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The gastrointestinal absorption of paracetamol in the rat

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The absorption of [^{3}H]paracetamol by rat small intestine, colon and stomach was studied in vivo and in vitro. Small intestinal in vivo studies, using a wide range of drug concentrations, showed that absorption was efficient and uniform throughout the small bowel, no site showing preferential absorption. Double reciprocal and direct plots indicated first order kinetics. The pattern was not observed when uptake was occurring from high concentrations of paracetamol in suspension. Gastric and colonic in vivo studies showed that there was appreciable absorption of [^{3}H]paracetamol from these sites. In vitro studies using everted intestinal sacs showed no effect on paracetamol transfer when the incubation temperature was lowered to 10 °C or when iodoacetate (5×10^{-2}M) and 2·4 dinitrophenol (5×10^{-4}M) was added to the incubation medium. There was, however, a significant reduction in transfer of paracetamol against a concentration gradient of 10 : 1 applied across the mucosa. These data suggest that the uptake of paracetamol is by a passive transport process and confirm the efficiency of paracetamol absorption observed indirectly by others.

Paracetamol is a weak acid (pK_a 9·5) of low molecular weight, soluble in both aqueous and lipid media; these properties indicate that under the conditions found in the gastrointestinal tract, rapid and efficient absorption might be expected. Although it is a widely prescribed analgesic, little attention has so far been paid to the mechanism by which it is absorbed from the gastrointestinal tract.

Studies in man, in which absorption was indirectly assessed by plasma concentrations of the drug, suggest that paracetamol is indeed rapidly and efficiently absorbed in that more than 70% of a 1·5 g oral dose can be recovered from the urine within 24 h (Heading et al 1973). Similar work suggests that the rate of absorption of paracetamol may be altered by dietary constituents (Jaffe et al 1971), the presence of gastric contents (McGilveray & Mattok 1972), and by drugs affecting the rate of gastric emptying (Nimmo et al 1973). Animal experiments (Weikel & Lish 1959) have not indicated a specific site for the absorption of paracetamol nor have they elucidated the mechanism of mucosal transfer of the drug but they have shown that it is well absorbed from the small intestine.

Paracetamol is not altered during transport across the intestinal mucosa into the systemic circulation (Levy 1971) and thus absorption may be studied by measuring disappearance from the intestinal lumen.

This report presents the results of in vivo and in vitro studies on the mechanism and site of absorption of [^{3}H]paracetamol by rat small intestine, stomach and colon.

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MATERIALS AND METHODS

[^{3}H]*N*-Acetyl-*p*-aminophenol (spec. act. 0·147, μCi nM^{-1}), labelled on the side chain was supplied by New England Nuclear Limited. Purity as assessed by thin layer chromatography exceeded 96%. Non-labelled paracetamol (Winthrop-paracetamol B.P.) and the tritiated drug were prepared as separate stock solutions in ethyl acetate.

Appropriate quantities of these solutions were evaporated under nitrogen and resuspended for further use in Krebs-Ringer buffer pH 6·6 to give a range of paracetamol concentrations from $1{\cdot}5 \times 10^{-1}$–$1{\cdot}98 \times 10^3$ μmol ml^{-1} (0·04 μCi ml^{-1}).

Concentrations of paracetamol above 130 μmol ml^{-1} exceeded aqueous solubility at 37°C and were prepared as suspension in 0·2% tragacanth, which, in control experiments, was shown not to affect absorption of paracetamol. Radioactive tissue samples and body fluids were processed by combustion in a Packard Tricarb Sample Oxidiser (Model 306), recovery always exceeded 95% (range 95–99%). The collected tritiated water was counted in a Packard Tricarb scintillation spectrometer (Model 3375) equipped with automatic external standardisation.

In vivo studies

(a) *Small intestine.* Sixty non-fasted female Wistar rats 150–200 g, were used. Light ether anaesthesia

was maintained throughout all the experiments. At laparotomy the entire small intestine was washed through with Krebs-Ringer buffer at 37 °C. Closed intestinal loops were tied at intervals along the intestine preserving the blood supply to each loop. The loops were injected with 0·2 ml of the test [^{3}H]paracetamol solution and replaced in the abdominal cavity for intervals of 1–30 min. At the end of the incubation periods the animals were killed by ventriculotomy, the loops removed and the radioactivity remaining in the loop wall and contents measured separately. Absorption was expressed as the difference in activity between the injected dose and that recovered from the intestinal loop and its contents. Studies were made of the effects of pH and increasing paracetamol concentration.

(b) *Colon and stomach.* Closed loops were constructed from proximal and distal colon, and the stomach was treated as a closed loop after tying ligatures at the cardia and pylorus. The experimental design was as used for the intestine.

In vitro studies

Using everted intestinal sacs (Wilson & Wiseman 1954) mucosal to serosal and serosal to mucosal transfer of paracetamol were studied. Further observations were made with a concentration gradient of paracetamol of 10:1 across the intestinal wall in each direction. The effects of metabolic inhibitors, 2,4-dinitrophenol and iodoacetate, and of decreased temperature (10 °C) on paracetamol transfer were also studied. Absorption was expressed either as the percentage of the administered dose or as μmol drug absorbed per intestinal loop.

RESULTS

(1) *In vivo studies*

(a) *Small intestine.* Using an incubation time of 30 min increasing the concentration of paracetamol injected from 1·5–100 μmol ml^{-1} resulted in a linear increase in net absorption from 0·25 ± 0·004 μmol to 14·7 ± 0·18 μmol per loop corresponding to an overall absorption of 70% of the injected dose (Fig. 1). When the amount of drug injected was increased further (Fig. 1) above a concentration of 130 μmol ml^{-1}, the solubility of paracetamol in Krebs-Ringer buffer pH 6·6 at 37 °C was exceeded and absorption from the tragacanth suspension became non-linear reaching a plateau such that at a concentration of 2000 μmol ml^{-1} only 66·5 ± 0·97 μmols of paracetamol were absorbed/loop, representing 16% of the injected dose.

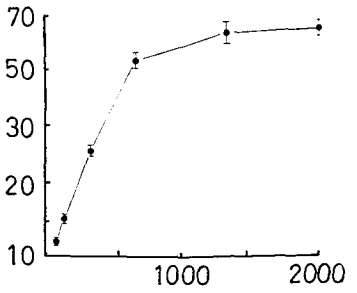

FIG. 1. Absorption of [^{3}H]paracetamol in vivo by rat small intestinal loops between concentrations of 1·5–2000 μmol ml^{-1} (abscissa). Volume of injected drug solution 0·2 ml; incubation time 30 min. Results are given as mean ± s.e.m. of 20 observations. Ordinate: absorption (μmol/loop).

Examination of the results in Fig. 1 from 1·5–100 μmol ml^{-1} both by a double reciprocal plot of concentration and rate of absorption and by the direct linear plot described by Eisenthal & Cornish-Bowden (1974) demonstrated uptake consistent with a passive transport process.

Alteration of pH of the incubation medium between 5·2 and 8·5 had no effect on absorption. Throughout the length of the small intestine from the ligament of Treitz to the ileo-caecal valve there was no selective site for absorption.

(b) *Colon and stomach.* Using a concentration of 50 μmol ml^{-1} and an incubation time of 30 min proximal colonic loops showed a net absorption of 29 ± 0·89% and distal loops 33·5 ± 0·99% of the injected dose. The difference between these values was not significant ($P < 0·1$). Under the same conditions absorption from the stomach was 22·5 ± 1·29% of the injected dose.

(2) *In vitro studies*

Fig. 2 summarizes the results of in vitro observations on the uptake of paracetamol by everted intestinal sacs. Transfer of labelled drug at a concentration of 10 μmol ml^{-1} with an incubation time of 1 h was measured in each direction across the intestinal wall.

Serosal to mucosal transfer was 1·79 ± 0·08 μmol/loop and mucosal to serosal transfer was 1·83 ± 0·13 μmol/loop; there was no significant difference between these results ($P > 0·1$).

Transfer of [^{3}H]paracetamol was then measured when a concentration gradient was established across the intestinal wall. When unlabelled paracetamol at a concentration of 100 μmol ml^{-1} was injected into isolated intestinal sacs and incubated for 1 h in Krebs-Ringer buffer containing [^{3}H]paracetamol at a

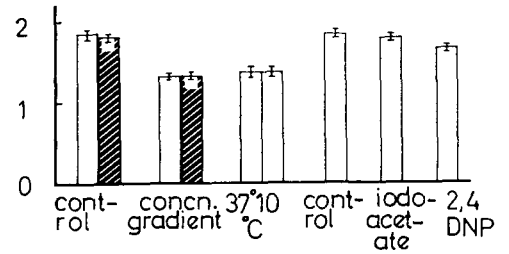

FIG. 2. In vitro uptake of [^{3}H]paracetamol (ordinate: μmol/sac) by everted rat small intestinal sacs. Open columns represent mucosal to serosal transfer and hatched columns serosal to mucosal transfer. Uptake has been studied in the presence and absence of a concentration gradient at different temperatures and in the presence of metabolic inhibitors. Results are the mean of six observations and the vertical bars represent s.e.m.

concentration of 10 μmol ml^{-1}; 1·41 $\pm$ 0·08 μmol of labelled drug appeared within the intestinal sac. When the experiment was repeated with the solutions reversed, 1·41 $\pm$ 0·1 μmol drug was transferred across the intestinal wall. There was, therefore, a significant reduction in the transfer of paracetamol across the mucosa against a concentration gradient when compared with the previous control experiments ($P < 0·05$). When the temperature of the incubation bath was lowered to 10 °C, the mean uptake of paracetamol was 1·43 $\pm$ 0·15 μmol/loop. In a simultaneous control experiment at 37 °C the mean uptake was 1·43 $\pm$ 0·04 μmol/loop ($P > 0·1$).

Transfer of drug in the presence of 2,4-dinitrophenol occurred at 1·69 $\pm$ 0·13 μmol/loop h^{-1} and in the presence of iodoacetate 1·79 $\pm$ 0·13 μmol/loop h^{-1}. In a simultaneous control experiment 1·83 $\pm$ 0·02 μmol/loop h^{-1} of paracetamol was transferred. There was no statistically significant difference ($P > 0·1$) between these results.

DISCUSSION

Studies of paracetamol absorption in man (Heading et al 1973; Jaffe et al 1971; McGilveray & Mattok 1972; Nimmo et al 1973) have, in general, been directed towards factors influencing absorption rather than identifying the mechanisms involved. Heading et al (1973) demonstrated the importance of the rate of gastric emptying on the rate of paracetamol absorption. In 1972, McGilveray & Mattok showed that fasting significantly increased the rate of absorption but not the total amount absorbed, when compared with ingestion of the drug after food. Jaffe et al (1971) demonstrated that meals containing large amounts of carbohydrate, particularly pectin, reduced the rate of paracetamol absorption as measured by urinary excretion of the drug, while high protein or fat diets had no effect.

We have shown that paracetamol is rapidly absorbed over a wide range of concentrations as long as the drug remains in solution, approximately 70% of the dose disappearing in 30 min. These results are in accord with those of Miller & Fischer (1974), who showed that 80% of a 25 mg kg^{-1} dose of paracetamol administered orally to rats was recovered in the urine within 24 h.

Our data indicate that paracetamol is absorbed by passive transport from the gastrointestinal tract of the rats as suggested by the following evidence:

(1) Absorption in vivo from intestinal loops was linear over a wide range of substrate concentrations (1·5–100 μmol ml^{-1}). Recalculation of the data by double reciprocal plot showed passive uptake by the mucosa in accordance with first order kinetics. This is in keeping with the data of Albert et al (1974) who showed that in man paracetamol absorption estimated by plasma concentrations, appeared to be a first order process.

(2) In our experimental conditions, paracetamol was well absorbed from all parts of the gastrointestinal tract including the stomach and colon, although the most efficient uptake of paracetamol occurred in the small intestine. No particular site of preferential absorption was demonstrated. These findings are at variance with the work of Weikel & Lish (1959) who fed paracetamol with a non-absorbable marker in a test meal to rats and determined the amount of drug remaining in the various parts of the gut at timed intervals. Their results suggested increasing efficiency of absorption of paracetamol from the stomach to the middle of the small intestine, thereafter absorption decreased. Their experimental design did not, however, permit a demonstration of the absorptive capacity for paracetamol of the various parts of the gastrointestinal tract, as each successive intestinal segment was presented with a lower concentration of the drug.

(3) The transport of paracetamol in vitro across the intestinal wall was ambidirectional and was not significantly altered by the presence of metabolic inhibitors or by low temperature. Transfer of paracetamol was significantly reduced in the presence of a concentration gradient.

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CORRIGENDUM

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Improved gas-chromatographic method and micro-extraction technique for the measurement of nicotine in biological fluids

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p. 75 right-hand column 3rd paragraph

To samples (100 μl) in a Dreyer tube were added an aqueous solution of quinoline (0·075 μg ml^{-1}; 100 μl) as internal standard, sodium hydroxide (5M; 400 μl) and di-isopropyl ether (**50** μl). After agitation on a Vortex mixer (1 min) the tube was centrifuged (1 min) and 5 μl of the organic layer was injected onto the gas chromatograph.