and co-workers (Osborne, Briel & Neuhoff, 1971; Briel, Neuhoff & Maier, 1972; Neuhoff, 1973) which has enabled amino acids, 5-HT and related substances to be estimated in characterized invertebrate neurones and milligram quantities of nervous tissue (Osborne 1973, Osborne & Neuhoff, 1973).

This method involves the extraction of amines and free amino acids from tissue. Dansyl chloride is then reacted with the extracted substance at alkaline pH to form highly fluorescent dansyl derivatives. The derivatives are then separated by two dimensional chromatography on 3×3 cm polyamide plates.

The sensitivity of this method has been considerably increased by the use of ^{14}C-dansyl chloride (98 mCi/mM, Schwarz/Mann, Orangeburg, New York). This enables autoradiograms of the plates to be prepared thereby often allowing the detection of substances which are present in quantities barely visible in UV light. It is also possible to mark the perimeters of the fluorescent spots, remove them from the plate using a microknife and determine the radio-activity of the dansyl derivatives in a liquid scintillation counter. This method detects as little as 1 picomole of an amino acid or 5-HT.

A practical demonstration of this method will be given. Detailed results which have been obtained from the use of the dansyl method to analyse characterized invertebrate neurones and defined areas of vertebrate brain tissue, will be presented.

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The use of model droplets in monoamine histochemistry and some problems of colour photomicrography

I. LASZLO (introduced by G. W. ASHCROFT) *M.R.C. Unit for brain metabolism, Pharmacology Department, Edinburgh University*

The relationship between the concentration of noradrenaline (NA) and that of 5-hydroxytryptamine (5-HT) and their fluorescence intensity and colour of fluorescence was investigated with the help of model experiments using droplets, the preparation and fluorescence characteristics of which are described.

5 μl droplets containing noradrenaline bitartrate (0·1 to 44 mg/ml) and 5-hydroxytryptamine creatinine sulphate (0·1 to 8·0 mg/ml) in 5 per cent albumin solution were dried on microscope slides at room temperature. In the case of 5-HT in order to increase the amount of this monoamine in the droplets, 5-hydroxytryptamine creatinine sulphate (8 mg/ml) was dried repeatedly on the same areas. The slides were treated with formaldehyde according to the method described by Falck & Owman (1965). Fluorescence was observed and fluorescence spectra were recorded with Zeiss microspectrophotofluorimeter (Laszlo, 1972). A direct relationship was found between the concentration of these monoamines and the intensity of fluorescence, when the latter was measured at the periphery of the droplets. The distribution of fluorescence intensity in these droplets was recorded and found to be fairly homogeneous. The colour of fluorescence of NA changes from green to yellowish green when its concentration increases, while the colour of fluorescence of 5-HT changes from green to yellow. The overlapping colour of fluorescence of these monoamines can be explained by their almost completely overlapping fluorescence spectra.

The large number of colours which occurred during these model experiments and in fluorescence microscopy raised the importance of an unambiguous description of

colours which appears possible by using Hickethier's system (Hickethier, 1970). Such description of colours which is based on a comparison to standard colours denoted by three digits numbers, may have several applications, e.g. in histochemical reactions and in photomicrography. It may be used for denoting colours in black and white photographs or in colour photographs when colour rendering is not perfect. One of the reasons which makes reconstruction of colours difficult in the case of fluorescent sections is that colour often changes during exposure (Laszlo, 1973).

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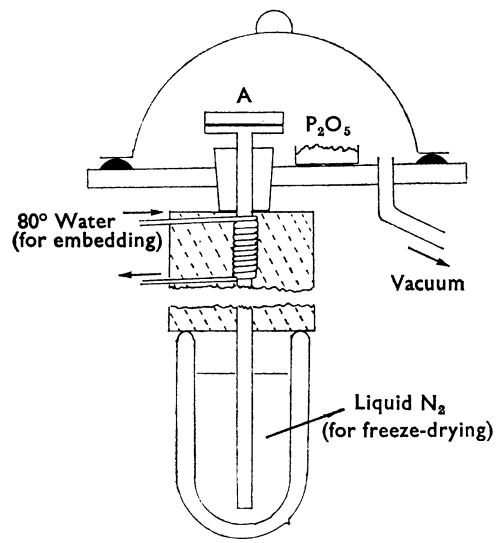
A simple, low-cost freeze-drier

R. TOWART (introduced by C. W. M. WILSON)

Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland

This apparatus was developed to provide an inexpensive method for the demonstration of biogenic amines by the fluorescence technique of Falk, Hillarp, Thieme & Torp (1962).

It is a much simplified version of the tissue drier described by Pearse (1968). A glass dessicator lid, on a base of $\frac{1}{2}''$ Perspex, sealed by a nitrile rubber gasket, forms the vacuum chamber. The drying module is a platform on top of a long brass rod which passes through a rubber bung in the base. The rod is insulated with expanded polystyrene, and its lower end is immersed in a flask of liquid nitrogen. An aluminium tissue holder (A) is placed on the drying platform. A metal lid filled with phosphorus pentoxide forms the water vapour trap.



The apparatus is also suitable for the vacuum embedding of treated tissue. A coil of copper tubing surrounds the rod immediately below the bung. When water at 80° C is circulated, sufficient heat is conducted to melt embedding wax in the tissue holder.

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