SCIENTIFIC MEETINGS

CHROMATOGRAPHY

Summary of a Lecture delivered by Professor A. H. Cook, D.Sc., Ph.D., at the Royal Institution

DR. COOK related how chromatography has helped in the isolation and purification of natural substances occurring in high dilution. This was especially so in recent years in the fields of hormones, vitamins, penicillin and vitamin B_{12}. Carotene, one of the first substances to be studied by the method, occurs at about 1 part in 1,000, lactoflavine is present at 1 part in 30,000 to 40,000 of culture medium and the anti-pernicious anæmia factor at about 1 in 80×10^6. Another difficulty in the study of natural products lies in the accompanying impurities which are very often similar in physical and chemical properties and indistinguishable by ordinary chemical or physical methods. Chromatography frequently provides a solution to such problems. When there are great differences in the extent to which certain substances are adsorbed some of them may pass straight through the column, but they are not lost as they appear in the liquid at the end of the column in an orderly fashion. This is the principle of the liquid chromatogram. Sometimes a zone may contain more than one substance when it will be necessary to fractionate them by repeating the process in a number of columns. An American apparatus provides for up to 200 repetitions. Colourless compounds are detectable by viewing them in ultra-violet light, when many fluoresce, by the addition of a suitable dyestuff whose relative behaviour on the column is known or by the conversion of the substance to a coloured or fluorescent derivative. Chromatography has been the key to the chemistry of the carotenoids. It has also played an important part in microchemistry (detecting 0·01 g.) and in the examination of wines, foodstuffs and drugs.

The degree to which a substance is adsorbed is related to molecular structure. The carotenoids which are characterised by a varying number of OH groups and conjugated or isolated double bonds, are a good example of this.

Oxygen atoms, double bonds (conjugated more than isolated bonds) are associated with increased adsorption. Thus fucoxanthin containing 10 double bonds and from 4 to 6 OH groups is most strongly adsorbed whilst α-carotene is about the least. The stereoisomeric methyl bixins give chromatograms which vary with the different molecular shapes of these compounds. This method of identification is much more sure than that of melting-points.

Recently the method of mechanically separating the zones on a chromatogram has been replaced by one where the zones are eluted into a special container where the liquid is observed either polaragraphically or by continuous measurements of the refractive index. In this way Tisellius and Claesson have separated mixtures of lauric, palmitic and myristic acids. Ion exchange is a development of chromatography in which the ionic charge of the column and eluent plays an important part. It has been used to study the nucleotides and amino-acids. Substances which form salts in a medium of acid *p*H may be separated on a cationic column and those forming salts in one of alkaline *p*H on an anionic column.

Another recent development is the partition method in which the column is packed with silica gel containing water. The passage of the zones

down the column depends on their relative partition coefficients between water and the organic solvents. Adsorption by the silica is sometimes a problem which may be avoided by the use of strips or sheets of filter paper. This is a very convenient way of testing urine and has led to the detection of cysteine which was not formerly suspected in abnormal urine. The strip of filter paper is spotted with a drop of solution on a line drawn near one end. It is then suspended vertically in a glass cylinder so that the spotted end is immersed in a trough or organic solvent saturated with water at the top of the cylinder. The atmosphere is kept saturated with organic solvent and water vapour by placing the cylinder in a shallow dish of the mixture. The movement of the zone of substance in solution relative to the distance moved by the advancing front of liquid is measured. Various reagents are used to detect the zones of substances present. By using a sheet of filter paper a number of solutions may be examined at the same time and, under standardised conditions, it is possible to use maps to identify the different substances by their relative positions. Amino-acids both in the free and bound forms, gramicidin, penicillin, purines, sugars and anthocyanins have all been studied by this method. Vitamin B_{12} was investigated by a modified method in which the chromatographic strip was laid across a seeded agar plate and the effect of the various zones on the bacterial growth was noted. Observations have been made on the products of photosynthesis in algae with the aid of radio-active particles. The cells were extracted with solvent and chromatograms prepared which were photographed on X-ray film. By comparison with chromatograms of known substances no less than 15 substances, hitherto unknown in photosynthesis, were detected. Partition chromatography has also shown the toxic factor in flour caused by treatment with nitrogen trichloride to be neither an amino-acid nor a protein. Because little change ensues in the nature of substances isolated by chromatography, it is indispensable in the investigation of natural products especially in such complex problems as the nature of bacterial toxins and the specificity of proteins. It has enabled us to speculate on the course of photosynthesis and the biogenesis of amino-acids.

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days after these compounds have been detoxicated or eliminated. The potentiating effect is due to the combined effect of the sulphone sensitisation and some element in fresh vegetables in the standard diet (fresh cabbage, lettuce, carrots, hay and oats). S. L. W.

Testosterone, Long-Acting Preparation of. E. Carlinfanti, F. D'Alò and L. Cutolo. (*Lancet*, 1949, **256**, 479.) To a solution of 1 g. of crystalline testosterone in 10 ml. of alcohol (96 per cent.) is slowly added, with constant stirring, 20 ml. of an equeous suspension of aluminium phosphate 7 mg./ml. The mixture is allowed to sediment and the supernatant fluid decanted off. The residue is made up to 40 ml. with saline solution. The preparation is stable and can be administered with a fine needle. Experiments were carried out on castrated guinea-pigs, one group receiving one injection of testosterone propionate in oil in a dose of 25 mg./100 g., and another group the same amount of pure testosterone adsorbed on to aluminium phosphate. It was found that one injection of the ester-in-oil preparation leads to a rapid rise and fall in the weight of the seminal vesicles, whereas the new preparation produces a greater and more continuous action, reaching a peak not earlier than 30 days after administration. S. L. W.