dimension, forced the separated proteins into a bed of agarose containing whole human anti-serum (Paines & Byrne) until the proteins were precipitated as an antigen-antibody complex. The plates were washed, dried at room temperature and incubated for 24 h in a buffered medium (pH 7·4) containing a ^{14}C-labelled drug. The concentration of the compound was calculated to provide a level equivalent to a therapeutic serum concentration of the unbound drug. The plates were washed with buffer to remove surface activity, dried, sprayed with a thin cellulose film to prevent chemography and autoradiographed against X-ray film for periods up to 100 days.

The binding of ^{14}C-diphenylhydantoin (^{14}C-DPH) has been studied in this manner, using a concentration of $3{\cdot}65\times10^{-6}$M, 25 nCi/ml in 0·02 M phosphate buffer at pH 7·4. The drug bound to nine different serum proteins, but showed high affinity for albumin and a protein in the β_2-region. This protein was subsequently identified at β_2-lipoprotein by lipid staining and use of monospecific antisera. The high affinity for these two proteins was confirmed by incubating first dimension agar strips with ^{14}C-DPH. The strips were then cut into a number of segments at right angles to the run and the activity of each segment was measured in a liquid scintillator counter. High activity was found in the segments corresponding to the albumin and β_2-lipoprotein peaks.

^{14}C-Phenobarbitone ($4{\cdot}74\times10^{-7}$M, 15 nCi/ml) incubated with second dimension plates gave autoradiographs that showed binding to 7 different proteins including albumin, transferrin, haemopexin, immunoglobulin A and β_2-lipoprotein. However, no particular protein had high affinity for the drug.

Studies with ^{14}C-acetylsalicylic acid ($4{\cdot}16\times10^{-5}$M, 0·65 μCi/ml) showed similar protein binding to that of phenobarbitone. Although binding to several β_2-globulins was observed, little or no binding to β_2-lipoprotein could be demonstrated.

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A technique for achieving greater stability of the brain for microiontophoretic studies of single cortical neurones

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In the course of our work with the tricyclic antidepressants using the microiontophoretic techniques (Bradshaw, Roberts & Szabadi, 1971), we needed to compare the effects of increasing doses of imipramine and desipramine on the responses of individual cortical neurones to noradrenaline and 5-hydroxytryptamine. The long time course of these responses, and their susceptibility to tachyphylaxis demand that successive drug applications are separated by 4–5 min. Furthermore, the effects of a single application of an antidepressant may persist for more than 30 min. Thus, a complete comparative dose-response study of the two antidepressants requires that the activity of a single neurone be recorded for several hours.

In order to achieve the great stability which is required for such studies, we had to reduce pulsation of the brain tissue to a minimum. At the same time, it was necessary to provide means for the continuous removal of excess c.s.f. since halothane anaesthesia increases c.s.f. production and thereby raises intracranial pressure. Conventional techniques try to solve these problems separately: pulsation is reduced either by mechanical stabilization of the area of recording (pressors, agar gel, paraffin wax, etc.) or by the application of bilateral pneumothorax and artificial ventilation; intracranial pressure is reduced by the intermittent or continuous withdrawal of c.s.f. from the cisterna magna. This latter procedure in itself enhances pulsation and promotes further c.s.f. secretion. We have found that these techniques could not provide the stability required for lengthy studies.

We have developed a new technique which offers the advantages inherent in recording from a practically closed skull, and at the same time, ensures means for continuous c.s.f. drainage. A small hole (less than 2 mm in diameter) is made in the skull with a dental burr. Then, under microscopic control, the dura is incised, and the incision dilated with a glass probe (the area of cortex exposed measures approximately 1 mm$\times$300 μ). The arachnoid is then ruptured, allowing c.s.f. to leak out, and the microelectrode is introduced under microscopic control.

This technique has several advantages. Firstly, it does not grossly interfere with the intricate physiological relationships between cerebral circulation, c.s.f. production and resorption, and the mechanical stability of the brain tissue. Thus movement of the tissue due to pulsation and raised intracranial pressure is minimized. This ensures a much more stable recording condition than is achieved by conventional techniques. Secondly, the incision in the dura allows a continuous drainage of c.s.f. It is not necessary, therefore, to provide further means for the removal of c.s.f. Thirdly, surgical intervention is reduced to minimum.

Using this technique, we are now able to study isolated single units of unchanging spike amplitude for two hours or more.

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Investigations on the histochemical demonstration of noradrenaline and 5-hydroxytryptamine in the area postrema of the rabbit by fluorescence microscopy

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Modifications of the method for the histochemical identification of noradrenaline (NA) and 5-hydroxytryptamine (5-HT) (Falck & Owman, 1965) are described. Brain tissue containing the area postrema was dissected from control and 5-HT treated animals (Dow, Laszlo & Ritchie, 1972), frozen in isopentane cooled with liquid nitrogen. The frozen tissue samples were stored in liquid nitrogen until freeze dried. Formaldehyde treatment was performed with paraformaldehyde equilibrated at 58% relative humidity, this degree of humidity having been found the most suitable for investigation of the area postrema by fluorescence microscopy. During vacuum embedding the original orientation of the tissue was maintained: