of riboflavin and the purification of flavin adenine dinucleotide.

K. YAGI
J. OKUDA
Y. MATSUOKA

Department of Biochemistry,
School of Medicine,
Nagoya University.
Oct. 17.

- ¹ Yagi, K., and Ishiguro, I., *Vitamins (Japan)*, 3, 29 (1950).
² Dimant, E., Sanadi, D. R., and Huennekens, F. M., *J. Amer. Chem. Soc.*, 74, 5440 (1952).
³ Whitby, L. G., *Biochem. J.*, 54, 437 (1953).
⁴ Cohn, W. E., and Carter, C. E., *J. Amer. Chem. Soc.*, 72, 4273 (1950).
⁵ Sheppard, C. W., Cohn, W. E., and Mathias, P. J., *Arch. Biochem. Biophys.*, 47, 475 (1953).
⁶ Warburg, O., and Christian, W., *Biochem. Z.*, 298, 150 (1938).
⁷ Crammer, J. L., *Nature*, 161, 349 (1948).
⁸ Yagi, K., *Vitamins (Japan)*, 6, 528 (1953).

Circular Chromatography of Terpenes on Adsorbent Coated Glass

THE 'chromatostrip' method of Kirchner, Miller and Keller¹ for the separation and identification of terpenes and related compounds is a useful technique in a field where chromatography has been little applied. The method consists essentially of coating glass strips with an adsorbent (silicic acid), oven-drying and cooling *in vacuo* over potassium hydroxide. The oil is spotted near one end of the strip, which is developed by capillary ascent in a similar manner to paper strips. Spots or zones are located by spraying with reagents, one of the most useful being concentrated sulphuric acid, with which most components of essential oils give characteristic colours. Kirchner *et al.* have indicated the usefulness of the method for two-dimensional chromatography, and Reitsema² has used glass plates on which a number of oils were directly compared.

In this laboratory the method has been adapted to the circular technique first described by Rutter³ and has resulted in better resolution of zones, with the added advantage that as many as six oils may be conveniently compared on one plate. 'Magnesol' (hydrated magnesium silicate) has given better results than silicic acid.

Circular glass plates (5½ in. diameter) are coated with the adsorbent in a similar manner to that described by Kirchner *et al.*¹, except that 'Magnesol' is used instead of silicic acid. The plates are dried for ½ hr. at 105° C. and cooled for ½ hr. under vacuum over potassium hydroxide. The plate is then spotted at its centre with the oil to be examined, and placed adsorbent side down over a 5-in. diameter Petri dish containing a few millilitres of solvent. In the centre of the Petri dish a ⅙-in. diameter cotton wick, held upright by passing it through a hole in a metal support, feeds the solvent on to the plate at the oil spot. A wooden jig is used for centring the wick and the plate accurately. The movement of the solvent can be seen through the glass and the position of the solvent front is marked with a glass pencil on the upper side of the plate. When measuring R_f values, the adsorbent is scraped off the glass above the pencil marks. Rate of development is controlled by the diameter of the wick and its degree of contact with the plate. About thirty minutes development gives good resolution for a radius of 2¼ in. from the point of contact.

The method has proved most useful in comparing the essential oils of hybrid *Eucalyptus* species with those of the parent trees.

L. H. BRYANT

Division of Wood Technology,
Forestry Commission of New South Wales,
96 Harrington Street,
Sydney.
Nov. 19.

- ¹ Kirchner, J. G., Miller, J. M., and Keller, G. J., *Anal. Chem.*, 23, 420 (1951).
² Reitsema, R. H., *Anal. Chem.*, 26, 960 (1954).
³ Rutter, L., *Nature*, 161, 435 (1948); *Analyst*, 75, 37 (1950).

Inverted Rhythmic Precipitation

MANY examples of rhythmic precipitation occur in geological and biological formations, where the separation of the rings of precipitate (of copper carbonate, calcium phosphate, etc.) decreases with the direction of diffusion of flocculating solution; and these have not generally been considered true cases of the Liesegang phenomenon¹. The laboratory techniques^{2,3} that are generally used for the study of rhythmic precipitation *in vitro* have always led to the typical spacing relation, for X_{n+1} , the position of the $(n+1)$ th ring,

$$X_{n+1} = (d_n - d_0) = ak^n \quad (1)$$

where d_0 , d_n are the distances of the first and $(n+1)$ th ring from the origin of diffusion⁴. With such techniques, X_n always increases geometrically with n , as the concentration of coagulating ion gradually decreases along the diffusion path^{5,6}. When the diffusing ion, on the other hand, acts as a peptizer, no precipitation is observed.

I have developed a new technique for obtaining inverse rhythmic precipitation, similar to that observed in many natural formations. The ammonia-peptized sol of an insoluble metal salt dispersed in a fluid gelatine suspension is coagulated by diffusing a mixed solution of acetic acid, together with a third peptizing ion of slower rate of diffusion, into the set gel (cf. ref. 6).

The concentration of peptizer is greatest at planes nearest to the origin of diffusion, and the separation of the rings now decreases with n .

The following results have been obtained with 0.005 *M* silver chromate sols peptized by 0.050 *N* ammonia, in 5 per cent dialysed gelatine gels. 1 c.c. of the mixed solution was diffused into 15 c.c. of gel contained in test-tubes, and the precipitation observed for six to eight days. Measurements of d were made with a cathetometer³.

Peptizing cation. (i) Precipitation with acetic acid plus copper nitrate solution. Over the range of concentrations, 2 *N* – 0.5 *N* acetic acid, 0.5 *N* – 0.1 *N* cupric ions, silver chromate is precipitated as a normal series of rings⁶, followed at the plane A (Fig. 1), about 2 cm. from the origin of diffusion (O), by a series of rings of large silver chromate crystals. These rings become closer together along the diffusion path; and at a plane B, at distance d_∞ from O, a band of flocculated precipitate is formed. The distance (X_n) of the n th ring from A is now given by the relation,

Fig. 1