

sensitivity of ferricyanide had already been recorded before, carried out some interesting experiments on this subject. Thus, on treating methaemoglobin in the dark with a solution of ferricyanide previously exposed to light, Bock's 'photomethaemoglobin' was immediately obtained, and was indistinguishable from cyanmethaemoglobin formed by treating methaemoglobin with cyanide. Haldane's conclusion as to the nature of 'photomethaemoglobin' was corroborated by Zeynek<sup>5</sup>.

On several occasions in the course of recent experiments, I have observed the formation of the cyanferriperoxidase complex during spectroscopic examinations of samples of pure peroxidase treated with ferri- or ferro-cyanide solutions freshly prepared, but not protected from light. These experiments suggest that if a haemoprotein compound, treated with ferri- or ferro-cyanide solutions, shows certain changes in its absorption spectrum, or in some of its other properties, which cannot be accounted for by simple oxidation or reduction reactions, it is advisable to compare the derivative thus obtained with the same haemoprotein solution treated with different concentrations of cyanide.

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<sup>1</sup> Baudisch, O., and Bass, L. W., *Ber. deutsch. chem. Ges.*, **55**, 2698 (1922).

<sup>2</sup> Bock, J., *Skand. Arch. Physiol.*, **6**, 299 (1895).

<sup>3</sup> Haldane, J., *J. Physiol.*, **25**, 230 (1900).

<sup>4</sup> Kobert, R., *Maly's Jahresh.*, 443 (1891).

<sup>5</sup> Zeynek, R., *Hoppe-Seyl. Z.*, **33**, 426 (1901).

### A New Technique of Chromatography and Ionophoresis on Ion-exchange Paper. Application to Separation of Barbiturate, Salicylate, Acetophenetidin and *p*-Acetylaminophenol

In a previous communication, we<sup>1</sup> showed that a mixture of salicylate, barbiturate, and acetophenetidin could be separated by a combination of chromatography and ionophoresis on a sheet of cellulosic anion-exchanger.

According to Stewart and Stolman<sup>2</sup>, a major metabolite of acetophenetidin in the human body is *p*-acetylaminophenol. When *p*-acetylaminophenol is subjected to chromatography on Whatman DE20 ion-exchange paper, it moves at approximately the same speed as phenobarbitone, so that a mixture of these two compounds is not resolved. However, if the paper is now subjected to ionophoresis, the *p*-acetylaminophenol moves more slowly than the barbiturate. This means that, by using our new technique<sup>1</sup> of chromatography and ionophoresis on ion-exchange paper, it is possible to obtain complete resolution of a mixture of salicylate, phenobarbitone, acetophenetidin and *p*-acetylaminophenol. The results are illustrated in Fig. 1.

It also has been observed that when the wet sheet (following ionophoresis) is viewed in the light from a mercury-vapour lamp emitting radiation at 2,537 Å., the *p*-acetylaminophenol shows up as a dark purple absorbing spot. However, after the paper has dried, the spot shows a very light blue fluorescence when viewed in the ultra-violet light. This phenomenon is observed when only *p*-acetylaminophenol is present. It occurs following chromatography in dilute

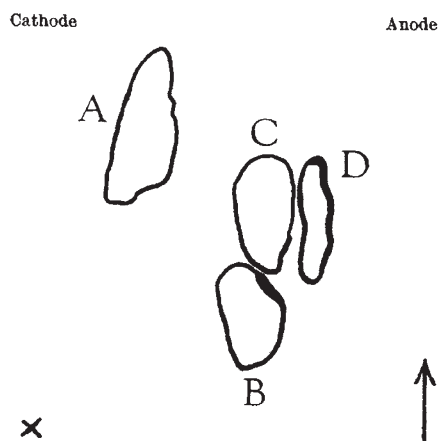


Fig. 1. Chromatography in 0.2 *N* ammonia (15 min.) followed by ionophoresis in 0.2 *N* ammonia (60 min.; constant current, 5 m. amp.; initial voltage, 250 V.) of a mixture of acetophenetidin (A), salicylate (B), *p*-acetylaminophenol (C) and phenobarbitone (D). Arrow indicates direction of ascending solvent-flow during chromatography; mixture applied at X

ammonia solution, but not when applied to the paper without ammonia treatment. Hence, the final dried sheet when examined in ultra-violet light shows the pale-blue fluorescent spot of *p*-acetylaminophenol differentiated quite clearly from the faster moving and dark purple absorbing spot of phenobarbitone.

The chromatography separation takes about 15 min., ionophoresis about 1 hr. 0.2 *N* ammonia solution is used as 'solvent'.

A full account of these procedures will be published elsewhere.

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<sup>1</sup> Street, H. V., and Niyogi, S. K., *Nature*, **190**, 537 (1961).

<sup>2</sup> Stewart, C. P., and Stolman, A., *Toxicology*, **1** (Academic Press, New York).

### Isolation of Beta-Alanine from *Iris tingitana* (var. Wedgewood)

BETA-ALANINE has often been reported (for example, Hulme and Arthington<sup>1</sup>) in plants on the basis of chromatographic evidence and is now considered to be a common plant constituent<sup>2</sup>.

Virtanen and Laine<sup>3</sup> isolated beta-alanine from the root nodule bacteria of peas, but there is no published report of the isolation of beta-alanine from higher plants. In the process of isolating beta-aminoisobutyric acid from iris bulbs<sup>4</sup>, beta-alanine has been obtained in crystalline form and characterized by classical physical and chemical methods.

Beta-alanine and beta-aminoisobutyric acid were separated from the other neutral amino-acids in the 80 per cent ethanol extract of iris bulbs (45 kgm.) on 'Dowex I' in the salt form<sup>5</sup>. The two beta amino-acids were separated from each other by chromatography with *n*-butanol/methanol/water (2 : 2 : 1) on a paper roll. Final purification was accomplished by crystallization from aqueous ethanol, resulting in 300 mgm. of material. Quantitative analysis of an extract of a different sample of Wedgewood iris resulted in a value of 0.045 μmole of beta-alanine per gm. of tissue fresh weight. The elemental analysis of the isolated material was 40.5 C, 7.93 H and 15.7 per cent N