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

by a method based on that of Pasquini & Soto (1972) using n-butanol-water. Following 15 min incubation suspensions were spun down and the resultant pellets resuspended in 1.5 ml 50% sucrose and extracted for 2 h at room temperature with 16 ml n-butanol-water. Experiments showed a large uptake into the organic layer in the ratio 9.7 : 1 compared to partition of organic solvent against an aqueous solution of CPZ of 1 : 1. Extraction of proteolipids from subfractions followed by incubation with ^{3}H-CPZ gave similar results. Subfractions prepared from rats which had been injected with ^{3}H-CPZ (8 mg/kg i.p.) also showed a preferential uptake into the organic layer when partitioned against water in the ratio 12.5 : 1. Phospholipids, phosphatidyl inositol and phosphatidyl ethanolamine in n-butanol-water (Blaustein, 1967) incubated with ^{3}H-CPZ followed by partition against water also gave a similar partition of about 10 : 1 in favour of the organic layer.

These results have led us to believe that the phenomenon of accumulation of CPZ in rat brain subfractions is a solubility effect of the CPZ in the proteolipid of the membranes.

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The effect of L-tryptophan on changes in motor activity caused by parachlorophenylalanine

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L-tryptophan administration increases brain 5-hydroxytryptamine (5-HT) turnover without effect on either motor activity (Modigh, 1973) or sensitivity to electric shock (Hole & Marsden, 1975). Intravenous infusion of L-tryptophan into normal human subjects also has relatively little influence on the results of objective psychological tests though EEG changes were noted (Greenwood, Friedel, Bond, Curzon & Lader, 1974). It is possible that L-tryptophan may have more striking effects when 5-HT metabolism is deficient. This would be consistent with the postulated 5-HT defect in depression and the reported beneficial effect of tryptophan on it (Coppen, 1972). We have studied the effect of L-tryptophan on motor activity in rats previously given p-chlorophenylalanine (PCPA) at a dosage that increased activity (Fibiger & Campbell, 1971) while only partially inhibiting 5-HT synthesis.

Male Sprague-Dawley rats (180-200 g) were housed in groups of three under a 12 h light-dark cycle at 24° C. After 4 days the following drug schedule was adopted: (1) PCPA (150 mg/kg i.p.) followed by L-tryptophan (150 mg/kg i.p.) 24 h later. (2) PCPA (150 mg/kg) plus vehicle (2.5% Tween in 0.9% saline) 24 h later. (3) Saline (0.9%) plus L-tryptophan (150 mg/kg) 24 h later. (4) Saline plus vehicle 24 h later. In one experiment L-tyrosine was given instead of L-tryptophan. Motor activity was measured simultaneously in control and treated rats with Animex DSE activity meters. Activity recording was started 15 min after the last injection (at 9.45 or 11.45 h) and continued for 120 minutes. At the end of this period the animals were killed and brain tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) measured (Curzon, Joseph & Knott, 1972).

L-tryptophan alone caused no significant change in motor activity while PCPA alone caused a significant increase which was almost completely reversed by L-tryptophan but not by L-tyrosine. L-tryptophan caused increases in brain tryptophan (+545%), 5-HT (+50%) and 5-HIAA (+108%). PCPA altered brain tryptophan by −26%, 5-HT by −59% and 5-HIAA by −66%. L-tryptophan markedly diminished the biochemical effects of PCPA, both 5-HT and 5-HIAA returning towards control values (−19% and −10% respectively). While L-tyrosine caused a significant increase in tyrosine (+76%) there was no significant change in brain tryptophan, 5-HT and 5-HIAA. Similarly, the tryptophan, 5-HT and 5-HIAA values in rats given PCPA were not significantly different from those in rats given PCPA and L-tyrosine.

Fibiger & Campbell (1971) showed that the effects of PCPA on motor activity were reversed