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THE EXCRETION OF ^{3}H-PAPAVERINE IN THE RAT

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Abstract—The excretion of ^{3}H-papaverine has been studied in the rat. After per oral as well as parenteral administration about 85 per cent of the administered radioactivity is excreted in faeces and urine in 4 days, and only negligible amounts of this radioactivity consist of unchanged ^{3}H-papaverine; most of the radioactivity is recovered in the faeces in the first 24 hr.

After an intravenous dose of ^{3}H-papaverine, about 70 per cent of the tritium is excreted in the bile in 6 hr. All this radioactivity is due to conjugated metabolites, which after hydrolysis with glusulase, give five peaks on thin layer chromatograms. After intraduodenal administration of these conjugated metabolites, a very small absorption occurs, while after administration of the hydrolysed metabolites about 60 per cent of the dose is excreted in the bile. After intramuscular injection of ^{3}H-papaverine radioactivity in the intestine follows quite good the time pattern of excretion of tritium in the bile. No significant difference was observed between control and bile cannulated rats with regard to the blood levels of radioactivity and ^{3}H-papaverine. These results suggest that the bile is the main route of excretion of papaverine metabolites and that enterohepatic circulation of these metabolites is not important.

AS EARLY AS 1915, Zahn[1] reported that in the urine of rabbit, cat and dog, no unchanged papaverine could be detected. Using a biological assay of papaverine, Levy[2] in 1945 found traces of unchanged papaverine in the rabbit, while Elek and Bergman[3] did not find papaverine in the urine in man. Axelrod *et al.*,[4] using a specific spectrophotometric method for measuring papaverine, and reported in detail about the drug's metabolic fate in animals and man; by enzymatic demethylation in the liver, papaverine is transformed to phenolic compounds; the glucuronides of the phenolic compounds found in the urine account for approximately 50–60 per cent of the administered dose of papaverine.

However, for a detailed study of the pharmacokinetics of papaverine, the spectrophotometric assay method is not sensitive enough. We therefore synthetized tritium-labelled papaverine; the results of a study concerning the biliary, faecal and urinary excretion after administration of the labelled compound in the rat are reported.

MATERIALS AND METHODS

^{3}H-papaverine. Specifically tritium-labelled papaverine was prepared from 6'-bromo-papaverine[6] by hydrogenolysis of bromine by tritium gas as indicated in Fig. 1. The specific activity of the labelled drug was about 1·6 Ci/m-mole; the radiochemical purity was more than 95 per cent. Immediately before use, the compound is diluted with cold papaverine hydrochloride to obtain a specific activity of 25 μCi/mg.

Animals and experimental procedures. Wistar male rats (200–300 g) were used. The dose of ^{3}H-papaverine was 5 mg/kg, being 125 μCi/kg.

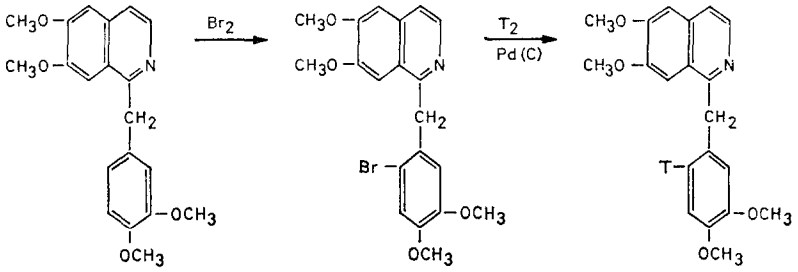

FIG. 1. Synthesis of tritium-labelled ^{3}H-papaverine.

For collection of urine and faeces, the animals were placed in individual metabolism cages. Standard laboratory chow and water were given *ad lib.* Papaverine was administered orally (via stomach tube), intraperitoneally or intramuscularly.

For collection of bile, the common bile duct was catheterized through a midline incision. This was done in anaesthetized rats (urethane, 1250 mg/kg i.p.) and in unanaesthetized rats; in the latter group, cannulation of the bile duct was done under ether anaesthesia and the animals were kept in restraining cages. ^{3}H-papaverine was given by the intravenous or intramuscular route. In a group of anaesthetized rats, biliary excretion was followed after intraduodenal administration of bile obtained from other papaverine-treated animals.

Finally some rats were decapitated at different times after intramuscular injection of ^{3}H-papaverine: the intestine was removed, and the first part of the small intestine, the second part of the small intestine and the large intestine, with their contents, assayed separately.

Blood samples were obtained in urethane-anaesthetized rats from the cannulated carotid artery.

Extraction of papaverine. Papaverine was extracted specifically (i.e. without its metabolites) as described by Axelrod *et al.*[4] a 100 μl aliquot of bile, urine or blood was pipetted in a 15 ml glass stoppered centrifuge tube containing 0·9 ml of water, 1 ml of NaOH 0·2 N, 1·5 g NaCl and 10 ml heptane containing 2% isoamyl alcohol. The mixture was shaken for 10 min, centrifuged and 5 ml of the organic layer used for counting. Recovery of the extraction procedure varies from 90–100 per cent.

Thin layer chromatography. 100 μl of bile was adjusted with 1 ml phosphate buffer 0·5 M, pH 7·4, and shaken with 10 ml chloroform. After centrifugation 8 ml of the organic layer was evaporated to dryness and the residue was dissolved in 1 ml of ethylalcohol.

An aliquot of 100 μl was spotted on Silica gel plates (250 μ thickness; E. Merck, A. G. Darmstadt, Germany). Ascending chromatography was performed with the solvent system chloroform–dioxane–ethylacetate–ammonia (25:60:10:5).

Some bile samples were incubated with glusulase (10,000 units, Serva), pH 5, 37°, for 20 hr, before extraction and thin layer chromatography.

Estimation of radioactivity. All radioactivity measurements were carried out on a Packard Tricarb Liquid Scintillation Spectrometer Model 3380, with external standardization. Quench correction was made with the Tricarb Absolute Activity Analyser model No. 544.

For assay of total radioactivity, urine (100 μl) or bile (50 μl) were pipetted as such into 10 ml Bray's solution. For counting the papaverine extracted from bile, blood or urine, 5 ml of the organic layer obtained was transferred to a vial containing 10 ml toluol scintillator. To blood samples of 100 μl, 0·2 ml 70% perchloric acid and 0·4 ml 30% H_2O_2 was added. After 3 hr at 75°, the colourless, clear solution was cooled and dissolved in 15 ml of a scintillator solution consisting of 0·7% BBOT 2·5-bis-2-(3-tert.-butylbenzoxasolyl) thiophene in a mixture of toluene–ethylcellosolve (6:4).

Faeces are dried, weighed and ground. 100 mg of the powder was treated as described for the blood samples. Small and large intestine, with their contents, were homogenized in 20 ml of water, and 200 μl of the homogenate was treated as described for the blood samples.

Muscular tissue was homogenized in 3 ml of water and treated likewise.

The zones of radioactivity on the thin layer plates were located, and the activity estimated roughly by a Packard Model 7201 Radiochromatogram Scanner.

RESULTS

Urinary and faecal excretion of tritium after oral, intraperitoneal and intramuscular administration. The results after oral, i.p. and i.m. dosing of ^{3}H-papaverine (5 mg/kg) are shown in Table 1. For the 3 routes of administration, around 85 per cent of the administered tritium is recovered from urine and faeces after 4 days; about 70 per cent was eliminated with the faeces. Most of the radioactivity is excreted in the first 24 hr; urinary excretion is most important in the first 2 hr after administration. Extraction of urine and feces with *n*-heptane, shows that only negligible amounts of unchanged papaverine-^{3}H (less than 0·1 per cent of dose) are present.

Biliary excretion. In rats dosed i.v. or i.m. with 5 mg/kg ^{3}H-papaverine, about 70% of tritium is excreted in the bile in the first 6 hr (Table 2). No significant difference could be found between anaesthetised and non-anaesthetised rats after i.m. injection. As shown in Fig. 2 radioactivity appears in the bile soon after i.v. administration of ^{3}H-papaverine; about 25% of tritium is excreted in the first 30 min; 45 per cent in the 1st hr. For the radioactivity, first order biliary elimination with a half time of 48 min and a rate constant of 0·0146 min^{-1} could be demonstrated. In the bile no unchanged papaverine was present as shown by the extraction procedure.

Biliary excretion after intraduodenal administration of bile from donor rats. Three different series of experiments were done. In a first series, bile from donor rats is administered intraduodenally: these donor rats have been given ^{3}H-papaverine 5 mg/kg i.m. and their bile is collected for 6 hr; about one-fifth of the bile collected is administered intraduodenally per rat. Only a small amount of radioactivity can be found in the bile of the receiver rats during the 6 hr after intraduodenal administration.

If on the other hand the bile from donor rats is incubated with glusulase during 20 hr before intraduodenal administration, the radioactivity collected in the bile of the receiver rats in 6 hr amounts to about 50–80 per cent. Finally in some experiments, to the bile of control rats ^{3}H-papaverine is added, and then given to receiver rats by intraduodenal administration; about 80 per cent of the total radioactivity given can be found in the bile in the first 6 hr.

Radioactivity in intestine after i.m. injection of ^{3}H-papaverine. Figure 3 shows the radioactivity of the proximal part of the small intestine, of the distal part of the small

TABLE 1. URINARY AND FAECAL EXCRETION OF TRITIUM AFTER ADMINISTRATION OF A SINGLE DOSE OF ^{3}H-PAPAVERINE (5 mg/kg) TO THE RAT

	Dose recovered (%)*					
	Oral		Intramuscular		Intraperitoneal	
	Urine	Faeces	Urine	Faeces	Urine	Faeces
Day 1	11.13 (6.10–14·65)	72.70 (66·90–74·40)	17·56 (12·57–22·91)	63·17 (60·00–76·00)	9·16 (8·08–10·71)	63·64 (35·78–77·10)
2	0·21 (0·16–0·28)	2·63 (1·27–3·83)	0·16 (0·13–0·19)	4·60 (2·03–9·80)	1·25 (0·28–3·11)	8·44 (0·68–28·40)
3	0·43 (0·23–0·70)	0·19 (0·06–0·37)	0·11 (0·07–0·19)	0·16 (0·09–0·22)	0·25 (0·21–0·28)	0·67 (0·08–1·60)
4	0·24 (0·19–0·34)	0·09 (0·06–0·11)	0·04 (0·02–0·05)	0·05 (0·01–0·06)	0·18 (0·17–0·20)	0·10 (0·03–0·22)
Total	12·01 (6·86–15·79)	75·62 (68·50–79·40)	17·87 (12·86–23·25)	67·98 (60·00–76·07)	10·85 (8·98–11·67)	72·85 (67·00–80·00)

* The figures are the mean recoveries for four animals; the numbers in parentheses indicate the range of recovery.

TABLE 2. BILIARY EXCRETION OF TRITIUM AFTER ADMINISTRATION OF ^{3}H-PAPAVERINE (5 mg/kg)

Mode of administration	Excreted after 6 hr (%)†		
Intravenous, anaesthetized*	72·00	73·00	74·10
Intramuscular, anaesthetized*	72·61	72·88	67·53
Intramuscular, unanaesthetized	66·26	67·61	68·56

* Urethane, 1250 mg/kg i.p.
† Individual values, three animals in each group.

intestine, and of the large intestine at different times after i.m. injection of ^{3}H-papaverine; measurement for the intestine was done with the contents included. Radioactivity in the whole intestine increased in function of time up to 3 hr after injection. After 30 min, radioactivity was highest in the first part of the small intestine, while after 1 hr radioactivity had almost completely migrated to the distal part of the small intestine; after 6 hr almost all the tritium was localized in the large intestine. The lower total radioactivity after 6 hr, as compared with 3 hr, could be explained by the fact that at that time some tritium had been excreted with the faeces.

Blood levels of total radioactivity and ^{3}H-papaverine in the rat. Blood levels of total radioactivity and ^{3}H-papaverine after intravenous administration of 5 mg/kg of ^{3}H-papaverine are shown in Fig. 4 for control rats and bile cannulated rats. The results of total radioactivity have been expressed in terms of micrograms papaverine per millilitre of blood because no information was available on the exact chemical nature of the total radioactivity in blood.

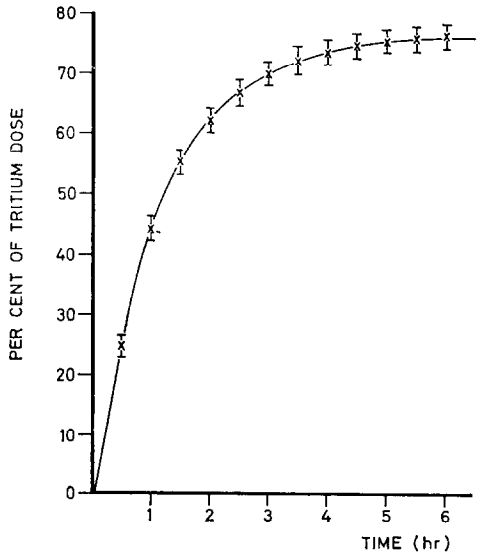

FIG. 2. Cumulative biliary excretion of tritium after intravenous administration of ^{3}H-papaverine (5 mg/kg) in the anaesthetized rat. Mean values are calculated for 8 animals (± S.E.M.)

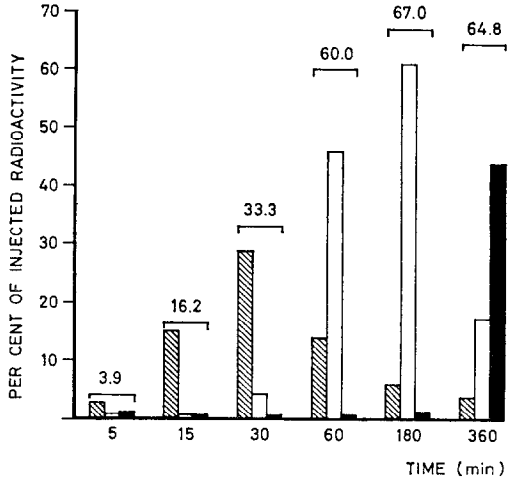

FIG. 3. Radioactivity in first part of small intestine (▨) in second part of small intestine (□), and in large intestine (■) at different times after intramuscular injection of papaverine-^{3}H (5 mg/kg) in the rat. Radioactivity is expressed in percentage of the administered dose; mean values for 4 animals are given. The figures in parentheses represent the activity for the whole intestine (i.e. sum of the three different parts).

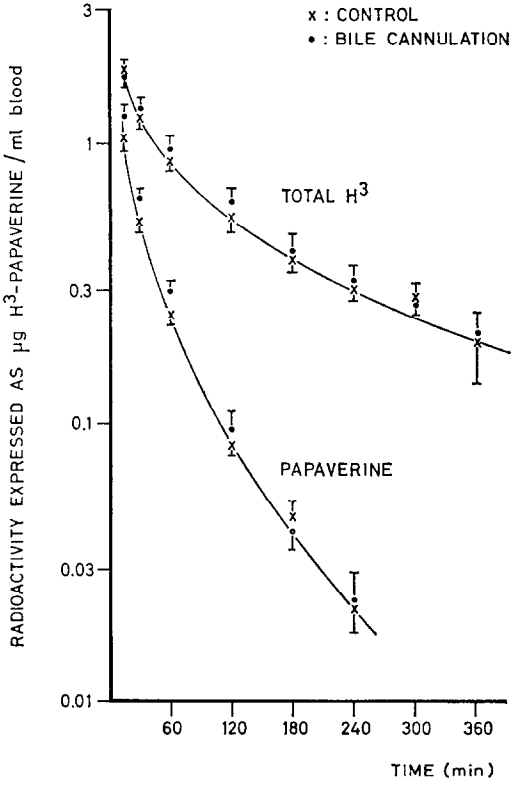

FIG. 4. Blood levels of total radioactivity and of ^{3}H-papaverine (both expressed as μg papaverine per ml of blood), after i.v. administration of 5 mg/kg of ^{3}H-papaverine are plotted against time for control rats and for bile cannulated rats. Rats were anaesthetized with urethane 1250 mg/kg i.p. Mean values (± S.E.M.) for 8 rats in each group.

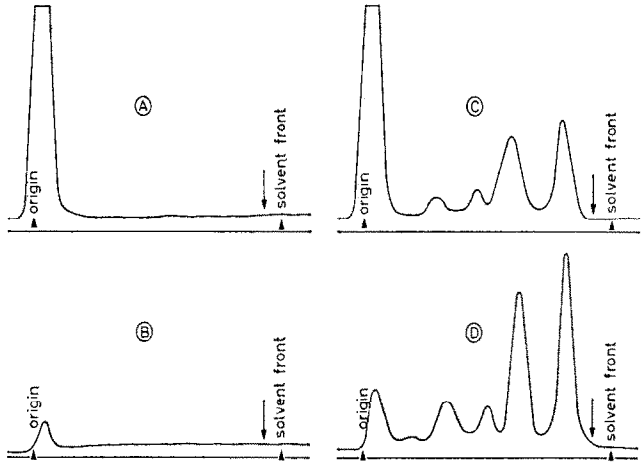

FIG. 5. Representative thin layer chromatography radioscans of ^{3}H-papaverine metabolites in bile samples developed in solvent chloroform–dioxane–ethylacetate–ammonia (25:60:10:5). A: Bile from rats given ^{3}H-papaverine, 5 mg/kg, i.v.; B: $CHCl_3$ extract from bile; C: as in A, but after 20 hr incubation with glusulase; D: $CHCl_3$ extract from bile C. Arrow indicates papaverine localization on the chromatogram.

No significant differences could be observed between the blood levels of the control and bile cannulated rats; this was also true for the total radioactivity and papaverine levels.

These results show also that the half-life of unchanged papaverine in blood of the rat is relatively short.

Thin layer chromatography. Bile samples collected from rats receiving ^{3}H-papaverine intravenously were subjected to thin layer chromatography. A representative experiment is shown in Fig. 5. While bile is spotted as such, the chromatogram produced a single radioactive peak at R_f 0·03, while unchanged ^{3}H-papaverine had a R_f value of 0·94. When bile was incubated with glusulase during 20 hr, and then spotted on thin layer chromatogram, five radioactive peaks can be observed with R_f values of 0·03, 0·32, 0·49, 0·66 and 0·82 respectively. The decrease of the 0·03 peak and the appearance of other peaks after incubation with glusulase suggest that the peak with R_f value of 0·03 is, for a large part, due to conjugated metabolites. After extraction of the hydrolysed metabolites with $CHCl_3$, as described in Materials and Methods, and thin layer chromatography of the $CHCl_3$ extract, the same five peaks can be detected. Here however the first radioactive peak (R_f 0·03) is very small, probably because the conjugated metabolites are practically not extracted by $CHCl_3$. This result was confirmed by the experiment in which the $CHCl_3$ extract of nonincubated bile gives only a very small peak at the start.

DISCUSSION

The results described show that in the rat after administration of papaverine labelled with tritium, radioactivity was rapidly excreted in the faeces and the urine; the finding that practically no unchanged product was excreted is in agreement with the results of Axelrod *et al.*[4] in the rat, and with findings in other species.[1–3] Most of the radioactivity was recovered from the excreta in the first 24 hr after drug administration for

different routes of administration; most of it was excreted via the faeces and only 10–17 per cent was eliminated in the urine. Axelrod *et al.*,[4] using a 10-times higher dose, recovered about 50 per cent of the dose given as urinary metabolites; in some animals we gave the 50 mg/kg dose: we found the same ratio between urinary and faecal excretion as with our lower dose. The fact that for the excretion pattern of tritium practically no difference was seen between oral or parenteral administration of ^{3}H-papaverine, proves the good absorption of papaverine from the gastrointestinal tract.

This was confirmed by the finding that, when ^{3}H-papaverine was injected intraduodenally, 80 per cent of the dose given was recovered in bile.

The bile is the main route by which papaverine is eliminated from the body. Indeed about 75 per cent of the radioactivity given can be found in the bile of the first 6 hr; approximately this amount is excreted in the faeces of the first 24 hr.

The chromatographic study of bile samples shows that no papaverine was present but that the radioactivity was due to metabolites conjugated to glucuronic acid or sulphate. This is in accordance with the findings of Axelrod *et al*[4]. who demonstrated that at least three phenolic compounds, formed by enzymatic demethylation of papaverine in the liver, were present in the urine in the conjugated form. These phenolic substances, only one of which has been identified by Axelrod *et al.*,[4] could be similar to those found in our experiments where bile was incubated with glusulase.

When bile from donor rats containing these conjugated metabolites was given intraduodenally to other rats, only a very small absorption occurred; if metabolites were given after incubation with glusulase, about 55 per cent of radioactivity is absorbed. These results suggest that enterohepatic circulation of the metabolites of papaverine in the rat is not important.

The fact that after parenteral administration of ^{3}H-papaverine the tritium content in the intestine remains constant from 60 min on, was consistent with a very small reabsorption of radioactivity.

This was confirmed by measuring blood levels of total radioactivity and ^{3}H-papaverine in control and bile cannulated rats. No significant difference could be found for these values between control and bile cannulated rats.

It has been suggested that molecular size and polarity are important properties for biliary excretion and that compounds with a molecular weight larger than about 300 are often excreted by the bile.[5] The fact that the phenolic metabolites of papaverine are conjugated to very polar compounds with a molecular weight above 350, can explain the importance of biliary excretion of these papaverine metabolites.

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