Kiriazis, W. C. and L. D. Friedman. Hiram College, Ohio. A modification in the technique for the paper chromatographic separation of pteridines.

In previous studies of separating pteridine substances in Drosophila melanogaster, workers have reported separating 10 substances by two-dimensional ascending chromatography (Narayawan and Weir, 1964, Genetics 50:387-392) and 17 substances by two-dimensional descending chromatography (Fox, 1956, Physiological Zoology 29: 288-298). These results were obtained through the use of the squashing technique in which approximately 10 to 15 heads or bodies of the flies were used. By using the whole fly, we have developed a method in which 10 to 23 spots, most of which are pteridines, can be separated in two-dimensional ascending chromatography. We are able to fractionate 200 Ore-R males and obtain 19 spots, and when 400 Ore-R males are used, 23 spots can be observed.

The two hundred whole flies were homogenized in 1 ml of two parts propyl alcohol to one part 1% ammonium hydroxide for thirty minutes in a Virtis 23 homogenizer. The homogenate was then centrifuged for thirty minutes at 4°C. The supernatant was removed and spotted on Whatman #1 chromatographic filter paper with a 1 µl pipette. Approximately 30 µl were used for the spot. The spot was allowed to dry, and the paper was then placed in a chromatographic jar (25 by 12) in a darkened room at 25 ± 1°C. The chromatogram was developed for 18 hours by ascending chromatography and dried at 25 ± 1°C in the dark. The solvent for the one-dimensional development was the standard two parts propyl alcohol to one part 1% ammonium hydroxide. For two-dimensional development, water-saturated collidine (2,4,6, trimethyl pyridine) in the ratio of three parts collidine to one hundred parts distilled water was used. The second development lasted ten hours. In both methods of development 700 ml of solvent was placed in the jar. The fluorescent pteridines on the chromatograms were then studied under an ultra-violet wavelength of 360 µm.

This modification of the standard method has two advantages. It separates substances which are in minute quantities in the fly, and it also produces a sufficient quantity of a particular pteridine in one spot that can be used for further quantitative analysis of the compound. By this method we have been able to separate four spots associated with drosopterin. In previous papers only three have been separated by ascending paper chromatography: drosopterin, isodrosopterin, and neodrosopterin. The fourth is of a darker red-orange fluorescence and has been confirmed by Gregg (personal communication) by descending chromatography and by Throckmorton (1962, The University of Texas Publication G205:415-487) by paper electrophoresis of the testes.

Slizynski, B. M. Edinburgh University, Scotland. Detaching of coverslips from albuminised slides.

When a squash preparation is made on an albuminised slide, the coverslip usually sticks at the edges of the preparation. It has been found that this can be avoided by making the albuminised area smaller than the coverslip. Albuminisation is done by a pipette: a drop of egg albumen solution is deposited on the slide (about the center) and dried up under cover. The best results (no protruding edges of the albumen) are obtained using a 1:200 solution of egg albumen in distilled water. The coverslip then detaches readily from the slide in 50-60% alcohol.

Sang, J. H. Sussex University, Brighton, England. Clearing Drosophila adults.

Scoring the numbers of melanotic tumors in Drosophila adults by dissecting them out is extremely laborious, and not 100 per cent accurate. On the other hand, the usual clearing procedures involve a complex routine, which is necessary for preserving the tissues but unnecessary for exposing these tumors. A 150 per cent solution of fructose (W/V), with a crystal of thymol per 25 ml. as antiseptic, proved ideal for the purpose, and flies immersed in the solution were completely cleared in 36-48 hours at room temperature. The solution can be used for other purposes where integrity of the tissues is not essential.