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A "Y" maze for Drosophila.

The apparatus was devised in this laboratory to investigate some behavioral preferences of D.m. It was constructed from three micro-sample containers sold by the Aloe Scientific Co., St. Louis, Missouri. The containers, Analocups™, (#V9327), measured approximately 25 mm in height and 11 mm in diameter with the base outside diameter slightly less than the inside diameter of the mouth. Thus the base of one cup could be fitted firmly into the mouth of another. The cups have conical bottoms,

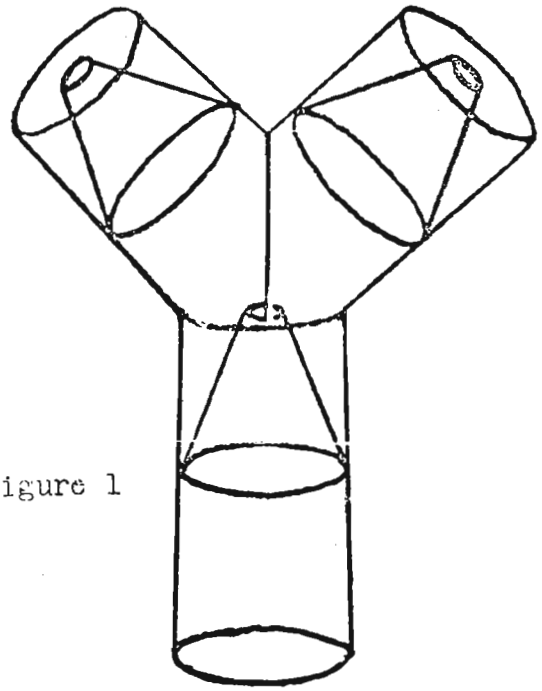

Figure 1

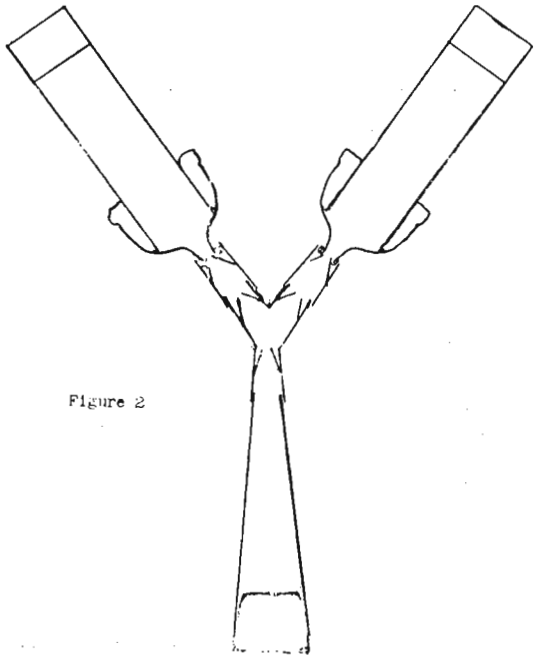

Figure 2

which when drilled with 2 mm openings formed one way traps. One cup with the base pointing upwards formed the stem of the maze, while two other cups formed the arms. The mouths of these latter cups were cut on one side at 45° angles to the axis and were glued together with acetate cement at their respective 45° angles to set the arms of the maze 90° apart. The other side of the mouth of each cup was sanded to allow proper seating on the base rim of the stem cup. A glass grinding wheel was used for the cutting because its fast, water cooled blade allowed a straight cut of the plastic cups without splintering, and the movable platform could be adjusted to cut the cups uniformly. Y mazes assembled in the above manner were consistently symetrical (Figure 1).

Flies were placed in a start chamber which was fitted into the stem of the Y maze and collected in glass shell vials (25 x 95 mm) connected to each arm of the maze. The start chamber was a section of a polypropylene cone (Poly Cone™, Cole-Parmer #36432) cut so that the small end of the cone would fit firmly into the stem of the Y maze. The floor of the large end of the cone was a rubber stopper. The conical nature of the chamber funneled the ascending flies into the maze. The collection vials, with 6 ml of food, were connected to the arms of the maze with an assembly of Analocups™, polyethylene thistle tube tops (Bel-Art, #H-14750) and polyurethane foam gaskets (Figure 2). The base of an Analocup™ was cemented on the the stem of a thistle tube top so that the assembly (vial, gasket, top) could be firmly connected to the arm of the maze. The assembled maze, start chamber, and collecting vials were supported by a test tube clamp inside a carboard box which, with fan and light, provided constant temperature and light.

Mazes with two color choices each were made by placing Contak™ self-sticking cellophane sleeves around the cylinder of each arm of the maze. The color choices were yellow-blue, green-blue, purple-red and orange-red. The mixed colors were made by combinations of the primary colors, yellow, red and blue. The color pairings were matched as closely as possible for light transmittance using a Science and Mechanics model A-3 light meter with Clairex™ CL-505L photo-conductive cell. The mazes could be oriented vertically or horizontally by

adjusting the test tube clamp.

The basic Y maze was also modified to provide a temperature choice apparatus by attaching 7 x 3.5 x 3 cm water compartments around each arm of the Y. A temperature difference in the two compartments was created by pumping water directly from constant temperature baths to each compartment.

Holmquist, G. University of Illinois, Urbana, Illinois. Removal of RNA from polytene chromosomes by lacto-aceto-orcein.

The lactic-acetic orcein (LAO)[1] squash method, as described by Nicoletti (DIS 33: 181), gives excellent band resolution of polytene chromosomes. After analyzing ^{3}H-RNA autoradiographs of salivary squashes which had been prepared by the Nicoletti method, it was found that LAO removes RNA from chromosomes. Since some workers have used LAO to stain RNA labelled material before autoradiography, the effects of this stain preparation on RNA should be understood.

Salivary glands were dissected into insect ringers solution containing ^{3}H-uridine (0.8 mC/ml, 24 C/mM) and incubated for ten min. before fixing in cold 45% acetic acid for 5 min. After fixation the glands were placed in LAO for 15 min. and squashed. The siliconized coverslip was removed by the dry ice method at specific times after squashing, the frozen slide with adhering material was immersed in 95% ethanol, rehydrated and autoradiographs were prepared and allowed to expose for two weeks. If the squashed preparation was allowed to differentiate in the stain for 2 days before coverslip removal, most of the radioactivity from ^{3}H-RNA was removed. After one day in stain, some RNA was removed and the remaining radioactive material was evident as a diffuse halo of silver grains surrounding each chromosome set. If the coverslip was removed immediately after squashing, most of the resulting silver grains appeared over the chromosomes. Thus, some component or combination of components in LAO appears responsible for the removal of RNA from chromosomes.

The following experiments were done in order to analyze the characteristics of the loss of chromosomal RNA. First the effect of 45% acetic acid on the molecular weight of newly formed RNA was analyzed, and this was followed by an examination of the effects of LAO and its components. To determine the effect of 45% acetic acid on chromosomal RNA, five gland pairs were incubated in ^{3}H-uridine as described above. One gland from each pair was fixed in alcoholic formalin and the RNA was extracted according to the SDS[2]-pronase and phenol method of Edstrom and Danholt (J. Mol. Biol. 28: 331-343, 1967). The remaining glands were fixed for 5 min. in cold 45% acetic acid, squashed, rinsed 5 min. in acetic acid and dehydrated in ethanol. The gland material on these slides was fixed in ethanolic formalin and digested from the slide in SDS-pronase solution. This digested material was extracted with phenol along with the digested material from the unsquashed glands and the RNA prepared by both methods was spun in a 5-20% sucrose gradient at 25,000 rpm in a SW 25.1 rotor for 6 hr. according to the conditions of Edstrom and Danholt. Recovery of labelled RNA was similar for both methods of extraction and radioactivity profiles from both extractions were indistinguishable with a peak at about 38s and material sedimenting as fast as 80s.

Since chromosomal RNA is not degraded or selectively removed by short treatments with cold 45% acetic acid, squashes prepared by this method were used as controls to test the effects of LAO components on squashed material. Salivary glands labelled with ^{3}H-uridine were squashed in cold 45% acetic acid, the coverslips were removed and different slides were subjected to the following conditions:
(1) 45% acetic acid at 3^{o}C for 5 min., (2) 45% acetic acid at 3^{o}C for 18 days, (3) 45% acetic acid plus 1% orcein at 3^{o}C for 18 days and (4) a 1:1 mixture of 85% lactic acid and glacial acetic acid for 3 days. The ^{3}H-RNA was retained as indicated by excellent autoradiographs with the first three conditions, but the fourth condition completely removed the chromosomal RNA. Apparently exposure of chromosomes to cold acetic or acetic-orcein solutions has no effect on RNA removal, while mixtures of lactic and glacial acetic acid do. Mixtures of lactic and glacial acetic acid should be avoided in studies of chromosomal RNA.

[1]LAO 2% by weight of powered orcein in equal parts of 85% lactic acid and glacial acetic acid. (Orcein Natural, G.T. Gurr Ltd., London, England.)

[2]SDS, sodium dodecyl sulfate.