genase, acid phosphatase, protein and hexosamine. Gastrin content was determined by biological assay using the perfused stomach of the anaesthetized rat (Amure & Ginsburg, 1964). In preliminary experiments the conditions of homogenization and centrifugation were used which, when applied to liver, would have yielded nuclear, mitochondrial plus microsomal and supernatant fractions. Although the mitochondrial plus microsomal fraction contained 20–33% of the total gastrin, large amounts of all the biochemical markers and of gastrin were found in the first sediment; this is probably due to the presence of mucus causing heavy cross-contamination of the fractions and incomplete cell disruption (Hubscher, West & Brindley, 1965). A gastrin-rich sediment was prepared by centrifugation of the "nuclei-free" supernatant at 20,000 *g* for 20 min which, after resuspension, was subjected to centrifugation in a modified density gradient (Baker, 1959). These experiments showed that the gastrin particle was distinct from mitochrondria (succinic dehydrogenase activity) but was not clearly separated from lysosomes (acid phosphatase activity).

Evidence for the occurrence in the gastrin-rich granules of a macro-molecule capable of forming complexes with gastrin was obtained from experiments in which the elution in molecular sieve chromatography (Sephadex G-50) of free gastrin and the gastrin activity of osmotically disrupted granules was compared.

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The role of protein synthesis inhibition in the prevention of morphine tolerance

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We have previously reported that tolerance rapidly develops to the analgesic effects of morphine-like drugs when these are infused intravenously into conscious rats at selected rates. We also showed that concurrent administration of actinomycin-D reduced or prevented the development of tolerance (Cox, Ginsburg & Osman, 1968). Since actinomycin-D inhibits DNA directed RNA synthesis and hence *de novo* protein synthesis, the effects on tolerance development of some other drugs which inhibit protein synthesis have been studied.

Cycloheximide, puromycin, 6-mercaptopurine (6MP) and 5-fluorouracil (5FU) all reduced the degree of tolerance produced by intravenous infusion of morphine HCl (7.5 mg/kg per hr), although it was necessary to inject 6MP and 5FU intracerebrally to demonstrate this effect. The effect of these drugs (in doses shown to be capable of preventing morphine tolerance) on the incorporation of ^{14}C-lysine into brain proteins has also been measured. Cycloheximide, which inhibits protein synthesis by hindering the movement of ribosomes along messenger RNA, produced an 18% inhibition of ^{14}C-lysine incorporation at a dose (50 μg/kg per hr, intravenously) which markedly reduced tolerance development. Cycloheximide at

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.