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DISCUSSION

POINTS TO BE CONSIDERED IN RUNNING CHROMATOGRAMS OF TISSUE EXTRACTS

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Figure 1 illustrates some of the practical points which must be considered when chromatograms of tissue extracts are run in phenol-HCl.

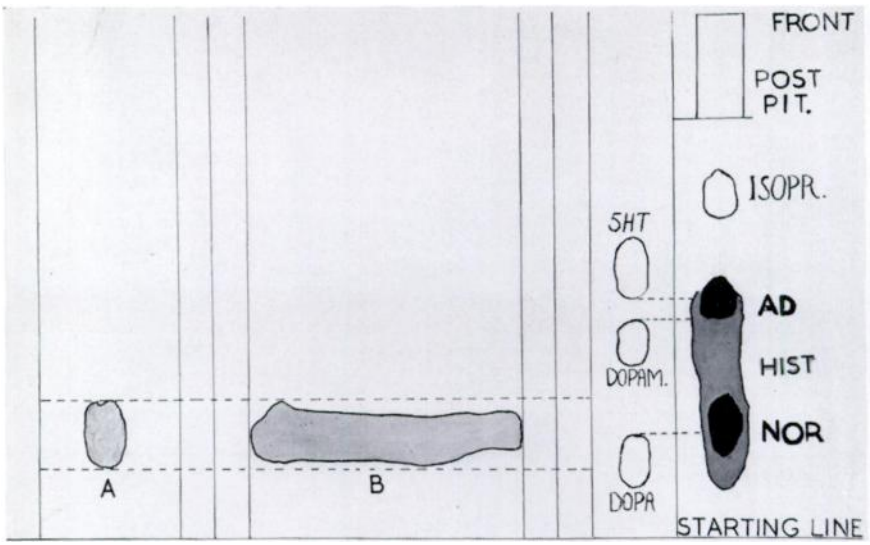

FIG. 1. Chromatograms of tissue extracts run in phenol-HCl.

A, on the left, is a control spot of norepinephrine, visualized by spraying the paper with a solution of ferricyanide. In section B, a brain extract to which norepinephrine had been added was applied to the base line as a horizontal band; after the chromatogram was developed and the paper sprayed the norepinephrine was found to be in precisely the region of the norepinephrine marker; thus the constituents of the brain extract did not modify the R_f value of the amine. The column on the right shows that the separation of norepinephrine, epinephrine, isoprenaline and the hormones of the posterior lobe of the pituitary is quite adequate in this system. Histamine, however, if present, would contaminate the lower part of the paper. The figure also shows that, in this solvent mixture, dopa is not satisfactorily separated from norepinephrine, and that the dopamine spot adjoins the epinephrine spot. When estimations are made by bioassay this does not create any difficulties, provided the quantities of dopa and dopamine present do not exceed those of norepinephrine and epinephrine by a factor of 100 or more: dopa has a biological activity only of about 1/100000 that of norepinephrine on the rat blood pressure, and dopamine has about 1/5000 of the activity of epinephrine on the rat uterus. On the pithed rat blood pressure, dopamine has approximately 1/150 of the activity of norepinephrine.

The separation of 5-hydroxytryptamine (5-HT) from epinephrine is not possible in this solvent system. If present in sufficient quantities, as for example in rabbit blood, it will interfere with the bioassay on the rat uterus by antagonising the action of epinephrine, and with the bioassay on the pithed rat blood pressure by mimicking the action of the epinephrine, although its pressor activity is much weaker. Differentiation between the two substances can, however, be achieved by the use of antagonists. Thus an amount of lysergic acid diethylamide, giving a bath concentration of 2.5 to 5×10^{-7}, may be added for 10 min

to the Ringer solution in which the uterus is suspended. To obtain reliable quantitative assays, however, it is desirable first to estimate the 5-HT concentration in the samples so that an equivalent amount of 5-HT can be added to the epinephrine standards.

When acetylcholine is the interfering agent, the uterus has to be treated with atropine for the assay of epinephrine. Stimulation of the uterus by carbaminoylcholine, as normally employed, has then to be replaced by stimulation with some atropine-insensitive agent; oxytocin, as discovered by Muscholl (to be published), is suitable for the purpose.

Isoprenaline is extremely active on the rat uterus, which it inhibits, and on the rat auricles, which it stimulates. In a small organ bath (2 to 6 ml), doses of 0.1 ng will produce an effect. The fact that it does not raise the blood pressure of the pithed rat distinguishes it from the other catecholamines.