Biochemical Pharmacology, Vol. 17, pp. 655–666. Pergamon Press. 1968. Printed in Great Britain

INVESTIGATIONS INTO THE METABOLIC FATE AND DISTRIBUTION OF HEPZIDINE MALEATE IN THE RAT AND THE MOUSE

W. HESPE, H. PRINS, W. F. KAFOE and W. TH. NAUTA

Research Department of N.V. Koninklijke Pharmaceutische Fabrieken v/h Brocades-Stheeman en Pharmacia, Looiersgracht 27–39, Amsterdam, The Netherlands

(*Received* 4 *November* 1967; *accepted* 7 *December* 1967)

Abstract—Metabolic studies with hepzidine maleate, labelled in two positions with ^{3}H and ^{14}C, respectively, showed that in rats, over 50 per cent of an orally administered dose of hepzidine maleate (10 mg/kg) is hydrolysed before absorption. The nonhydrolysed part of the dose is absorbed to a large extent. An important biotransformation of hepzidine is *N*-demethylation; it involves about 20 per cent of a 10-mg/kg oral dose and over 37 per cent of an equal dose, given i.p. On the other hand, *N*-demethylation of the hydrolytic product 1-methyl-4-piperidinol was very slight. Excretion of ^{14}C radioactivity in the expired air shows good first-order kinetics, from which a half-life of about 4·2 hr could be evaluated for hepzidine maleate in the rat. Next to urinary excretion, biliary excretion plays an important role in the elimination of hepzidine maleate and metabolites. In the urine the hydrolytic products of hepzidine, especially 1-methyl-4-piperidinol and its metabolites (probably conjugates) are predominant. There is evidence that in bile the hydrolytic product 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ol and its metabolites (probably conjugates) are predominant, but in contrast to urine a substantial amount of the hepzidine is excreted in bile as such and/or in some form which still possesses the intact hepzidine structure. After i.p. administration of hepzidine maleate-^{14}C to female mice (50 mg/kg) autoradiographic distribution studies show the radioactivity to attain considerable levels in most organs, while blood levels stay relatively low. Highest accumulations of radioactivity are found in the liver, lungs, hypophysis, Harder's gland, submaxillary gland, mucous glands in tongue, palatum and pharynx, bone marrow, lymphoid tissue, urinary bladder wall, urine, bile, intestinal contents, and specified zones in the kidneys and adrenals. To a lesser degree there is also a pronounced penetration of radioactivity into the CNS, where hippocampus, cerebral cortex and thalamus show a higher degree of radioactivity than other brain areas. In most organs maximum levels are observed at 1 hr after administration. No specific long-lasting accumulations of radioactivity have been observed, except in the urinary bladder wall, which still shows a high level of radioactivity at 16 hr after administration.

FROM a series of dibenzo[*a*,*d*]cyclohepten-5-yl ethers, prepared by Van der Stelt *et al.*[1, 2] the compound hepzidine maleate* was selected for clinical investigation on account of the results of pharmacological[3] and toxicological[4] studies. In order to provide a basis for research on the mechanism of action and also to improve insight into pharmacological, toxicological, and clinical findings, an investigation into the

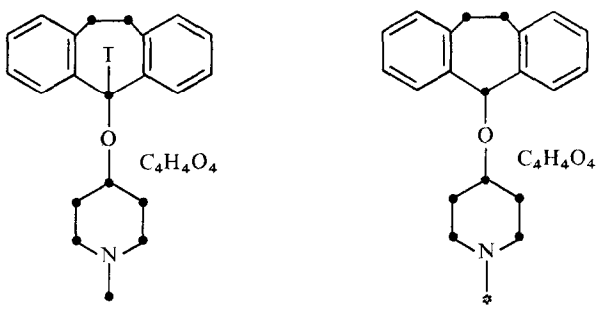
* Nonproprietary name, accepted by the WHO for 4-[(10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-yl)oxy]-1-methylpiperidine hydrogen maleate.

metabolic fate of the compound was started. The present paper reports part of the results obtained, including data about absorption, biotransformation and elimination of hepzidine maleate by rats, and its pattern of distribution in mice.

MATERIALS AND METHODS

^{14}C and ^{3}H-labelled hepzidine maleate

We used hepzidine maleate (denoted further HM) labelled with ^{14}C and ^{3}H, respectively.



The tritiated compound was synthesized by catalytic reduction (Pt) of 10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5-on with tritium, conversion of the alcohol obtained to the chloride, and subsequent etherification with 1-methyl-4-piperidinol.

The ^{14}C-labelled compound was obtained by a reductive *N*-methylation of *N*-demethyl hepzidine with formaldehyde-^{14}C. Both compounds were purified to a satisfactory level of chemical and radiochemical purity, which was checked by means of thin-layer chromatography. Specific activities obtained were 7·6 mC/g for the tritiated and 2·0 mC/g for the ^{14}C-labelled compound.

Animal studies

Male albino rats (TNO-Wu strain), weights between 130 and 360 g, and virgin female mice (TNO-Swiss) of about 20 g were used in the experiments.

The substance was administered by gastric tube or injected i.p. as a solution in a citric acid/phosphate buffer (pH = 7·4).

For the absorption and excretion experiments (urine, faeces, respiratory air, bile) with rats previously described procedures were applied.[5, 6]

To study the distribution of radioactivity 50-mg/kg doses of the ^{14}C-labelled compound were administered i.p. to a number of mice. At time intervals of 1, 2, 4, 8 and 16 hr after administration, respectively, one of the mice was anaesthetized with ether and killed by immersion in a solid carbon dioxide–acetone mixture. The distribution of radioactivity was studied by employing the macroautoradiographic technique described by Ullberg,[7] in which 30 μ sections of the whole animal, taken and dried in a freezing room, are brought into contact with a photographic emulsion (Gevaert Structurix X-ray Film). The pictures in the present article are prints of the autoradiograms: A higher intensity of white corresponds to a higher level of radioactivity.

Radioactivity measurements

A Packard Tri Carb Liquid Scintillation Spectrometer 314EX was used for all radioactivity measurements. Samples of urine or ethanolic extracts (0·5 ml) were mixed with 10 ml of a scintillation cocktail according to Bray[8] in plastic counting-vials. Expiratory carbon dioxide was bound into an ethanolamine-containing scintillation cocktail from which 15-ml aliquots were measured. Bile was collected directly into the polyethylene vials and weighed, after which 10 ml of the mentioned Bray-cocktail was added.

The faeces, after grinding and in some cases pretreatment by 2 hr refluxing with hydrochloric acid, followed by alkalinization, were extracted by repeated stirring with fresh amounts of ethanol for 20-min periods. After the faecal mass had settled the clear solutions were decanted and combined.

The different parts of the intestinal tract and their contents, after having been cut into small pieces, were extracted with ethanol in the same way. Corrections for quenching were made by the internal standard method.

Analytical procedures

To determine the nature of the radioactive products in the collected urine, successive extraction procedures were employed and the concentrated extracts analysed by TLC.

The procedure mainly used was extraction of the urine, which had first been saturated with sodium sulphate before its being alkalinized to pH = 12, with dichloroethane, followed by a second extraction with dichloroethane, carried out on the acidified extraction residue (pH < 1). In some of the experiments these treatments were preceded by incubation of the urine or urinary extracts with β-glucuronidase (Sigma or N.B.C.) or β-glucuronidase-arylsulphatase (Sigma L-1) for 24 hr at 37° and optimal pH.

To determine the nature of the radioactive products in the alcoholic extracts of the different parts of the gastrointestinal tract of rats, these extracts were evaporated to a small volume and then directly subjected to TLC.

TABLE 1. THIN-LAYER CHROMATOGRAPHIC DATA OF HEPZIDINE MALEATE AND OF SOME OF ITS MAIN METABOLITES

Solvent systems	R_f Value ranges for			
	Hepzidine	*N*-Demethyl-hepzidine	DBL	1-Methyl-4-piperidinol
n-Butanol (100)—ammonia S.D. 0·91 (8)	0·67–0·78	0·41–0·48	0·80–0·89	0·25–0·34
Chloroform (50)—acetone (40)—diethylamine (10)	0·67–0·76	0·38–0·46	0·68–0·78	
Alcohol (99)—70% ammonia (1)	0·49–0·59	0·21–0·30	0·78–0·86	0·11–0·18
Reagents	Dragendorff	Dragendorff; 2,4-dinitro-benzene	Sulphuric acid	Dragendorff; Gibbs' reagent

The separations were carried out on Silica gel G according to Stahl. Table 1 lists the solvent systems employed in the chromatographic separations and the corresponding R_f value range for hepzidine and three potential metabolites which were available in a pure form. For the identification of the spots use was made of these reference

substances and of colour reagents—also listed in Table 1—while the chromatograms were scanned for radioactivity by means of the Berthold Dünnschicht scanner.

RESULTS AND DISCUSSION

Absorption; stability of hepzidine in the gastrointestinal tract

Table 2 shows the levels of radioactivity established in the different parts of the gastrointestinal tract of rats at different time intervals after oral administration of 10 mg/kg of HM-^{3}H. The gradual lowering of the total amount of radioactivity determined (last column) proves that the administered radioactivity is rather well absorbed, especially if we take into account that the biliary excretion of radioactive material (Fig. 5) makes a substantial contribution to the radioactivity present in the intestine.

TABLE 2. RADIOACTIVITY RECOVERED FROM THE GASTROINTESTINAL TRACT OF RATS AT DIFFERENT TIME INTERVALS AFTER ORAL ADMINISTRATION OF 10 mg/kg OF HM-^{3}H

Interval after admin. in hr	Percentage of administered radioactivity recovered (each figure represents the mean of two animals)				
	Stomach	Small intest.	Caecum	Colon	Total alim. tract
0·5	48·6	19·3	0·0	0·1	68·0
1	24·7	32·8	0·1	0·7	58·3
2	18·6	22·4	0·1	0·1	41·2
4	2·3	26·4	18·8	0·1	47·6
8	0·2	9·7	25·5	0·2	35·6

TLC revealed, that in the stomach and its contents 10,11-dihydro-5*H*-dibenzo [*a*,*d*]cyclohepten-5-ol (= DBL) was the predominating radioactive product as early as 30 min after administration, although intact hepzidine could be demonstrated as well. In the small intestine, at the same time, only hepzidine was found. At 120 min after administration the same procedures showed hepzidine to be nearly absent in the stomach, while in the small intestine DBL now had become clearly present. The conclusion can be, that hepzidine undergoes hydrolysis in the stomach, which in view of its established sensitivity to acid[9] is not surprising. Next to unchanged hepzidine radioactive DBL can be regarded as involved in the absorption process, therefore. The following findings provide evidence that, within the intestinal tract, part of the radioactive material is present in the form of conjugates with glucuronic and/or sulphuric acid (a) after evaporation to dryness of the alcoholic extracts from the different intestinal parts, suspension of the residue in water, and treatment with β-glucuronidase/arylsulphatase, the radioactive yield of an extraction with dichloroethane was considerably higher than without the enzyme treatment in a comparable experiment (b) as a consequence of the treatment, especially the amount of DBL detectable on the chromatograms from the dichloroethane solutions, was increased.

The origin of conjugated products can be the bile; this assumption is supported by the finding that the amount of conjugates is highest in the small intestine, yet it can not be excluded that they are partly formed before absorption, because the gastrointestinal mucosae are capable of conjugating foreign compounds.[10, 11]

Elimination

The overall results of the excretion studies, in rats, with the two differently labelled hepzidine molecules are summarized in Table 3, and also the results from a comparable experiment with the metabolite 1-$^{14}CH_3$-4-piperidinol, synthesized from hepzidine by (chemical) hydrolysis. Also included are the results from comparable experiments in which amounts of urine or bile, collected from HM-^{14}C-treated rats, were readministered to rats.

The cumulative time course of urinary and respiratory excretion of the administered radioactivity is depicted in Figs. 1–3 for the radioactivity administered in the form of ^{14}C- and in the form of ^{3}H-labelled hepzidine maleate, respectively: both are fairly rapidly eliminated from the animal body. Within 24 hr by far the largest part of the urinary and respiratory excretion of radioactivity is shown to be completed (Figs. 1–3).

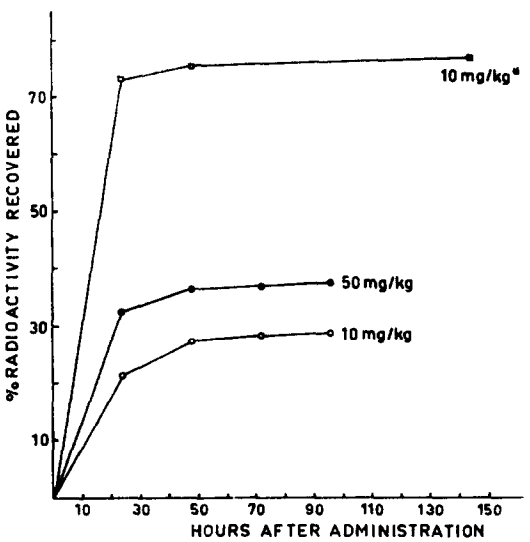


FIG. 1. Mean cumulative course of radioactivity in the urine, in percentage of the administered radioactive dose, given orally in the form of HM-^{3}H.
* Bile duct ligated.

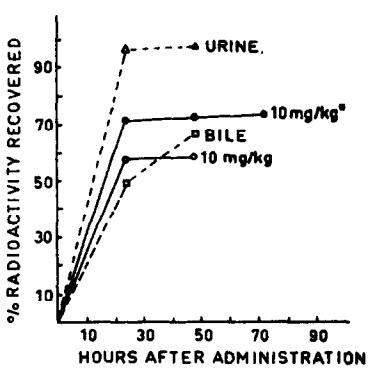


FIG. 2. Mean cumulative course of radioactivity excreted in the urine, in percentage of the radioactive dose, given orally either as HM-^{14}C, or bile-^{14}C, or urine-^{14}C.
* Bile duct ligated.

TABLE 3. MEAN RADIOACTIVITY RECOVERED FROM URINE, FAECAL EXTRACTS, AND EXPIRED AIR AFTER ADMINISTRATION OF HM-^{3}H, HM-^{14}C, 1-$^{14}CH_3$-4-PIPERIDINOL, AND OF ^{14}C-RADIOACTIVE BILE AND URINE TO RATS WITH AND WITHOUT BILE DUCT LIGATION

Substance	Dose (mg/kg)	Route of admin.	Number of animals		Mean percentage of administered radioactivity recovered						
			Bile duct ligated	Bile duct not ligated	Urine Interval (hr)	%	Faecal extr. Interval (hr)	%	Expiratory air Interval (hr)	%	Total %
HM-^{3}H	50	or.		2	0–96	37·3	0–24	23·0			60·3
HM-^{3}H	10	or.		2	0–96	28·9	0–24	30·1			59·0
HM-^{3}H	10	or.	2		0–120	76·7	0–120	7·0			83·7
HM-^{14}C	10	or.		4	0–48	58·3	0–48	5·9	0–7	15·8	80·0
HM-^{14}C	10	or.	4		0–48	73·5	0–48	1·0	0–7	11·5	86·0
Bile-^{14}C	2*	or.		2	0–48	66·1	0–48	16·6	0–7	8·1	90·8
Urine-^{14}C	2†	or.		1	0–48	97·0	0–48	2·5	0–7	0·8	100·3
1-$^{14}CH_3$-4-piperidinol	3·1‡	or.		1	0–48	97·5	0–48	0·3	0–7	0·3	98·1
HM-^{14}C	10	i.p.		2	0–48	21·4	0–48	6·1	0–7	37·2	64·7

* The figure represents HM-equivalents, administered in 1·8 ml of bile, collected over a period of 0–2 hr from 3 donor rats and pooled; dose to the donor rats: 50 mg/kg of HM-^{14}C, or.

† The figure represents HM-equivalents, administered in 1 ml of concentrated urine, collected over a period of 0–24 hr from 2 donor rats and pooled; dose to the donor rats: 10 mg/kg of HM-^{14}C, or.

‡ Equivalent to 10 mg/kg of HM.

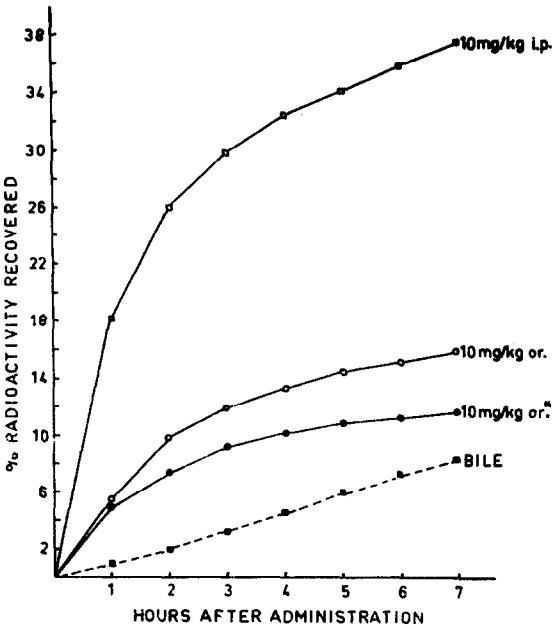


FIG. 3. Mean cumulative course of the radioactivity excreted in the expiratory air, in percentage of the radioactive dose, given orally either in the form of HM-^{14}C or bile-^{14}C.
* Bile duct ligated.

The same was established for the faecal excretion. The amount of radioactivity excreted in the expired air (Table 3) following administration of the ^{14}C-labelled compound points to a pronounced *N*-demethylation, in which mainly hepzidine itself seems to be involved since the *N*-demethylation of 1-methyl-4-piperidinol is very slight (Table 3).

A semilogarithmic plot of the amount of nonexcreted radioactivity in the expired air (putting the total excreted amount, obtained by extrapolation of the cumulative curves, at 100) against time shows good first-order kinetics, starting 2–3 hr after administration (Fig. 4).

According to Wilbrandt,[12] and other authors, the biological half-life of a substance can be evaluated from such plots: for hepzidine in the rat this leads to a figure of about 4·2 hr. Hepzidine is *N*-demethylated following intraperitoneal administration to a much greater extent than after oral dosage (Table 3, Fig. 3) presumably on account of hydrolysis in the stomach, which causes less hepzidine to be available for *N*-demethylation.

The difference between the administered radioactive dose and the sum of the percentages recovered from urine and expired air can be regarded as the true excretion percentage in the faeces. That the experiments did not fully account for this amount is presumably due to incomplete extraction (for similar results see Emmerson and Anderson[13]). In view of the good absorption of radioactivity after oral administration of labelled hepzidine the faecal excretion must be mainly the result of the established high affinity to bile (Fig. 5). This is indirectly confirmed by the rise in urinary excretion following bile duct ligation, which involves about 15 per cent in the case of HM-^{14}C and nearly 50 per cent in the case of HM-^{3}H (Table 3). The fact that

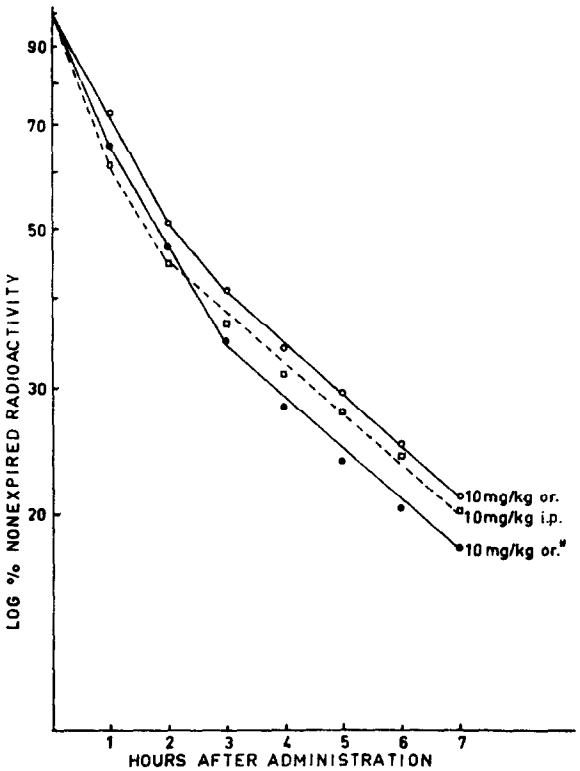


FIG. 4. Logarithmic time course of nonexpired radioactivity in percentage of total expired radioactivity.
* Bile duct ligated.

readministration of bile, collected from HM-^{14}C-treated animals, again leads to excretion of radioactivity in urine, expired air (Table 3; Figs. 2–3) and bile (Fig. 5) points towards an enterohepatic circulation of radioactivity in some form.

Biotransformation; qualitative aspects

It has been discussed above that after oral administration to rats hepzidine is partly hydrolysed to DBL and 1-methyl-4-piperidinol and partly converted into *N*-demethyl hepzidine. Hydrolysis seems to occur exclusively or mainly in the stomach; the site of *N*-demethylation is undoubtedly the microsomal system of the liver. Readministration, to rats, of collected radioactive bile, gives rise to a distribution pattern of radioactivity over urine, faeces, bile, and expired air which closely resembles the pattern after direct administration of HM-^{14}C (Table 3, Fig. 5). This suggests that the ^{14}C-radioactivity in the bile is mainly due to hepzidine itself or some unstable (conjugation) product from which within the gastrointestinal tract, hepzidine comes free. After readministration of collected radioactive urine to rats, the administered radioactivity is nearly completely eliminated in the urine (Table 3), suggesting that the administered urine contains little or no unchanged hepzidine; the greater part of the urinary ^{14}C-radioactivity seems to be attributable to 1-$^{14}CH_3$-4-piperidinol and further metabolites of this compound. Chromatographic analysis of the urine points into the same direction, although the presence of hepzidine and *N*-demethyl hepzidine could

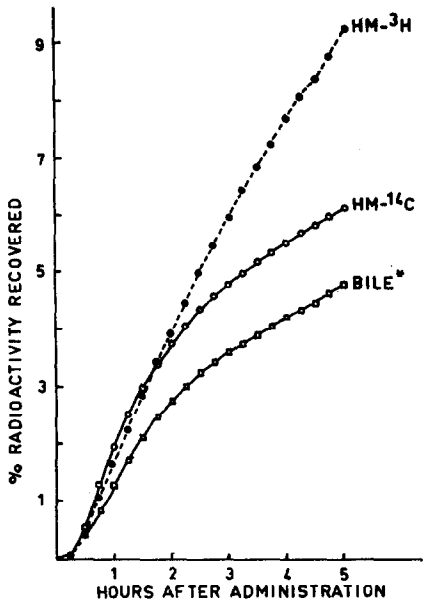


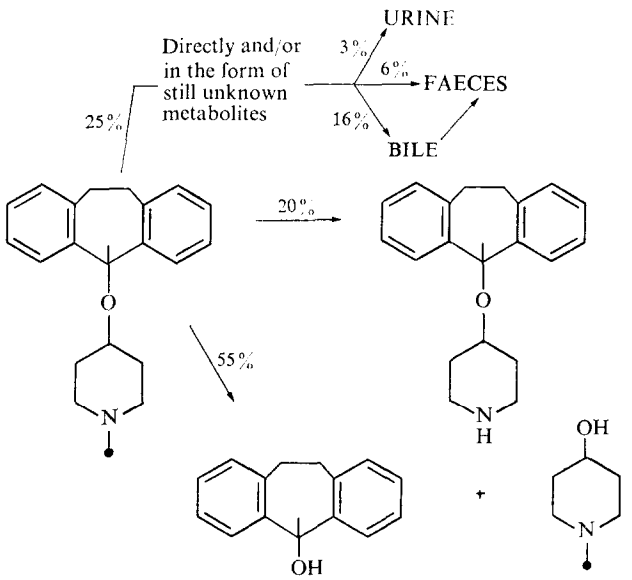
FIG. 5. Cumulative excretion of radioactivity in bile after oral administration of HM-^{14}C (mean of 4 rats), HM-^{3}H (mean of 2 rats), or bile (mean of 2 rats) to bile fistula rats under phenobarbital narcosis.

* Oral dose of 1 mg/kg of HM equivalents in 0·71 ml of bile, collected from 3 donor rats over a period from 0–2 hr and pooled; dose to the donor rats 50 mg/kg of HM-^{14}C given orally.

be demonstrated. Since the yields of extraction of radioactivity from urine rise with the polarity of the solvents used and treatment with β-glucuronidase/arylsulphatase considerably increases the extraction yield it may be concluded that a large part of the urinary radioactivity consists of glucuronic acid and/or sulphuric acid conjugates. DBL should be among the compounds conjugated, because conjugation is a common reaction for secondary aromatic alcohols of various types.

Biotransformation; quantitative aspects

Since the elimination of 1-$^{14}CH_3$-4-piperidinol and its metabolites in bile is negligible, all ^{14}C-radioactivity eliminated in bile must be due to hepzidine and unknown metabolites, still possessing the intact structure and the label. The rise in urinary excretion after bile duct ligation can be considered as an accurate measure for the radioactivity eliminated into the bile under normal circumstances. In this respect it is remarkable that bile duct ligation did not lead to a rise in excretion in the expiratory air. It follows from Table 3, therefore, that the biliary elimination of hepzidine as such and/or in the form of still unknown metabolites involves about 16 per cent of the administered ^{14}C-dose. The figures listed for the faecal excretion of radioactivity refer to incomplete faecal extraction. For a better insight into the real faecal excretion percentage of radioactivity we should subtract the sum of the percentages excreted by the other routes from the administered dose, which is put to 100 per cent. The total ^{14}C-elimination in the expired air can be obtained by extrapolating the cumulative respiration curves (Fig. 3). Calculated in this way an amount of 22 per cent of the administered ^{14}C-dose is found to be eliminated into the faeces. If we compare this figure with the 16 per cent for the biliary ^{14}C-excretion it is evident that by far the



SCHEME 1. Metabolic fate of an oral 10-mg/kg dose of hepzidine maleate in the rat.

largest part of faecal ^{14}C-excretion originates from biliary excretion. The remaining 6 per cent excreted into the faeces can be ascribed to direct faecal elimination of non-absorbed material. Urinary excretion following 10 mg/kg of HM-^{3}H accounts for 29 per cent of the administered dose; after an equal dose of HM-^{14}C this is 58 per cent (Table 3). This means, that at least 29 per cent of the administered ^{14}C-radioactivity present in urine must be ascribed to ^{14}C-products which do not contain the aromatic part of hepzidine. The most likely candidate is 1-methyl-4-piperidinol and possible further metabolites of this compound; in rats, at least about 30 per cent of an administered dose of 10 mg/kg of hepzidine maleate must undergo hydrolysis, therefore. Chromatographic analysis of urine, in which only minor amounts of hepzidine are found, shows that the real percentage of hydrolysis may be much higher than this minimum value, which is based on the improbable assumption that all urinary tritium is present in the form of hepzidine itself. However, in the urinary extracts from the HM-^{3}H experiments, DBL and *N*-demethyl hepzidine prevailed; the hepzidine accounted for only about 10 per cent of the radioactivity of the analysed material. This would mean that about 3 per cent of the administered hepzidine is excreted in urine as such, and 55 per cent of the urinary ^{14}C-excretion is due to 1-methyl-4-piperidinol and its metabolites. These analytical results point to hydrolysis of over 50 per cent of the administered dose, therefore. Also, comparison of the amounts of radioactivity excreted by the respiratory route following oral and i.p. administration, respectively, of an equal dose of HM-^{14}C (Table 3) leads to an estimate of the amount hydrolysed of over 50 per cent; after i.p. administration respiratory excretion is more than twice as high as after oral administration, over the same period of time (Fig. 3), a tendency which can be expected to hold also for the extrapolated total excretion.

Summarizing, these quantitative considerations lead to Scheme 1 for the metabolic fate of hepzidine maleate in the rat.

Distribution

After administration of HM-^{14}C (50 mg/kg, i.p.) to female mice the autoradiograms showed radioactivity in nearly all organs, indicating a large volume of distribution of the compound and its radioactive metabolites; representative examples of the distribution are shown in Figs. 6–9.

The blood level of radioactivity, which is relatively low, attains a maximum the first two hours after administration. The highest accumulations of radioactivity are found in the liver, lungs, hypophysis, Harder's gland, submaxillary gland, mucous glands in the root of the tongue, palatum and pharynx, bone marrow, lymphoid tissue, urinary bladder wall, urine, bile, intestinal contents, and specified zones in the kidneys and adrenals. Although to a lesser degree, there is also a pronounced penetration of radioactivity in the CNS, where hippocampus, cerebral cortex and thalamus show a higher degree of radioactivity than other brain areas (Fig. 7). In most organs the maximum levels are observed at one hour after administration. The fairly rapid elimination found in rats is confirmed by the high concentration of radioactivity in the excretory organs in combination with the fairly rapid decrease of overall radioactivity in the animals with time, as can be judged from the autoradiograms obtained at different time intervals. At 16 hr by far the largest part of the radioactivity has left the animal body; no specific long lasting accumulations of radioactivity have been observed, with the exception of the urinary bladder wall, which still shows a high level of radioactivity at 16 hr after administration. The zone in which the radioactivity in the kidney is concentrated is located in the inner cortex (Fig. 8) and probably corresponds to what is called zone 2 in the rat kidney by Krakusin and Jennings,[14] an area which consists mainly of the straight portions of the proximal tubules and, to a lesser extent, of ascending limbs of Henle's loops.

The adrenals show a higher concentration of radioactivity in the cortex than in the medulla, especially in a zone which approximately marks the corticomedullar border line.

The high levels of radioactivity found in bile (Fig. 9) point to biliary excretion as an important source of the intestinal radioactivity. In how far the HM-^{14}C itself or radioactive metabolites are involved in the distribution pattern which emerges from this study is difficult to judge. We believe, that the distribution picture is rather representative for hepzidine itself, especially at the early time intervals, because we circumvented two of the main metabolic transformations to be expected from the experiments in rats: first, intraperitoneal administration avoids the important primary hydrolysis which would be anticipated following oral administration, although diffusion into the stomach, observed during this investigation, might give rise to the formation of hydrolytic products to a limited extent. Secondly, the choice of the label prevents the *N*-demethylated compound from taking part in the radioactivity distribution.

REFERENCES

1. C. VAN DER STELT, A. F. HARMS and W. TH. NAUTA, *J. medl Pharm. Chem.* **4**, 335 (1961).
2. C. VAN DER STELT, A. B. H. FUNCKE, H. M. TERSTEEGE and W. TH. NAUTA, *Arzneimittel-Forsch.* **16**, 1342 (1966).
3. A. B. H. FUNCKE, W. J. LOUWERSE, H. M. TERSTEEGE, A. F. HARMS and W. TH. NAUTA, *Archs Int. Pharmacodyn. Thér.* **167**, 334 (1967).
4. D. MULDER and C. J. VAN EEKEN, *Archs Int. Pharmacodyn. Thér.* **162**, 497 (1966).
5. W. HESPE, A. M. DE ROOS and W. TH. NAUTA, *Archs Int. Pharmacodyn Thér.* **156**, 180 (1965).

6. W. HESPE, A. M. DE ROOS and W. TH. NAUTA, *Archs Int. Pharmacodyn. Thér.* **164**, 397 (1966).
7. S. ULLBERG, *Acta Radiol. suppl.* **118**, (1954).
8. G. A. BRAY, *Analyt. Biochem.* **1**, 279 (1960).
9. A. M. DE ROOS, R. F. REKKER and W. TH. NAUTA, *Pharm. Acta Helv.* **38**, 569 (1963); *Pharm. Weekblad* **102**, 851 (1967).
10. K. HARTIALA, *Biochem. Pharmac.* **6**, 82 (1961).
11. J. H. STEVENSON and G. J. DUTTON, *Biochem. J.* **82**, 330 (1962).
12. W. WILBRANDT, *Schweiz. med. Wschr.* **94**, 737 (1964).
13. J. L. EMMERSON and R. C. ANDERSON, *Toxic. appl. Pharmac.* **9**, 84 (1966).
14. J. S. KRAKUSIN and R. B. JENNINGS, *Archs Path.* **59**, 471 (1955).

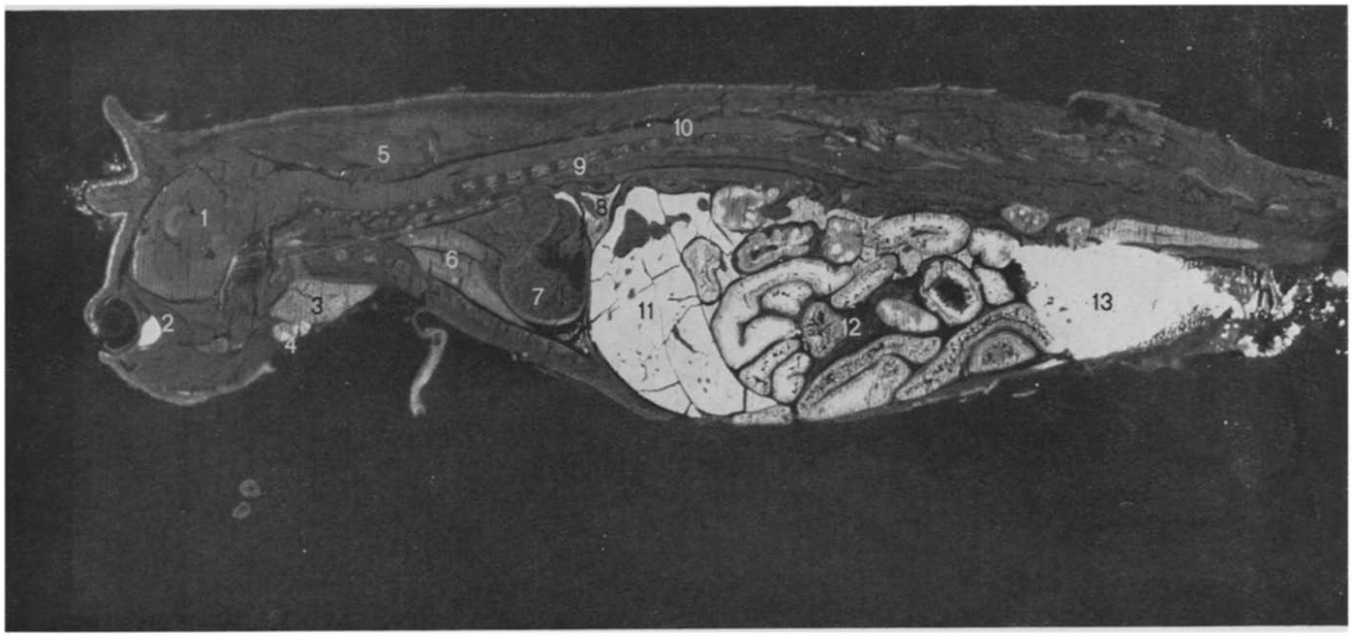


FIG. 6. Distribution of radioactivity in a mouse, 2 hr after administration of HM-^{14}C, 50 mg/kg, i.p.; 1 = brain; 2 = Harder's gland; 3 = salivary glands; 4 = lymph node; 5 = brown fat; 6 = thymus; 7 = blood; 8 = lung; 9 = vertebrae; 10 = spinal cord; 11 = liver; 12 = intestine; 13 = urine.

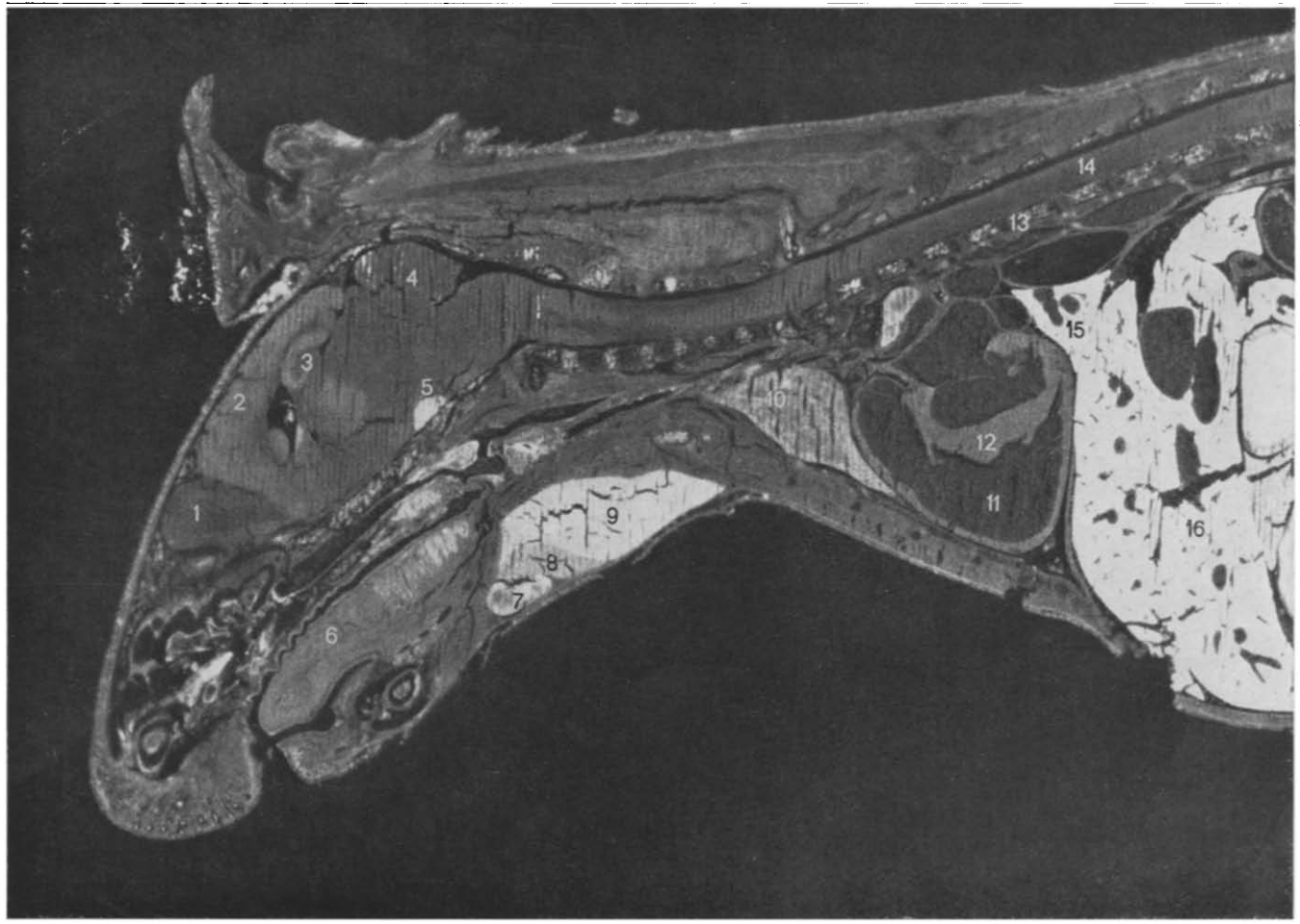


FIG. 7. Enlarged detail of the distribution pattern of radioactivity in a mouse, 1 hr after administration of HM-^{14}C, 50 mg/kg, i.p.; 1 = olfactory bulb; 2 = cortex; 3 = hippocampus; 4 = cerebellum; 5 = hypophysis; 6 = tongue; 7 = lymph node; 8 = sublingual gland; 9 = submaxillary gland; 10 = thymus; 11 = blood; 12 = myocard; 13 = vertebrae; 14 = spinal cord; 15 = lung; 16 = liver.

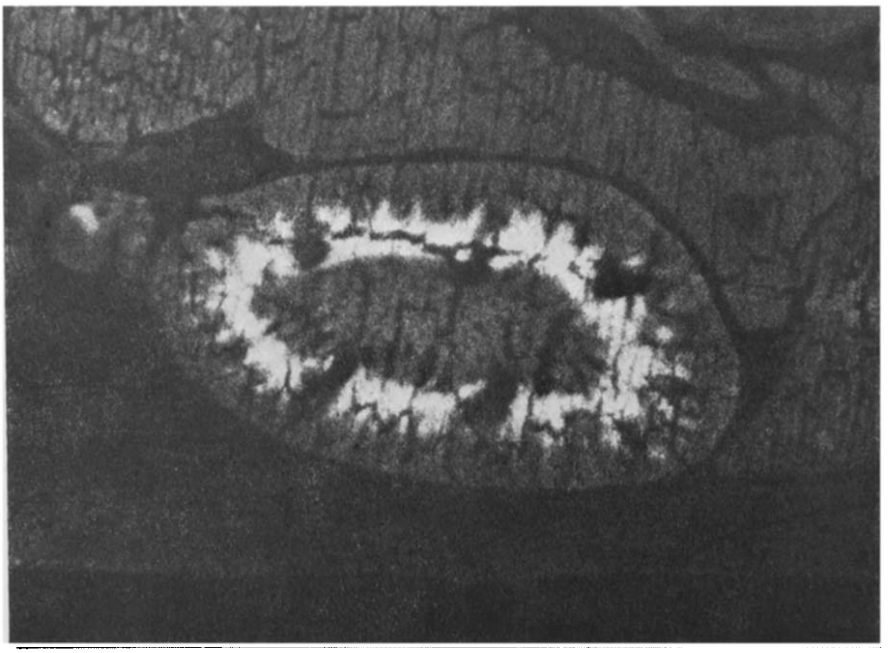


FIG. 8. Detail of the distribution pattern of radioactivity in a mouse 1 hr after administration of HM-^{14}C, 50 mg/kg, i.p., showing the pattern of radioactivity within the kidney.

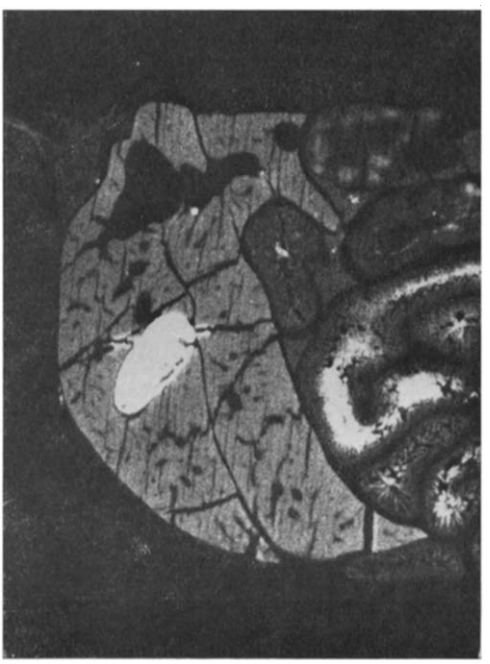


FIG. 9. Detail of the distribution pattern of radioactivity in a mouse, 2 hr after administration of HM-^{14}C, 50 mg/kg, i.p., showing the liver and bile bladder.