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Destruction of chlorpromazine during absorption in the rat *in vivo* and *in vitro*

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Summary

1. Concentrations of total radioactivity in plasma of rats given intravenous and oral ^{35}S-chlorpromazine, were similar. Concentrations of unchanged drug, however, were lower after oral doses.

2. Chlorpromazine circulated in solution through isolated loops of rat intestine was rapidly absorbed by the tissue. Measurements of glucose transport and histological examination indicated that the tissue was intact. In these *in vitro* experiments some of the chlorpromazine was converted to products, which together with unchanged drug, were partly retained in the intestinal wall and partly transferred to the serosal side of the tissue. Observations at three concentrations supported the hypothesis that transfer of unchanged drug occurred by passive diffusion.

3. Conversion of chlorpromazine to metabolites in the intestine *in vivo*, would account for the differences in concentrations of chlorpromazine and total radioactivity in plasma after oral doses.

Introduction

It is commonly supposed that orally administered chlorpromazine is rapidly and well absorbed in man and animals, although there appears to have been no systematic investigation of the absorption phenomenon (Goodman & Gilman, 1965; Shepherd, Lader & Rodnight, 1968). Results of recent studies of chlorpromazine concentrations in plasma of man and animals after parenteral and oral doses suggested that incomplete absorption of oral doses occurs; concentrations of unchanged drug after injected doses were 3–10 times those after oral doses (Curry, Davis, Janowsky & Marshall, 1970). In contrast, urinary excretion studies in man indicated complete absorption, either as unchanged drug or metabolites; excretion rates of metabolites after oral and intramuscular doses were similar (Hollister, Curry, Derr & Kanter, 1970). One possible explanation for these apparently inconsistent sets of data would be that chlorpromazine is converted to absorbable metabolites during the process of absorption. This report describes experiments designed to examine this possibility.

Methods

In vivo *experiments*

Male Sprague-Dawley rats weighing 150–250 g were fasted for 24 h before use. They were then given intravenous (tail vein) or oral (stomach tube)

doses of ^{35}S-chlorpromazine hydrochloride (Radiochemical Centre, Amersham, England) (approximately 5 mCi/mg) (10 mg/kg in 0·9% NaCl; concentration of solution 2 mg/ml). At chosen intervals (Figs. 1 and 2) the rats were anaesthetized lightly with ether, and blood (up to 3 ml) was drawn by cardiac puncture and placed in tubes containing heparin (lithium salt). Plasma was separated by centrifugation.

In vitro *experiments*

Lengths (30 cm) of jejunum were isolated from male Sprague-Dawley rats weighing 150–250 g (technique of Fisher & Parsons, 1949). Krebs bicarbonate fluid (60 ml, pH 7·4, 37° C) containing ^{35}S-chlorpromazine hydrochloride (approximately 1 mCi/mg) (50, 100 and 200 μg/ml) was circulated by a gas lift (95% O_2/5% CO_2) through the lumen of each preparation. The serosal surface was bathed with Krebs bicarbonate fluid (25 ml) containing no chlorpromazine hydrochloride. The Krebs solution contained (g/l.): NaCl, 6·9; KCl, 0·35; $CaCl_2$, 0·14; KH_2PO_4, 0·16; $MgSO_4 \cdot 7H_2O$, 0·15; and $NaHCO_3$, 2·1. Both solutions contained glucose (5 g/l.). Samples of luminal and serosal solutions were taken at intervals (Fig. 3). At the end of the experiments, the tissue samples were homogenized with 4 volumes of 0·05 N HCl.

Chemical methods

In the *in vitro* experiments, chlorpromazine was assayed specifically by gas chromatography (Curry, 1968). Total radioactivity in plasma, luminal and serosal solutions, and in the tissue homogenates, was determined by direct sampling of 0·5 ml of the solutions. For the specific assay of chlorpromazine by radioactivity measurements (*in vivo* experiments), 1 ml samples of these materials were examined by selective solvent extraction (Curry, Derr & Maling, 1970).

All measurements were expressed as chlorpromazine hydrochloride by comparison with solutions of known strength and specific activity. Radioactivity was determined by liquid scintillation spectrometry. Standard quenching corrections were made. The liquid scintillation fluid, 15 ml per vial, consisted of toluene containing: 2,5-bis-[5′-tert-butylbenzoxazolyl (2′)]-thiophene (BBOT), 0·4%; naphthalene, 0·8%; and cellosolve, 40%. The gas chromatographic method analysed chlorpromazine with a recovery of 100% and a standard deviation of 6%. The extraction and radioactivity measurements also analysed chlorpromazine with a standard deviation of 6%; recovery was 72% and appropriate corrections were made in the calculations. Single assays of radioactivity and chlorpromazine in each sample were carried out.

Glucose in the luminal and serosal fluids was determined using glucose oxidase, peroxidase and a chromogen as supplied in a Biochemica Test Combination (Boehringer Mannheim GMBH).

Histology

Tissues were fixed in 10% formol saline and representative sections were stained with haematoxylin and eosin.

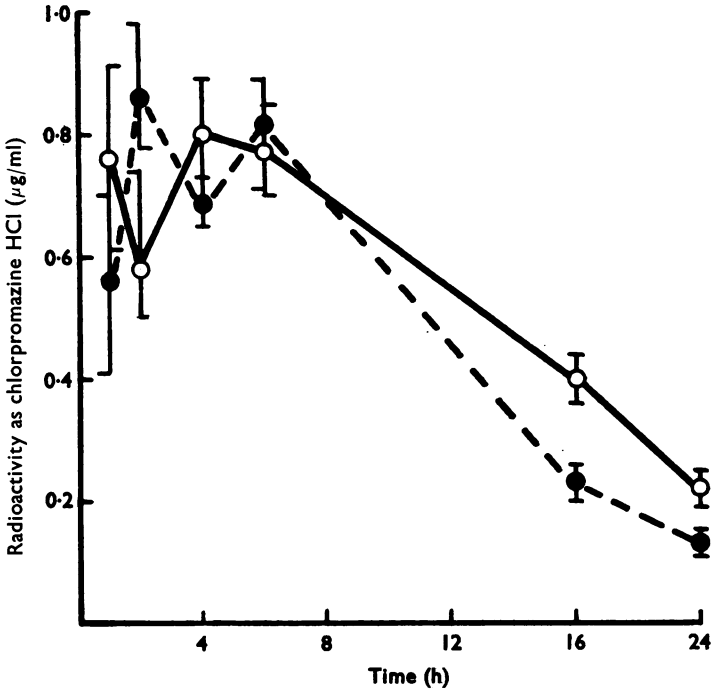

FIG. 1. Total radioactivity (expressed as chlorpromazine hydrochloride) in rat plasma after intravnous (○——○) and oral (●- - -●) administration of ^{35}S-chlorpromazine hydrochloride (10 mg/kg). Each point is the mean of values from four–six rats ± S.E.

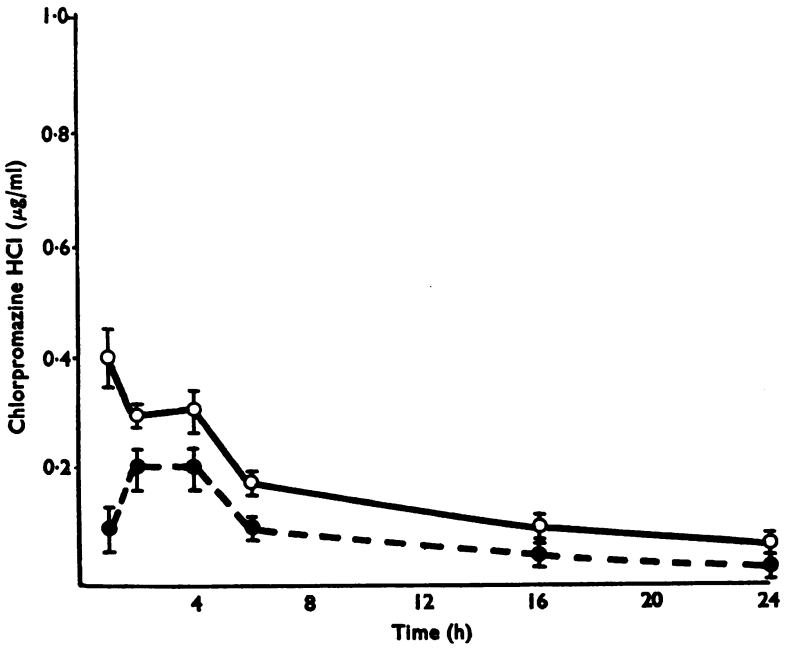

FIG. 2. Chlorpromazine (expressed as hydrochloride) in rat plasma after intravenous (○——○) and oral (●- - -●) administration of 10 mg/kg. Each point is the mean of values from four–six rats ± S.E.

Results

Concentrations of radioactivity and chlorpromazine in plasma

Plasma radioactivity concentrations were comparable after intravenous and oral doses (Fig. 1). This indicated that the orally administered radioactivity was absorbed into the general circulation rapidly and probably completely. Plasma chlorpromazine concentrations were lower after the oral doses (Fig. 2). This indicated that the orally administered chlorpromazine was not absorbed completely into the general circulation as unchanged drug. It appears that chlorpromazine was chemically or biochemically changed during transfer from the intestinal lumen to the general circulation, and that the products of this change were transferred rapidly and probably completely.

Chlorpromazine absorption in vitro

Radioactivity and unchanged chlorpromazine concentrations declined rapidly in the luminal solutions in the *in vitro* experiments (Fig. 3). The radioactivity and the chlorpromazine were both partially localized in the intestinal material and partially transferred to the serosal solutions (Fig. 3). Unchanging concentrations in the luminal solutions were reached in approximately 30 min although unchanging concentrations were not reached in the serosal solutions during the 60 min of the experiment. Serial samples of the intestinal material were not possible, so it is not known whether an unchanging concentration was reached in the tissue.

Concentrations of radioactivity were higher than concentrations of chlorpromazine by variable proportions. The difference was smallest in the luminal solutions ; at

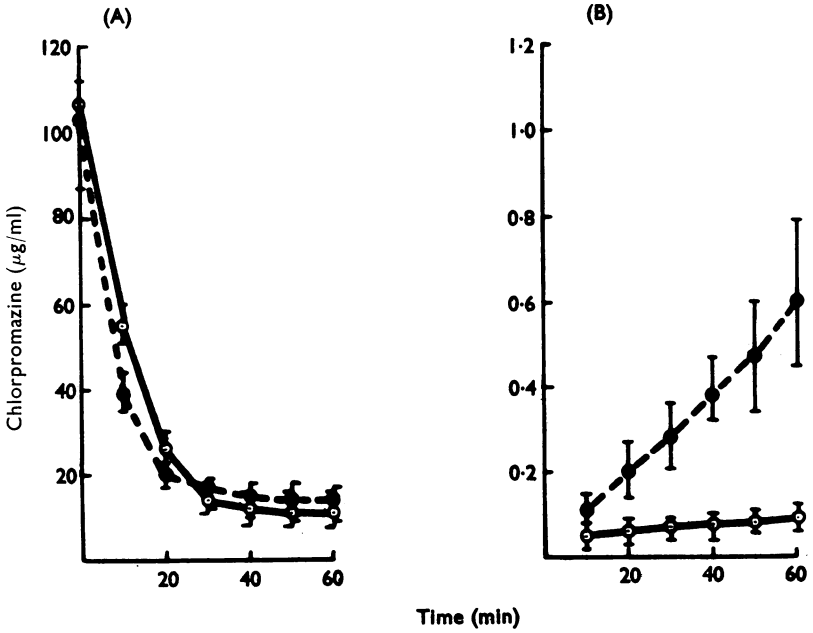

FIG. 3. (A) Disappearance of radioactivity (●– – –●) and chlorpromazine (○——○) from luminal solutions of ^{35}S-chlorpromazine hydrochloride (100 μg/ml) circulated through loops of rat intestine. Each point is the mean of values from four experiments ± S.E. (B) Appearance of radioactivity and chlorpromazine in the serosal solutions in the same experiment.

the two higher substrate concentrations, all the luminal radioactivity was in the form of unchanged chlorpromazine. At the other extreme, radioactivity appeared in the serosal solution much more rapidly than did unchanged chlorpromazine; at the end of the experiments at three substrate concentrations, a mean of 24% of the serosal radioactivity was present as unchanged drug, with a lower percentage at the lower substrate concentrations, and a higher percentage at the higher substrate concentrations.

Concentrations of unchanged chlorpromazine, at the end of the experiments, in the luminal solutions, intestinal material, and serosal solutions, were proportional to the amounts of chlorpromazine originally present. The mean concentration ratios for intestinal to luminal, and intestinal to serosal distributions were 7·5 and 893 respectively (Table 1).

The amounts of chlorpromazine and total radioactivity in the three compartments (luminal solution, intestinal wall and serosal solutions) in the experiments at the various concentrations were totalled. To account, at this point, for all the radioactivity included in each experiment, it was necessary to include radioactivity adsorbed on the surface of the apparatus. At the end of each experiment, the adsorbed chlorpromazine was washed out of the apparatus with 0·1 N HCl, and assayed for total radioactivity and unchanged chlorpromazine as described for luminal and serosal solutions (above). Adsorbed radioactivity constituted a considerable proportion (>50%) of the total radioactivity present. It was entirely accounted for as unchanged drug. The sum of the radioactivity in the four compartments (washings, luminal solution, intestinal wall and serosal solutions) was equal to the amount of radioactivity originally present as chlorpromazine.

For each experiment, the difference between radioactivity present initially and that in unchanged chlorpromazine was recovered in the metabolites formed. The range of amounts of metabolites was not great, even over a range of doses (3–12 mg); proportional conversion to metabolites varied inversely with substrate amount (Table 2).

TABLE 1. *Concentrations of chlorpromazine in the luminal and serosal solutions, and in the intestinal wall, at the 60 min interval in absorption experiments* (in vitro) *with three different substrate amounts*

Substrate amount (mg)	Chlorpromazine concentration (μg/ml)		
	Luminal solution	Serosal solution	Intestine
3	5·6±0·2	0·03±0·01	34·6± 3·4
6	15·1±1·2	0·13±0·02	107·0±19·4
12	24·4±1·6	0·22±0·03	198·0±35·0

Each figure is the mean of values from four experiments±S.E.

TABLE 2. *Amounts of radioactive metabolites formed from chlorpromazine during the experiments shown in Table* 1

Substrate (amount in mg)	Metabolites (amount in mg)	Proportion converted to metabolites (%)
3	0·49±0·10	16·3±3·3
6	0·60±0·18	10·0±2·9
12	0·35±0·10	2·9±0·9

Each figure is the mean of values from four experiments±S.E.

Histology and glucose absorption

Histological examinations of sections of tissues prepared after experiments *in vitro* for 1 h with or without chlorpromazine indicated that the mucosa and villi were intact (Fig. 4). The histological appearance was similar to that reported by Fisher &

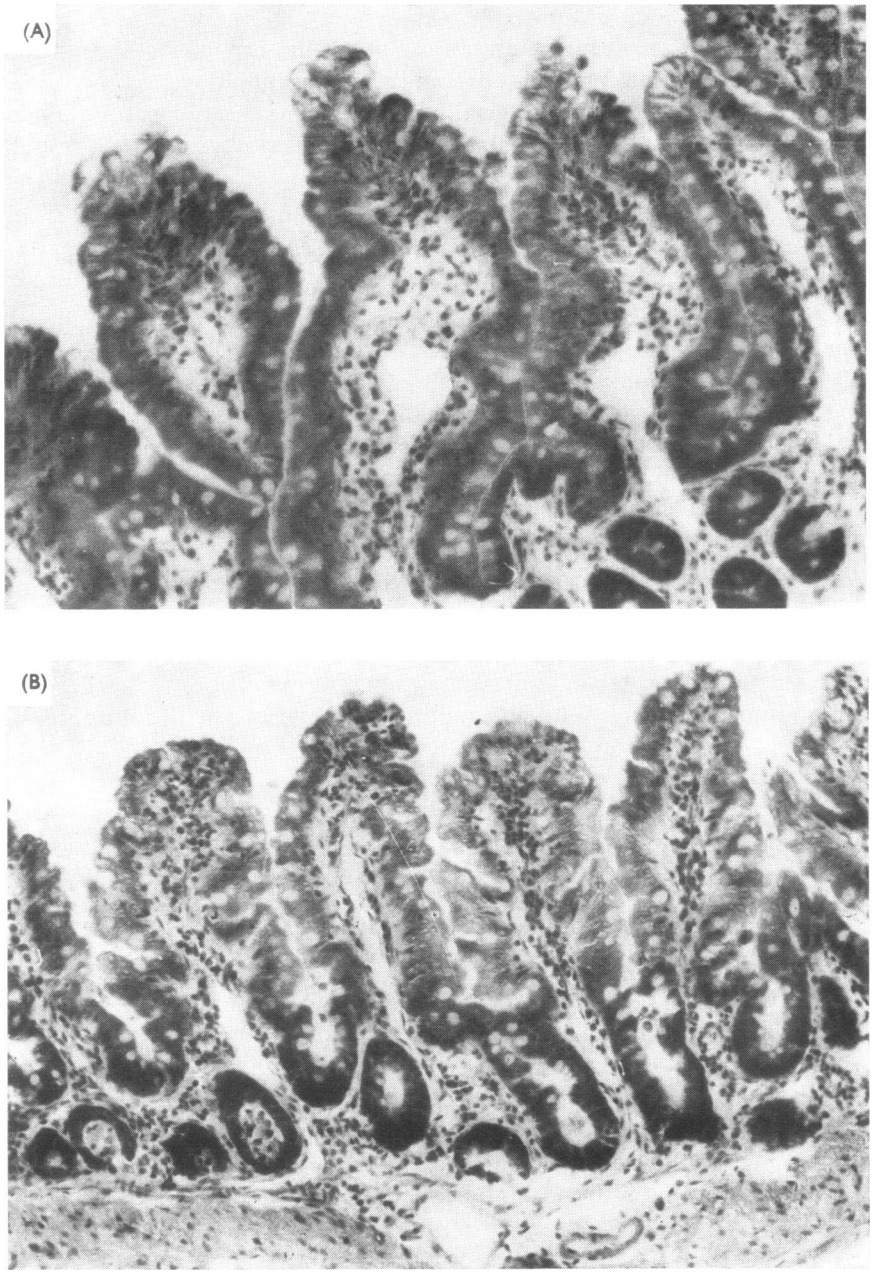

FIG. 4. Rat small intestine showing appearance of the tissue after survival for 1 h. In (A) the luminal fluid contained no chlorpromazine. In (B) it contained 200 μg/ml of chlorpromazine. It is apparent that the drug did not damage the mucosa or the villi.

Parsons (1949), there being some evidence of disengagement of cells from the mucosa at the tips of the villi. This has been associated with the normal, continual replacement of the mucosa.

The ability of the intestine to absorb glucose from the lumen and actively transfer it against a concentration gradient to the serosal fluid was further evidence of structural and functional integrity of the mucosa. Thus the initial concentrations of glucose in the luminal and serosal fluids were equal; at the end of the experiments the glucose content of the luminal fluid decreased and that of the serosal fluid increased so that the final concentration of glucose in the serosal fluid was 2–3 times that in the luminal fluid. The rate of absorption of glucose from the luminal fluid containing various concentrations of chlorpromazine and the rate of transfer into the serosal fluid were, with one exception, similar to measurements carried out in control experiments with no chlorpromazine (Table 3).

Discussion

The area under a graph of concentration of drug in plasma against time is proportional to the dose administered. This proportionality has been described as 'the law of corresponding areas', and used to assess proportional absorption of orally and parenterally administered acetylsalicylic acid (Harris & Riegelman, 1969). Application of this 'law' to the radioactivity and chlorpromazine data obtained in these experiments indicated that: (1) the orally administered radioactivity was rapidly and completely absorbed, since the areas under the graphs of concentration in plasma against time after oral and intravenous doses were similar; and (2) the orally administered chlorpromazine was not completely absorbed as such, since the area under the graph of concentration in plasma against time after the oral dose was less than 50% of the area under the corresponding graph after the intravenous dose. Since the radioactivity was part of the chlorpromazine molecule, the compound had obviously been metabolized during transfer from the gastro-intestinal lumen to the general circulation. Transfer of the products of this metabolism occurred freely.

There are several possible sites *in vivo* for metabolism during transfer between the gastro-intestinal lumen and the general circulation: (1) in the lumen, with or without assistance from intestinal flora; (2) in the intestinal wall; and (3) in the hepatic portal system. Complete transfer of chlorpromazine to the general circulation as unchanged drug when administered intraperitoneally in rats had already been demonstrated (Curry *et al.*, 1970). It is noteworthy that in both these and our experiments, almost identical techniques were used, and almost identical reference data (chlorpromazine concentrations in plasma after intravenous doses) were obtained. Complete transfer after intraperitoneal administration seemed to rule out

TABLE 3. *Rates of absorption of glucose from the lumen of the rat intestine* in vitro *and rates of transfer of glucose into the serosal fluid at various luminal chlorpromazine concentrations*

Initial concentration of chlorpromazine in lumen fluid (μg/ml)	Rate of absorption of glucose from lumen fluid ((mg/cm)/h)	Rate of transfer of glucose into serosal fluid ((mg/cm)/h)
0	4·11±0·17	2·00±0·23
50	3·72±0·20	2·47±0·21
100	3·92±0·13	2·66±0·12*
200	3·60±0·36	1·33±0·27

Data are reported as mean values±S.E. of five experiments. *Significantly different from control (*t* test; $P<0·05$).

(in the present context) metabolism of chlorpromazine during absorption through the hepatic portal circulation, as a proportion of the intraperitoneally administered dose would have reached the general circulation by this route, and would therefore have been exposed to similar destructive factors.

The *in vitro* experiments demonstrated the occurrence of decomposition without the presence of a hepatic portal system. It is thought that decomposition occurred in the intestinal wall by biochemical mechanisms not involving intestinal flora, for the following reasons: (1) only very small quantities of products were found in the luminal solutions, so that if they were formed in these solutions, they were absorbed remarkably rapidly into the intestinal material; (2) the possibility of participation by bacterial flora was reduced by starving the rats overnight in the *in vivo* experiments, and by diluting the luminal contents considerably in the *in vitro* experiments; (3) formation of products (Table 2) had the characteristics of a saturable, enzyme catalysed process, rather than the characteristics of chemical decomposition. Further experiments will be needed to confirm the nature of the mechanism of chemical change in chlorpromazine, and the knowledge that decomposition took place in living, intact intestinal material will be an important factor in the planning of these experiments.

Unchanged chlorpromazine was apparently transferred between the intestinal lumen and the serosal solutions by passive diffusion. This is shown by the remarkably consistent proportionality of the final concentrations of the drug in luminal and serosal solutions, and in the intestinal material. Passive diffusion of chlorpromazine would be in accordance with general concepts of drug absorption (Binns, 1964; Brodie, 1967). Concentrations in the intestinal material remained high, presumably because of drug binding. Concentrations in the serosal solutions were low. A more rapid transfer is presumably achieved *in vivo* as the result of having: (1) a much larger 'pool' (the whole body); and (2) plasma protein, to provide competing binding sites on the serosal side. Chlorpromazine is firmly bound to plasma protein and extensively localized in tissues (Curry, 1970; Curry *et al.*, 1970). Plasma concentrations of unchanged drug indicated that absorption had ceased to occur within 4 h of administration of the oral doses.

These studies should be considered in the interpretation of the chlorpromazine concentrations in human plasma mentioned in the **Introduction**. Metabolism of the type discussed, if it occurred in man, could be the reason for concentrations of unchanged chlorpromazine in plasma after oral doses being lower than those after parenteral doses. Transfer of the products to the general circulation after formation would account for the 24 h excretion rates of metabolites after the two doses being similar, since both oral and intravenous doses are eventually completely metabolized.

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