200 μg/kg per hr produced a 70% inhibition. Puromycin inhibits the transfer of amino-acids from amino-acyl transfer RNA to the polypeptide chain which is forming on the ribosome. An intracerebral dose of 100 μg which almost completely prevented any fall in the analgesic activity of morphine during infusion, inhibited the uptake of ^{14}C-lysine by 40%.

Actinomycin-D, 6MP and 5FU had less effect on lysine incorporation. These drugs inhibit or modify the synthesis of RNA and for this reason we have also measured their effect on the incorporation of 6-^{14}C-orotic acid into brain RNA. Actinomycin-D at a dose (20 μg/kg per hr, intravenously) which completely prevented tolerance development during a 7 hr infusion reduced the uptake of ^{14}C-orotic acid by 17%. Dose schedules of 6MP and 5FU which slowed down the rate of acquisition of tolerance reduced the incorporation of orotic acid into RNA by approximately 15%.

These results provide further evidence that the development of tolerance to morphine in rats is causally related to a modification of protein synthesis in the brain which probably involves derepression of DNA.

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Magnetic resonance studies of anaesthetics in cyto-membranes

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We have previously described the nuclear magnetic relaxation (NMR) of several anaesthetics in a number of membrane preparations, and concluded that the characteristic changes observed with increasing anaesthetic concentration might reflect the changes in membrane structure associated with anaesthetic action (Metcalfe, Seaman & Burgen, 1968; Metcalfe & Burgen, 1968).

A difficulty with this approach is that it is not possible to distinguish directly the contributions of lipid and protein to the overall interaction. More direct evidence of this kind can be obtained by the technique of electron spin resonance (ESR). A simple ESR spectrum is obtained from the unpaired electron of the nitroxide (>N→O) group, which can be introduced into a wide range of structures including analogues of anaesthetics, lipids, or covalent protein reagents. The ESR spectra of these "spin labels" inserted into the membrane may provide information about the rotational motion of the label, the dielectric nature of its micro-environment, and about the orientation of the label in the membrane.

The effects of anaesthetics on a range of spin labels in erythrocyte membranes were found to be consistent with the conclusion from the NMR studies that there is a progressive fluidizing of the membrane lipids as the anaesthetic concentration is increased, until protein binding sites are exposed which were previously inaccessible.

By designing the spin labels so that the nitroxide group will be located at different regions in the membrane structure, detailed information about the localization of anaesthetics can be obtained.

Evidence from both NMR and ESR experiments suggests that molecules such as cholesterol have the effect of increasing the order or packing of the membrane. The transition from a fluidizing action to an ordering effect may be observed in ascending the *n*-alkyl alcohol series, so that both kinds of structural perturbation may be associated with anaesthetic action.

All these effects have been demonstrated directly on spin labelled nerves by Hubbell & McConnell (1968), who are responsible for this novel method of probing membrane structure and to whom I am most grateful for the opportunity of taking part in the work presented here.

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The state of quaternary ammonium ions in solution

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The rotational correlation times (the mean time taken for a molecule to rotate 1 radian in solution) of drugs can be estimated from their nuclear magnetic spin-lattice relaxation times. We have measured these for the N-methyl protons of alkyltrimethylammoniums and alkyldimethylammoniums and for some bisquaternary ammonium compounds by the method of rapid adiabatic passage (Pople, Schneider & Bernstein, 1959). Theoretical relaxation rates calculated on the assumption that the molecules behave as rigid entities in which no rotation about bond axes is permitted agree excellently with the experimental values. Rotation about C–C or C–N bond axes makes no measurable contribution to the relaxation process. This means that in these compounds the energy barrier to bond rotation is large enough to make alterations of conformation slow compared with the rotational correlation time. The population of conformations should therefore be regarded as stable in the context of discrete collisions with the receptor. By contrast in choline methyl ether and homocholine methyl ether rotation about the O bond is relatively free and this is also true of rotation of the acetyl group in acetylcholine and methacholine. These differences in ease of rotation are important in interpreting relaxation rates of drugs when they are interacting with binding sites.

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