s is 10.5% in X-bearing and 10.1% in Y-bearing genomes, but the frequency of T(X;A)s is 3.3% and that of T(Y;A)s is 12.4%. Thus T(Y;A)s were much more frequently recovered than T(X;A)s under exactly comparable circumstances. The lack of T(X;3)s is to be noted, however, and is unexplained, but even if there had been an equal number of T(X;3)s as of T(X;2)s, the frequency of T(X;A)s would still have been considerably less than that of T(Y;A)s. The greater recoverability of Y translocations might be ascribed to a higher frequency of induction due to the heterochromatic nature of the Y, but most likely it is due to a higher frequency of loss of X translocations.

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