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.