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A method for providing intermittent intravenous injections in unrestrained animals

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As part of an investigation into the pharmacological effects of nicotine in relation to tobacco smoking, a method has been devised for intermittent intravenous administration of drugs to unrestrained Squirrel monkeys. The injector system is self contained and can be carried permanently, or occasionally, by the animal. The injector, constructed largely of perspex, contains a 20 ml chamber for solution for injection; the solution is maintained at a constant positive pressure by liquefied gas ('Arcton' 114—I.C.I. Ltd.) contained inside a rubber balloon within the chamber. A battery powered circuit operates a relay at pre-selected intervals (usually 30 or 60 sec) which momentarily opens a valve allowing a small volume (between 0·005–0·010 ml) of solution to be ejected from the device. The solution is injected into the animal via a silicone rubber cannula permanently implanted into an external jugular vein. The injector is fitted to an aluminium backplate attached to leather harness which is worn permanently by the animal.

The device has been used to investigate the effects of small doses of nicotine on the performance of trained monkeys on several operant conditioning schedules, but could be adapted to provide intermittent intravenous injections to other species of experimental animal.

Two dimensional immunoelectrophoresis of human serum proteins for the investigation of protein binding of drugs

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Two dimensional immunoelectrophoresis has been used by Freeman & Pearson (1969) to study the binding of ^{125}I-thyroxine to human serum proteins. Using the method described by Clarke & Freeman (1968) the present study has extended this work to the investigation of the protein binding of a variety of drugs.

Pooled human serum (4–6 μl) was separated on agarose strips by electrophoresis in barbitone buffer (pH 8·6, 0·03 M, ionic strength 0·035) with the addition of calcium lactate (1·8 mM). A second dimension electrophoresis, perpendicular to the first

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.