KCl. The homogenate was centrifuged at 10,000 g for 20 min, followed by a further centrifugation of the supernatant at 140,000 g for 90 min, to obtain the microsomal pellet. The pellet was then resuspended in buffer and, following a further centrifugation at 140,000 g for 45 min, it was finally suspended in 0.1 M phosphate buffer pH 7.6. Cytochromes P-450 and b_5 were determined by the method of Omura & Sato (1964) and NADPH-cytochrome c reductase by the method of Phillips & Langdon (1962). The metabolism of foreign compounds was measured over 30 min at 37°C using the incubation conditions described by Mazel (1971).

The concentration of cytochrome P-450 was 9 times lower than that found in liver (intestine 0.046 ± 0.004 nmol.mg protein^{-1}; liver 0.43 ± 0.05), of cytochrome b_5 was 5 times lower (intestine 0.080 ± 0.014 nmol.mg protein^{-1}; liver 0.42 ± 0.08) and of NADPH-cytochrome c reductase was $2\frac{1}{2}$ times lower (intestine 57.4 ± 5.1 nmol.mg protein^{-1} min^{-1}; liver 142 ± 3.10). Pretreatment with phenobarbitone (75 mg/kg daily, i.p.) over a period of 3 days lowered slightly the mean concentrations of cytochrome P-450 (0.031 ± 0.006 nmol.mg protein^{-1}) and NADPH-cytochrome c reductase (44.3 ± 4.1 nmol.mg protein^{-1} min^{-1}) but appeared to increase the concentration of cytochrome b_5 (0.108 ± 0.013 nmol.mg protein^{-1}). The 10,000 g supernatant was shown to be N-demethylate, ethylmorphine and aminopyrine.

This evidence suggests the presence of a mixed-function system in rat intestinal tissue similar to that found in liver.

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The acetylation of HC-3 by choline acetyltransferase

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The main action of HC-3 on cholinergic transmission is presumed to be an inhibition of the uptake of choline into the presynaptic nerve terminal (MacIntosh, Birks & Sastry, 1956). It has also been suggested that HC-3 might itself be incorporated into the nerve terminal where it may be acetylated by choline acetyltransferase (ChAc) and subsequently released as a false neurotransmitter (Rodriguez de Lores Arnaiz, Zieher & de Robertis, 1970; Hemsworth, 1971).

The acetylation of HC-3 by ChAc *in vitro* has been studied in a number of laboratories with conflicting results (Diamond & Milfay, 1972). The present experiments were therefore performed to investigate these differences in the acetylation of HC-3. Several different established methods for determination of ChAc activity were utilized to detect any acetylated product that might be formed by enzymatic acetylation.

The observed acetylation of 10^{-3}M HC-3 by soluble rat brain ChAc, compared with the acetylation of choline at the same concentration, varied between 0 and 27% depending on the procedure used for the isolation of the acetylated product.

When the reineckate method, first described by McCaman & Hunt (1965), was used for isolation of the radioactive acetylated products of incubation, HC-3 was shown to be acetylated at 26% the rate of acetylation of choline. When the sodium tetraphenylboron extraction method of Fonnum (1969) was used, HC-3 was shown to be acetylated 11% compared with the acetylation of choline. The ion-exchange method used by Diamond & Kennedy (1968) was also used for isolation of any acetylated reaction products, however with this method no acetylation of HC-3 was observed. A mercuric potassium iodide extraction procedure described by Glover & Green (1972) was also utilized for determination of radioactive acetylated product and the acetylation of HC-3, in comparison with choline, using this method was shown to be 21%.

These variations in the acetylation of HC-3, in comparison with the acetylation of choline, appear to arise as a result of differences in the recoveries of acetyl HC-3 and acetylcholine when different methods are used for the isolation of the acetylated radioactive products of incubation. Identification of acetyl HC-3 and acetylcholine was made by paper electrophoresis and the results verify that HC-3 can be acetylated by ChAc *in vitro*.

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Mechanism of accumulation of chlorpromazine in subfractions of rat brain

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Our previous findings have shown that subcellular fractions of rat brain prepared from a 10,000 g pellet are capable of accumulating chlorpromazine (CPZ) when incubated with ^3H-CPZ *in vitro* giving similar results to the ^3H-CPZ found after preparation of subfractions following i.p. injection *in vivo*. A time course study for the accumulation in subfractions prepared from cortex, mid-brain and hind-brain showed that an equilibrium situation was rapidly achieved which did not alter appreciably after the first few minutes of incubation. Further experiments have been performed to investigate the mechanism of this accumulation. Subcellular fractions were prepared on a discontinuous sucrose gradient by a method based on that of Marchbanks & Whittaker (1967) and incubated for 15 min in ^3H-CPZ which had been diluted down with different concentrations of unlabelled CPZ. Pellets were collected by rapid centrifugation and activity measured by liquid scintillation counting. Results showed that the amount of CPZ accumulated increased with increasing concentration up to the highest concentration studied of 10^{-3} M, and furthermore, preincubation of fractions in 10^{-3} M CPZ, followed by subsequent incubation in different concentrations of ^3H-CPZ as before, did not alter the amount accumulated. The amount of protein in the incubation did not appear to be a limiting factor. The possible pH dependence of the accumulation was investigated by altering the pH of the medium and this showed that, although the difference in the amount accumulated between the individual pH points tested (pH 5, 6, 7 & 8) was not significant, the difference between the accumulation at the highest and lowest pH was significant at the level $P < 0.001$.

To investigate the dependence of this accumulation on the lipid content of the subfraction, proteolipids were extracted from subfractions which had been incubated in ^3H-CPZ