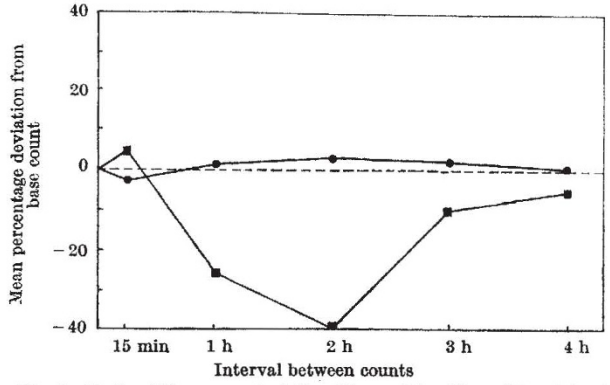

Fig. 1. Eosinophil response to tail cutting and handling of two inbred strains of mice. ■, *C57BL*; ●, *C3H/2*

of eosinophils per 32 mm² of the Fuchs–Rosenthal counting chamber was recorded for each animal according to the procedure of Speirs and Meyer[3].

Mean percentage deviation from baseline measures for each strain and each testing period is given in Fig. 1. An analysis of variance of these deviations indicates that the *C57BL/Crgl* strain responds to tail cutting and handling with significant changes in blood eosinophils ($P < 0.01$), with the greatest fall occurring 2 h after the initial count and recovery almost complete by 4 h. The *C3H/Crgl/2* strain, during this same 4 h period, shows no significant variation in eosinophils.

Clearly, the *C57BL/Crgl* strain shows significant eosinopenia at 2 h and is more responsive to tail cutting and handling than is the *C3H/Crgl/2* strain. Thus, the earlier failure to corroborate the findings of Wragg and Speirs[2], of eosinopenia at 3·5 h after the initial blood count, would seem to be due to the fact that the *C57BL/Crgl* subline completes the eosinophil cycle more quickly than the *C57BR/cd* subline investigated by these other workers. Presumably, genetic separation accounts for these differences in sensitivity to experimental manipulations. Historically, these sublines have been separated for many generations[4]: the first generation separation of *C57BL* and *C57BR* occurred about 1920. Ample time has elapsed between separation of lines for systematic genetic differences to appear.

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MISCELLANEOUS

Thin-layer Chromatography of Relatively Voluminous Samples

The principle of this method is that a relatively voluminous sample (50–100 μl.) is easily applied over a large area of chromatographic substrate and can then be swept into a narrow band by an advancing solvent front. This narrow band is used as the starting line for conventional thin-layer chromatography. The width of the band compares favourably with the diameter of spots applied by the time-consuming, conventional method of repeated application of microlitre amounts. We have used the method for the chromatography of amino-acids in aqueous solvents on 250-μ thick layers of both silica and cellulose, and the method should have general applicability.

The sample is liberally applied in a continuous streak over about 3–10 cm² of the starting region of the thin layer. The solvent is quickly evaporated with the help of a hair-drier (cold air only) to prevent appreciable spreading and particularly to ensure that the sample does not reach that part of the thin layer which will be dipped into solvent in ascending chromatography. A solvent is chosen which can be readily evaporated and in which all components of the sample have an R_F value near unity. This solvent is allowed to ascend through the region over which the sample has been applied, and thereby sweeps the sample forward at the solvent front. The usual techniques and precautions of ascending thin-layer chromatography are used. After about 1 min the solvent front has risen above the region over which the sample was applied. The plate is lifted out and quickly dried with a hair-drier. The sample has now been concentrated into a thin starting line. A second sweep with this solvent is similarly made to ensure that no material is left behind and the sample is then ready for chromatography by the usual methods.

Good starting lines 3–20 cm long have been made by this method, both on silica gel and on cellulose. One large plate may be divided into several independent chromatographic lanes by scribing 1–2 mm wide lines clear of substrate material in the direction of chromatographic development. The sample should be applied as evenly as possible and it is important not to damage the chromatographic substrate layer during application. Although one may apply numerous confluent spots with a micropipette the following method is better and faster. A length of disposable inert surgical tubing of about 1 mm internal diameter is attached to a Hamilton 100 μl. syringe (Hamilton Co., Inc., Whittier, California). The sample is drawn into the surgical tubing by means of the syringe, and can then be expelled in a smooth, gentle flow over the thin-layer plate. As liquid does not enter the syringe it can be used repeatedly with new lengths of tubing. The connexion between the syringe and tubing is made by a small rubber stopper. One end of the stopper is drilled to fit firmly over the syringe nozzle. The surgical tubing fits snugly into a coaxial hole in the other end of the stopper. Both 'Kel-*F*' and polyethylene tubing have been used, and insertion into the rubber stopper is facilitated by cutting the tubing at an angle of 45°. With a trace of paraffin lubricant on the plunger of the syringe, a precision of better than 1 per cent of sample volume was found routinely, without any special precaution. Glass melting point tubes could be used in place of the surgical tubing.

Bush[1] originated the 'running up' technique for paper chromatography of steroids in volatile organic solvents. The applicability of this technique to thin-layer chromatography appears to have gone unnoticed: due to the thinness of the chromatographic layer much less volatile solvents, and in particular water, can also be used. The method of handling small volumes of fluid has been adapted from Yphantis[2]. We thank Dr. K. Lunan for introducing us to thin-layer chromatography, and the U.S. Public Health Service for financial support (grant *GM*–06965).

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