n-butanol saturated with aqueous acetic acid solution, and containing 1 or 2 moles of acetic acid per litre of the phase) may be removed afterwards, together with much of the aqueous phase, by exposing the paper sheets to the air for many hours or by drawing over them in a drying cabinet a current of warm air (up to 60° C.) for an hour or so. We prefer to remove most of the volatile material by a brief run in the cabinet, and complete the removal by exposing the sheets to air at room temperature for several hours.

We spray the dried sheets with a suitable *p*H indicator solution and then re-dry them. A solution of 40 mgm. of brom-phenol-blue (or of brom-cresol-green) in a mixture of 95 ml. of ethanol and 5 ml. of water and adjusted to a purple tint corresponding with *p*H 5·0 in water (or blue with the second indicator, corresponding with *p*H 5·5 in water) is satisfactory for most purposes. The positions of the individual acids are then revealed by yellow spots on a purplish-blue (or greenish-blue with the second indicator) ground.

The spots have slight tails but are reasonably well defined. The R_F values (that is, the ratios of the excursions of the spots from the initial positions, to that of the front of the mobile phase from the same positions) appear to decrease slightly with decrease of concentration of the acids.

By excising the areas of paper enclosing the spots, steeping in water and titrating with dilute alkali between selected *p*H values, we have obtained rough estimates of the quantities of the acids in the solutions initially applied. Blanks are determined on excised areas of paper (over which the mobile phase has run) which are free from the spots.

The aqueous phase is established in the paper by exposing it in the chromatography tank to vapour from the aqueous volatile acid solution which has been used to saturate the mobile phase. The mobile phase is run over the paper from a trough in the manner described by Consden, Gordon and Martin[1] (that is, by capillarity). We have used Whatman No. 1 filter papers (22 in. × 18 in.). The volumes of acid solutions applied as initial spots have been 0·02 ml. Mixtures of 20 μgm., each of malic, tartaric and citric acids, have been resolved with ease. The period of a chromatographic run has varied from 18 to 40 hours, at about 17° C.

In our view, the essential chromatography problem was to suppress the ionizations of the acids in both phases, particularly in the fixed phase wherein (water comprising most of the phase) they would be extensive at low concentrations in the absence of a high concentration of hydrogen ions from another source. Whether adsorption of the acids by the paper constituted a serious problem in our initial experiments, we do not know. This problem, to whatever extent it may have existed, was probably solved jointly with the ionization problem, at least to the present state of satisfaction, by 'swamping' with the volatile acid.

A limitation to this application of partition chromatography is imposed by the fact that the acids concerned in the study should be of sufficiently low volatility to remain on the paper during exposure in the chromatography tank and during the subsequent removal of the volatile acid.

Work is proceeding with organic acids of general interest in plant and animal metabolism, on the use of other mobile phase solvents and formic acid as the volatile acid, and on 'two-dimension' partition-chromatographic[1] separations. It is our intention to publish a more detailed report of the work later.

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[1] Consden, Gordon and Martin, *Biochem. J.*, **38**, 224 (1944).
[2] Partridge, *Nature*, **158**, 270 (1946).

Mercurochrome as an Indicator in Acid–Alkali Titrations

It has been found that, in an alkaline solution, mercurochrome (disodium-dibromo-hydroxy mercury fluorescein) exhibits fluorescence which disappears as soon as the solution reaches the neutralization point during its titration with an acid. It then becomes markedly red as the solution becomes acid, there being now no fluorescence.

This change was equally clearly marked in the case of natural waters even with low alkalinity, and the results were identical with those obtained when methyl orange was used as an indicator. When solutions of potassium hydroxide, sodium carbonate or sodium bicarbonate were titrated against a standard acid, the use of mercurochrome as an indicator gave exactly the same results as methyl orange.

Even when the titration of sodium carbonate with an acid was begun with phenolphthalein as an indicator and completed with mercurochrome, instead of methyl orange, the results were the same.

The same change in the reverse order was observed with equal exactness while passing from an acid solution, through the neutralization point to alkaline solution.

One drop of a 1 per cent aqueous solution of mercurochrome was employed throughout.

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A New Colour Reaction for the D Vitamins

While making a comparative study of the colour reaction of steroids and triterpenes, we have found a hitherto undescribed reaction produced by the D vitamins, producing a clear and limpid blue colour which we think might be used for quantitative estimations. We carry out Pettenkofer's reaction for the biliary acids as follows: to 1–2 mgm. calciferol is added 1–2 mgm. saccharose, and the whole is dissolved completely in 1–2 c.c. of absolute ethanol. Then we add two drops of concentrated sulphuric acid, giving a red colour; then six drops of sulphuric acid are added, giving a stark blue colour. The test-tube is shaken to mix the two layers. The colour diffuses throughout the liquid, and the definitive colour is a pale limpid blue.

The intensity of the colour increases within the first fifteen minutes; thereafter it becomes stabilized as a sky-blue colour. The coloration obtained seems to be in a direct relation to the concentration, that is, according to Beer's law.

Ergosterin in similar conditions gives a clear violet colour.

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