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The effect of trypsin on "survivability" of imaginal disks of D. melanogaster.

In connection with the works concerning dissociation of imaginal disks subjected to the action of trypsin solution, it was of interest to discover "survivability" of these disks under conditions of different concentrations of trypsin solution. For this purpose leg imaginal disks were chosen from the larvae of D. melanogaster of the "Berlin wild" line at the age of 72 hours and were put into trypsin solutions of different dilutions. 0.25% trypsin solution was used as an initial material to prepare the working solutions of 1:1, 1:2, and 1:8 in physiologic Ringer's solution (NaCl 7.500 g, KCl 0.287 g, $CaCl_2 \cdot H_2O$ 0.287 g, distilled water to 1.0 liter). After 5 minutes' treatment of imaginal disks by trypsin solution at room temperature they were washed for one minute with 20% solution of bull serum prepared on the basis of Ringer's solution. Using the standard techniques of transplantation of imaginal disks (1), the latter were transplanted into larvae of the same age and line. In the control, selected disks of the same age were exposed for 5-10 minutes in Ringer's solution and then transplanted into the larval hosts.

For further analysis only those larvae were used that survived for 4 hours after transplantation, since the mortality in the first hours is presumed to be conditional on the imperfection of surgical techniques (2). The larval hosts with implanted disks were placed in tubes with the standard forage for drosophila (agar, raising, treacle) and kept at room temperature. Part of these larvae were allowed to pupate and imagos emerged. The adult flies were dissected and the presence of disk-implants was established. These disks were examined under a microscope to determine the elements of leg tissue. Results are given in Table 1.

Table 1. "Survivability" of leg imaginal disks after treatment with trypsin solution of different dilution.

Dilution of 0.025% trypsin solution	Number of surviving larvae	Number of adult flies (%)	Number of "survived" disks with regard to imago (%)	Number of "survived" disks with regard to larvae (%)
1:1	48	28.6	0.0	0.0
1:2	114	10.5	8.3	0.9
1:8	337	23.7	56.2	16.0
Control	791	40.0	55.0	22.3

This table shows that the increase of dilution of trypsin solution led to the increase of "survivability" of disks, both with regard to adult flies and with regard to surviving larvae; but at the same time this increase did not yield a concrete result in respect to the number of flies concerning surviving larvae. We are inclined to explain all this by unregistered technical conditions of operation rather than by peculiarities of the given trypsin dilution. In the selected disks we did not discover any significant qualitative difference (for example, appearance of allotypical elements) between the experimental and control groups.

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Manifestation of mutation singed on the homoeotic limbs, caused by the action of homoeotic mutations Nasobemia and aristapedia at different temperatures.

The mutation singed (ns,1-21.0) (twisted bristles) is manifested unequally on bristles of different sizes. Specifically, its expressivity is more marked on large bristles in comparison with small ones. As a result of the effects of some mutations on the homoeotic structures (1,2,

3,4) it was interesting to find out whether the feature of behavior of mutation sn on the homoeotic structures is retained at different temperatures. For this purpose homoeotic mutations Nasobemia (Ns,3-48.0) and aristapedia (ss^a,3-58.5) (its three alleles: ss^{ak}, ss^{ax} and ss^{a40a}) (transform antennae to the legs of mesothoracic type) were used. The number of large bristles was significantly more in mutants Ns. The double mutants sn;Ns and sn;ss^a obtained at 17, 25 and 28°C and single mutants sn, Ns and ss^a were studied by binocular microscope (increase 12.5 x 4.0). 100 individuals of every genotype were tested to estimate penetrance, 64 to estimate expressivity.

The effect of temperature on the degree of twisting of bristles in mutants sn was not detected. Twisting of bristles in mutants Ns and ss^a was not found at different temperatures. Twisting of bristles on thoracic legs and antennal homoeotic limbs was observed at the interaction of homoeotic mutations Ns and ss^a (its three alleles) with the mutation sn at all temperatures (16, 25 and 28°C). The complete penetrance of this effect was observed. The degree of its expression on homoeotic structures was identical to that on the corresponding segments of thoracic legs. In particular, twisting of bristles was significantly more marked in double mutants sn;Ns on the homoeotic structures than in mutants sn;ss^a. It can be explained by the predominance of large bristles on homoeotic limbs in mutants sn;Ns. The effect of temperature on the degree of twisting of bristles in double mutants was not found. The coincidence of expressivity of mutation sn on homoeotic structures with that on the corresponding segments of thoracic legs independent of temperature points out the morphogenetic relationship of elements (bristles) of homoeotic and normal structures.

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Kemphues, K.J. and T.C. Kaufman, Indiana University, Bloomington, Indiana. Two-dimensional gel analysis of total proteins from X/0, X/Y, X/Y/Y, X/Y^S and X/Y^L testes from D. melanogaster.

An attempt to identify proteins specific to the Y chromosome of D. melanogaster utilized the NEPHGE two-dimensional gel electrophoresis system of O'Farrell (1977), as modified by Waring (1978), to display total proteins extracted from testes. These gels offer the best one-step method for visualizing a large array of proteins. In these experiments, resolution of more than 300 spots from total proteins of [^{35}S] methionine labeled adult testes was possible.

Careful comparisons of autoradiographs of gels of labeled proteins from X/0, X/Y, X/Y/Y, X/Y^S and X/Y^L testes were made. These comparisons revealed no consistent differences in the two-dimensional pattern of proteins from testes of the various genotypes, other than an increase in the intensity of labeling of five spots from X/Y/Y testes relative to other genotypes. No protein differences, either qualitative or quantitative, were found between the autoradiographs of X/0 and X/Y testes.

One basic protein of 55,000 daltons, however, did fail to appear in gels of testes from two of the four X/0 stocks examined. A two-dimensional gel analysis of protein from X/Y testes at various developmental stages (24 hour intervals, starting at 72 hours after egg deposition) showed that the 55,000 dalton protein was not synthesized until late in development (220 hours; late pupa). The late synthesis of this protein suggests that some triggering event is necessary prior to synthesis. Because X/0 spermatids degenerate before maturation (Kiefer 1970), it is possible that the 55,000 dalton protein appears in some X/0 testes and not in others because this degeneration could begin either before or after this triggering event due to differences in genetic backgrounds or culture conditions.

These results indicate that if the Y chromosome fertility factors are expressed as proteins, the detection of these proteins will require more elaborate techniques than those described here. These experiments also demonstrate that the Y chromosome functions in a subtle manner which does not involve the regulation of synthesis of the major protein components of spermiogenesis. However, it may be possible that the Y specific proteins contain no methionine and that other labeling procedures will allow for their detection.

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