differential two-column working are both expensive and difficult. In any case, physical detectors are frequently of comparatively low sensitivity compared with many colorimetric chemical methods. The low specificity of physical detection methods can be advantageous when subsequent radioactivity measurements are required, in that all the components separated by the column can be seen, but at other times the controlled specificity of colorimetric chemistry can simplify the chromatographic method. A carefully designed chemical-analysis system can achieve response times of the order of 3–5 s with total liquid flow rates as low as 0.5 ml/min. This compares favourably with direct physical methods. At the same time it will generally show no response to the eluting solutions and therefore will measure the eluted components against a stable base-line. This makes high-sensitivity working much easier and, as many suitable colour reactions are extremely sensitive, detection limits can be extremely low.

Flexible-gradient elution combined with a fast-response chemical-detection system offers great possibilities for extending chromatographic technique. This is well illustrated by a system developed in this laboratory, where a programmed continuously varying gradient of borate–acetate is used to elute a column (18 cm × 0.2 cm) of Dowex 1 (micro-spherical beads) at 60°C. With a flow rate of 0.4 ml/min ribose, glucose, fructose, glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, ribose 5-phosphate, fructose 1,6-diphosphate, glucose 1,6-diphosphate, UDP-glucose, ADP, ATP and UTP are successively eluted in under 4 h. The flowing-stream chemical-detection system, based on the orcinol reaction, gives a detection limit of about 50 nmol for each of the components.

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Thin-Layer-Chromatographic Equipment

By J. P. LEPPARD (*Wolfson Bioanalytical Centre, University of Surrey, Guildford, Surrey, U.K.*)

T.l.c. is a widely used technique for qualitative analysis of reaction mixtures, quality control, screening and the like. In many cases routine t.l.c. of a large number of samples is required, which leads to practical problems with regard to efficient use of laboratory personnel and to their morale. Also, although t.l.c. is a very useful technique for qualitative analysis, it has been found difficult to use it to obtain quantitative results in a reasonably straightforward manner. A number of research workers and manufacturers

have therefore designed equipment to mitigate either or both of these handicaps. Since the solvent development of the plates is generally straightforward, and a wide range of commercially prepared plates is now available, most of the problems of quantitative t.l.c. arise either in the application of the sample to the plate or in evaluation of the separated spots of material after development. Innovations have therefore been made particularly in the production of sample-applicators and of plate-scanners (since direct quantitative determination *in situ* presents the greatest attractions in terms of simplicity and speed).

Sample-applicators range from relatively inexpensive controlled-bore capillary tubes, through various modified syringes, to 'sophisticated' (and expensive) semi-automatic machines. The latter, which make use of several methods of sample transfer such as electrostatic precipitation or 'firing' on to the plate, may work well, but are often useful only for preparative t.l.c. or will not cope with more than one or two different samples at a time; moreover, they may be tedious to clean. In our laboratory a machine of unorthodox type has been designed, initially intended mainly to relieve the tedium of repetitive application of an array of different samples (as would be required, for example, in laboratories concerned with quality control or screening programmes). It consists essentially of a reciprocating drive that moves mounted pick-up arms into a row of sample cups and then on to the t.l.c. plate. Up to 12 samples can be 'spotted' simultaneously, and up to 99 aliquots can be 'spotted' without intervention of the operator. Timers allow for drainage of the sample on to the plate and for drying of the spots, and the machine has exceeded expectations by giving a precision of 2–3% even with solvents as volatile as chloroform.

Many densitometric plate-scanners are now available, mostly at prices well above £1000; some are self-contained and some are for attachment to a spectrophotometer. Many of the instruments depend on a simple and well-established design principle, involving linear traverse of the lanes on the t.l.c. plate by a slit of light, usually with reflectance measurement; others involve techniques such as 'flying spot', double-beam and even double-wavelength scanning. Most of the more expensive scanners measure in various modes (transmission, reflectance, fluorescence, fluorescence quenching), all with visible-light illumination and some with u.v. light also. A common feature of the more refined instruments is that they measure the intensity of the reflected, transmitted or emitted light over the whole area of the spot and subsequently present an integrated value for the spot. In our laboratory a scanner has been produced that makes use of a rather different principle of quantitative determination that is well founded but has been rather neglected: it depends on the measure-

ment of spot area, this being related to the amount of material present (Purdy & Truter, 1962).

Among other devices that have been investigated in this Centre, mention should be made of a machine that uses a flame ionization detector for detection and quantitative determination of t.l.c. spots on quartz rods coated with silica gel (Padley, 1969). Good results were obtained with sample loadings of 1–10 µg,

the main difficulties encountered being concerned with application of the sample without dislodging the coating and retention of developing solvent on the coating after supposed drying.

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PHARMACOLOGICAL BIOCHEMISTRY GROUP COLLOQUIUM ON 'MERCURY INTOXICATION'

Clinical Symptoms of Mercury Poisoning in Man

By P. LESLEY BIDSTRUP (11 *Sloane Terrace Mansions, Sloane Terrace, London SW1X 9DG, U.K.*)

Mercury vapour, inorganic compounds of mercury and organic compounds of the aryl series, such as phenylmercuric acetate, produce symptoms and signs that differ significantly from those caused by absorption of short-chain alkylmercury compounds (ethyl and methyl derivatives).

Mercury and the inorganic compounds. The soluble inorganic mercury compounds, particularly HgCl_2 , are irritating to skin and mucous membranes (Sollman, 1957), and absorption from the skin may be sufficient to cause systemic poisoning. Some people are particularly susceptible, collapse and serious illness resulting from therapeutic doses of Hg_2Cl_2 (calomel) (Martindale, 1958). Slow absorption of insoluble compounds through the skin may occur, and mercury dermatitis in the red areas of tattoo [HgS (cinnabar)] has been reported (Keiller & Warin, 1957).

Since HgCl_2 has become less popular as an agent for homicide or suicide, acute systemic mercury poisoning is seldom seen. Characteristic features were coagulation, irritation and superficial corrosion of tissues at points of contact, rapidly fatal circulatory collapse or delayed death from renal tubular necrosis. Haemorrhages, congestion and ulceration of the large intestine were found also at necropsy. Pink disease (acrodynia) of infants is attributed by some authorities to hypersensitivity to inorganic mercury present as Hg_2Cl_2 in anthelmintics and teething powders (Warkany & Hubbard, 1953). Exposure to high concentrations of mercury vapour results in lung irritation (Matthes *et al.*, 1958).

Metallic mercury and its inorganic compounds are used in many industries. In the early days acute or sub-acute poisoning, characterized by stomatitis, gingivitis, excessive salivation, loss of teeth, gross tremor with ataxia and serious mental disturbance, was not uncommon, for example, among mirror-

makers, mercury miners and others. Today, chronic mercury poisoning is seen among persons employed in a variety of occupations: the manufacture and servicing of scientific equipment, including thermometers, barometers and mercury-arc rectifiers, the cleaning and packing of mercury or mercury compounds and the manufacture of dry-cell batteries are examples. The common symptoms are erethism (manifested by nervousness, irritability, a tendency to blush readily and change of temperament) and tremor. The pathological lesion has not been identified. Absorption is mainly from inhalation of vapour, droplets or dust, but occurs also through the skin. Symptoms subside on removal from exposure; recovery may be hastened by administration of *N*-acetyl-D-penicillamine 250mg four times daily (q.d.s.) by mouth.

Proteinuria may occur, as may the nephrotic syndrome. The latter is relatively rare, considering the numbers of persons at risk of absorption of mercury. The mechanism is not understood; hypersensitivity has been postulated, but it could well be an as yet unidentified abnormal metabolic pathway. Other signs of mercury poisoning are usually absent from those who develop the nephrotic syndrome. The clinical manifestations of inorganic-mercury poisoning are discussed and occupations entailing exposure to mercury described by Bidstrup (1964). Goldwater (1964) reports on studies of the absorption and excretion of mercury in man. The excretion of mercury in the urine collected over 24h is a valid index of absorption, particularly on a group basis, but is not diagnostic of poisoning. 'Normal' output in non-exposed persons is less than 25 µg/l; in those at risk of absorption considerable fluctuation in mercury excretion occurs from day to day.

Organic compounds of mercury. Because the short-chain alkyl compounds of mercury, such as the ethyl and methyl derivatives, have proved to be extremely toxic to man and animals, it has been usual to condemn all organic mercury compounds. In fact, the aryl compounds, such as phenylmercuric acetate, have been used extensively in the past as fungicidal