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Excretion of ^{14}C-edrophonium and its metabolites in bile: role of the liver cell and the peribiliary vascular plexus

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Summary

1. The metabolism and biliary excretion of ^{14}C-edrophonium chloride was studied in Wistar rats.

2. Approximately 5% of the dose was recovered from bile in 6 hours. Most of the radioactivity was eliminated as ^{14}C-edrophonium glucuronide. Small amounts of the unchanged drug were also detected in bile, particularly during the first hour after administration of the drug.

3. The concentration of ^{14}C-edrophonium glucuronide in bile was approximately 15–20 times its concentration in plasma.

4. In contrast, the concentration of unchanged ^{14}C-edrophonium was similar in bile and plasma.

5. Evidence is presented that unchanged ^{14}C-edrophonium is transferred from plasma to bile via the peribiliary vascular plexus.

Introduction

Although quaternary amines are widely used in biology and medicine, little is known of the factors governing their metabolism and elimination. Some quaternary amines (for instance, hexamethonium, decamethonium, neostigmine, gallamine, and dimethyltubocurarine) are only present in trace amounts in bile (Levine, 1960; Lüthi & Waser, 1965; Calvey, 1966; Feldman, Cohen & Golling, 1969; Meijer & Weitering, 1970). On the other hand, benzomethamine, oxyphenonium, procainamide ethobromide, and tubocurarine are more extensively excreted (Levine & Clark, 1955, 1957; Schanker & Solomon, 1963; Cohen, Brewer & Smith, 1967), and as much as 30% of the dose may be detected in bile. In many cases, it is not possible to differentiate between the excretion of quaternary compounds and their metabolites or conjugates. In addition, many quaternary amines have profound effects on systolic and diastolic blood pressure and hepatic blood flow; such factors may readily influence the biliary excretion of these compounds. Schanker (1965) suggested that quaternary amines with two polar groups at opposite ends of the molecule are excreted in bile in small amounts, while compounds with one or more non-polar rings at one end of the molecule are extensively eliminated. More recent evidence implies that certain bisquaternary amines are excreted in high concentrations in bile, and suggests that lipid solubility may be an important determinant of hepatic transport (Meijer & Weitering, 1970).

The present experiments are concerned with the biliary excretion of metabolites of the phenolic quaternary amine edrophonium chloride (ethyldimethyl(3-hydroxy-

phenyl)ammonium chloride) in the rat. This compound is occasionally used in man in the diagnosis of various neuromuscular disorders, but there is no published work on its biotransformation or elimination. Although most of the quaternary amine was excreted in bile as a glucuronide conjugate, small amounts of unchanged edrophonium were also detected. Evidence is also presented that the unchanged drug and its main metabolite are transferred into bile at different sites and by different physiological mechanisms.

Methods

Radioactive materials

Ethyl[1-^{14}C]dimethyl(3-hydroxyphenyl)ammonium chloride (specific radioactivity 10·0 mCi/mmol) was obtained from the Radiochemical Centre (Amersham, Bucks), dissolved in a solution of 4-chlorocresol (0·25% w/v in deionized water), and stored at −10° C. Standard solutions of tri[^{14}C]methyl(3-hydroxyphenyl)ammonium chloride were prepared from the iodide salt (specific radioactivity 10·2 mCi/mmol; The Radiochemical Centre) by ion exchange chromatography on columns (7 cm × 0·5 cm) of Amberlite CG-50, 200 mesh (B.D.H. Ltd., Poole, Dorset). The columns were eluted with 0·1 M HCl.

Drugs

Heparin sodium (Evans Medical, Liverpool) was obtained commercially. 3-Dimethylaminophenol and edrophonium chloride were kindly donated by Roche Products Ltd., London W.1.

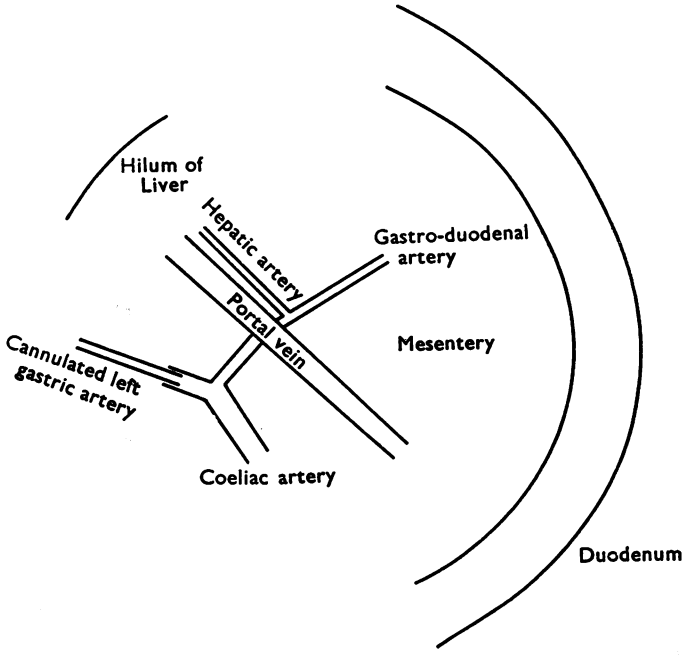

FIG. 1. Diagrammatic representation of the technique used for introduction of ^{14}C-edrophonium chloride into the hepatic arterial circulation of the rat. The duodenum is displaced to the left of the animal.

Experimental procedure

Wistar rats (150–250 g) of either sex (Carworth Europe, Alconbury, Huntingdonshire) were anaesthetized with urethane (14% w/v in water; 10·0 ml/kg, i.p.). A polyethylene cannula was placed in a femoral vein, and respiration was assisted (when necessary) by means of an endotracheal tube. The abdomen was opened and a polyethylene cannula (0·26 mm internal diameter) was inserted in the common bile duct approximately 1 cm above its entry into the duodenum. A polyethylene cannula was sometimes placed in a common carotid artery.

In some experiments, a 22 gauge needle attached to a length of fine polyethylene tubing filled with heparin sodium (10 mg/ml) was inserted approximately 5 mm into the portal vein near the hilum of the liver. The other end of the polyethylene tubing was attached to a needle and syringe. In other studies, the main trunk of the hepatic artery, the left gastric artery, the splenic artery and the gastroduodenal artery were dissected out near the hilum of the liver. The left gastric artery was cannulated in a retrograde direction until the tip of the cannula was approximately 1 mm from the main trunk of the coeliac artery (Fig. 1). The gastroduodenal artery and the splenic artery were ligated, and a cotton thread was placed loosely around the main trunk of the coeliac artery. This experimental technique permitted the introduction of drugs via the cannula in the left gastric artery. The main hepatic arterial circulation was temporarily occluded by means of the cotton thread while the drug was injected through the arterial cannula. On loosening the cotton thread, the drug was 'washed in' and the hepatic arterial circulation was re-established.

In quantitative studies, ^{14}C-edrophonium chloride (usually 2·0 μmol/kg in 0·9% NaCl) was injected into a femoral vein over a 1 min period. Such doses have only slight effects on systolic or diastolic blood pressure; under these conditions, the drug is unlikely to influence liver blood flow. Bile was usually collected for 1–6 h after administration of the drug. Volumes were calculated by dividing the weight of each specimen by a previously determined value for the specific gravity of bile (1·011).

In other experiments, samples of bile were collected at 10 min intervals after injection of ^{14}C-edrophonium chloride. Samples of carotid arterial blood (0·2 ml) were removed at the midpoint of each collection of bile, and plasma was obtained after centrifugation. Small amounts of heparin (10 mg/ml) were used to prevent coagulation in the carotid arterial cannula. After assay of radioactivity, ^{14}C-edrophonium was separated from its metabolites in bile and plasma by paper chromatography.

Other studies were concerned with injection of the labelled drug into the femoral vein, the portal vein, or the hepatic artery by means of the techniques described above. Bile was collected at 5 min intervals for 20 minutes. In some experiments, specimens of bile collected from a cannula 25 cm long (dead space=13·5 μl) were directly applied to paper chromatograms and resolved as described below.

Identification of metabolites

Samples of bile were applied as a narrow band to one end of a sheet of Whatman no. 1 paper (40 cm × 50 cm). Labelled components were resolved by descending chromatography in solvent system A (see below) for 16 h at room temperature. A strip of paper (1 cm × 50 cm) was removed from the edge of the dried radiochroma-

togram and cut transversely into serially numbered squares (1 cm × 1 cm). Each square was transferred to a glass vial and radioactivity was detected by scintillation counting. Part of the remaining paper corresponding to the main peak of radioactivity was removed and rechromatographed in warm deionized water. Eluate was collected as the solvent front reached the end of the paper strip. Specimens of the eluate (0·5 ml) were added to β-glucuronidase (Ketodase, Warner-Chilcott, 15,000 Fishman units), incubated at 38° C for 36 h, and reduced in volume in a a current of nitrogen before paper chromatography.

Samples were developed by the descending technique on Whatman no. 1 paper. The following solvent systems were used: A, butan-2-ol-water-ethanol-acetic acid (32:12:8:1, by vol.); B, butan-2-ol-ethanol-water-aq. NH_3 of sp. gr. 0·88 (80:20:19:1, by vol.); C, butan-1-ol-water-ethanol-aq. NH_3 of sp. gr. 0·88 (90:25:10:1, by vol.); D, 2-methylpropan-1-ol-water-aq. NH_3 of sp. gr. 0·88 (100:15:2, by vol.); E, butan-2-ol-water-acetic acid (100:25:4, by vol.). After resolution, the radioactive peaks were determined by scintillation counting. Thin layer chromatograms (TLC aluminium sheet Polyamide 11 F254; Merck, Darmstadt) were activated (110° C for 30 min) prior to development in solvents A and B. Radioactive zones were detected by directly adding strips (0·5 cm wide) of the chromatogram to scintillation fluid. All samples were concurrently chromatographed with authentic standards of ethyl[1-^{14}C]dimethyl(3-hydroxyphenyl) ammonium chloride, tri[^{14}C]methyl(3-hydroxyphenyl)ammonium chloride, and 3-dimethylaminophenol. Radioactive standards were identified on paper and thin layer chromatograms as described above; standards of 3-dimethylaminophenol were located with ultraviolet light (254 nm) and sometimes developed with Dragendorff's reagent.

In some studies, samples of bile (0·6 ml) were adjusted to pH 5·0 (0·1 M HCl) and added to 0·2 M sodium acetate buffer, pH 5·0 (4·0 ml) or β-glucuronidase (20,000 Fishman units). Control specimens were prepared by the addition of β-glucuronidase to acidified bile containing glucaro-(1→4)-lactone (5 mM; Calbiochem). In both instances the final volume was approximately 4·7 ml. In parallel experiments, sulphatase (20 units; Sigma Chemical Company) was added to samples of acidified bile (0·6 ml) containing glucaro-(1→4)-lactone (5 mM). After incubation at 38° C for 48 h, the specimens were resolved by paper chromatography.

Measurement of radioactivity

Specimens of bile or plasma were directly added to scintillation fluid of the following composition (per litre): 2,5-diphenyloxazole (4 g), 2-ethoxyethanol (250 ml) and toluene (750 ml). Radioactivity in samples of bile or plasma (usually 25 μl) was counted at an efficiency of approximately 90% in a Unilux Nuclear Chicago liquid scintillation spectrometer. Counting efficiency was determined by the channels ratio method, using a ^{133}Ba external standard. Similar efficiencies (±1%) were obtained when n-hexadecane-1-^{14}C (The Radiochemical Centre) was used as an internal standard.

Paper and thin layer chromatogram strips were usually added to scintillation fluid containing (per litre): butyl-PBD (6 g), Triton-X-100 (333 ml) and toluene (667 ml). In some experiments paper chromatograms of radioactive bile were scanned in a Tracerlab 4π radiochromatogram scanner.

Results

Separation of metabolites

When bile collected after administration of ^{14}C-edrophonium chloride was resolved by paper or thin layer chromatography, radioactivity was mainly detected in two zones of the chromatogram (M1 and M2). M1 was identified as ^{14}C-edrophonium glucuronide (ethyl[1-^{14}C]dimethyl(3-oxyphenyl)ammonium glucuronide) by elution from paper chromatograms and hydrolysis with β-glucuronidase; R_f values after treatment with the enzyme were similar to authentic ^{14}C-edrophonium chloride (Table 1). Hydrolysis of M1 by β-glucuronidase was inhibited by the specific inhibitor glucaro-(1→4)-lactone; under these conditions, the radiochromatograms closely resembled those obtained when untreated bile was resolved (Table 2). M2 was readily identified as the unchanged quaternary amine (Table 1). Small amounts (less than 1%) of another unidentified metabolite (M3) were identified in some paper chromatograms. Authentic ^{14}C-edrophonium was readily separated from its C- and N-dealkylated analogues (trimethyl(3-hydroxyphenyl)ammonium and 3-dimethylaminophenol) in the solvent systems used (Table 1). Dealkylated metabolites of ^{14}C-edrophonium, or their glucuronide and sulphate conjugates, were not identified in bile.

TABLE 1. *Resolution of phenolic quaternary amines by paper or thin layer chromatography*

	Paper chromatography R_f values in solvent					Thin layer chromatography R_f values in solvent	
	A	B	C	D	E	A	B
^{14}C-Edrophonium	0·76	0·29	0·36	0·30	0·65	0·87	0·73
^{14}C-Trimethyl(3-hydroxyphenyl)ammonium	0·69	0·21	0·24	0·20	0·52	0·82	0·64
3-Dimethylaminophenol	0·91	0·95	0·91	0·91	0·85	—	—
Bile							
M1	0·14	0·03	0·05	0·02	0·04	0·63	0·11
M2	0·76	0·32	0·40	0·35	0·67	0·91	0·68
M1 after treatment with β-glucuronidase	0·71	0·34	0·37	0·34	0·58	0·87	0·75

Standard solutions of the quaternary amines or bile samples were resolved by descending paper chromatography or ascending thin layer chromatography.

TABLE 2. *Identification of M1 as ^{14}C-edrophonium glucuronide*

Sample	Proportion of total radioactivity (%) Edrophonium glucuronide (M1)	Edrophonium (M2)
Rat bile	98·4	1·6
Rat bile incubated with β-glucuronidase	3·0	97·0
Rat bile incubated with β-glucuronidase and glucaro-(1→4)-lactone	98·7	1·3

Values represent the proportion of the total radioactivity (corrected for background) detected as M1 and M2 on paper chromatograms.

Excretion in bile

Administration of ^{14}C-edrophonium chloride did not significantly affect the rate of biliary secretion. Table 3 shows the excretion of the unchanged drug and its glucuronide metabolite in bile. Only small amounts of radioactivity were eliminated, and less than 5% of the dose was recovered in bile in 6 hours. During the first hour, almost one-tenth of the radioactivity was eliminated as the unchanged drug; in contrast, only trace amounts of ^{14}C-edrophonium were subsequently excreted in bile. Thus, after the first hour, almost all the radioactivity in bile was eliminated as ^{14}C-edrophonium glucuronide.

Bile : plasma concentration gradient

The concentration gradient of ^{14}C-edrophonium and ^{14}C-edrophonium glucuronide between bile and plasma is shown in Table 4. Values were calculated during the first hour after administration of the drug; it was assumed that the mean concentration of edrophonium and its conjugate in 10 min collections of bile was representative of the level at the mid-point of each sample. The concentration of ^{14}C-edrophonium glucuronide in bile was always much greater than the plasma concentration, and the maximal gradient, which was attained 25 min after injection of the drug, was $21{\cdot}5 \pm 2{\cdot}5$ (mean $\pm$ S.E.M.). In contrast, the concentration of unchanged ^{14}C-edrophonium in plasma and bile was similar, particularly in the later stages of the experiment. The initial variability in the gradient (from $0{\cdot}8 \pm 0{\cdot}2$ at 5 min to $2{\cdot}3 \pm 0{\cdot}5$ at 15 min) may be related to the rapid exponential decline in the plasma concentration of edrophonium.

TABLE 3. *Excretion of radioactivity in bile as ^{14}C-edrophonium and ^{14}C-edrophoniumglucuronide*

Hour	% of dose	Excretion of radioactivity in bile % excreted as	
		Edrophonium	Edrophonium glucuronide
1	$1{\cdot}2 \pm 0{\cdot}7$	$8{\cdot}7 \pm 4{\cdot}1$	$90{\cdot}6 \pm 4{\cdot}2$
2	$2{\cdot}5 \pm 1{\cdot}3$	$1{\cdot}1 \pm 0{\cdot}4$	$98{\cdot}1 \pm 0{\cdot}8$
3	$3{\cdot}3 \pm 1{\cdot}7$	$0{\cdot}9 \pm 0{\cdot}3$	$98{\cdot}3 \pm 0{\cdot}4$
4	$4{\cdot}0 \pm 2{\cdot}0$	$0{\cdot}9 \pm 0{\cdot}3$	$98{\cdot}3 \pm 0{\cdot}4$
5	$4{\cdot}5 \pm 2{\cdot}2$	$0{\cdot}6 \pm 0{\cdot}2$	$98{\cdot}5 \pm 0{\cdot}6$
6	$4{\cdot}7 \pm 0{\cdot}7$	$0{\cdot}7 \pm 0{\cdot}2$	$98{\cdot}2 \pm 0{\cdot}9$

Values representing the % of the dose eliminated in bile were summated at hourly intervals. Results for the excretion of ^{14}C-edrophonium and ^{14}C-edrophonium glucuronide correspond to the proportion of each compound detected on paper chromatograms. Values, expressed as the mean $\pm$ S.D., represent the results of at least eight experiments.

TABLE 4. *Bile:plasma concentration gradient of ^{14}C-edrophonium and ^{14}C-edrophonium glucuronide*

Time (min)	Bile : plasma concentration gradient	
	^{14}C-edrophonium	^{14}C-edrophonium glucuronide
5	$0{\cdot}8 \pm 0{\cdot}2$	$14{\cdot}6 \pm 1{\cdot}5$
15	$2{\cdot}3 \pm 0{\cdot}5$	$16{\cdot}4 \pm 2{\cdot}2$
25	$2{\cdot}2 \pm 0{\cdot}3$	$21{\cdot}5 \pm 2{\cdot}5$
35	$1{\cdot}6 \pm 0{\cdot}3$	$16{\cdot}0 \pm 3{\cdot}1$
45	$1{\cdot}4 \pm 0{\cdot}3$	$15{\cdot}6 \pm 3{\cdot}3$
55	$1{\cdot}2 \pm 0{\cdot}1$	$14{\cdot}1 \pm 3{\cdot}2$

Specimens of blood were removed at the mid-point of each 10 min collection of bile. Values represent the mean $\pm$ S.E.M. of at least five experiments.

Route of administration

The influence of the route of administration on the elimination of the unchanged drug in bile is shown in Table 5. When ^{14}C-edrophonium chloride was injected into the hepatic arterial circulation, 0·3% of the dose was recovered from bile within 20 minutes. By comparison, less than 0·1% was recovered when the compound was infused into the portal or the femoral vein. Despite the small amounts of unchanged drug excreted, the differences observed were statistically significant ($P<0·05$). In other experiments in which ^{14}C-edrophonium chloride was directly injected into the hepatic artery, the unchanged drug was always detected in bile within 2–3 minutes.

Discussion

Only small amounts of ^{14}C-edrophonium and its metabolites were eliminated in bile, and less than 6% of the dose was recovered in 6 hours. Thus, the quantitative excretion of the drug in bile is similar to most other low molecular weight quaternary amines (Levine, 1960; Lüthi & Waser, 1965; Calvey, 1966). Many of these compounds are rapidly eliminated from the body by renal tubular secretion (Peters, 1960); concurrent studies have shown that approximately 50% of parenterally administered ^{14}C-edrophonium is excreted in urine in 24 h, both as the unchanged drug and as its glucuronide conjugate (Back & Calvey, unpublished). The present paper is concerned with the relatively insignificant proportion of the drug present in bile, because these experiments throw some light on the different means of excretion involved in the elimination of the unchanged drug and its glucuronide conjugate.

Identification of the major biliary metabolite of ^{14}C-edrophonium as a glucuronide conjugate (M1) was based on its elution from paper chromatograms and subsequent incubation with β-glucuronidase (and the addition of the specific inhibitor of β-glucuronidase, glucaro-(1→4)-lactone). Identification of the conjugate was somewhat incomplete since it was not possible to compare directly the R_f values of M1 and authentic edrophonium glucuronide in the various solvent systems used. However, no methods for the synthesis of quaternary amine conjugates were traced in the chemical literature, and attempts to synthesize authentic edrophonium glucuronide were unsuccessful.

Dealkylated metabolites of ^{14}C-edrophonium (or their glucuronide and sulphate conjugates) were not identified in bile. In contrast, demethylated conjugates of its analogue ^{14}C-trimethyl(3-hydroxyphenyl)ammonium have been isolated (Somani, Wright & Calvey, 1970; Calvey, Somani & Wright, 1970). These differences may be related to the failure of edrophonium to penetrate the lipoprotein barrier imposed

TABLE 5. *Effect of hepatic arterial (HA), portal venous (PV), and systemic venous (SV) administration on the excretion of unchanged ^{14}C-edrophonium in bile*

Time	% of dose excreted				
(min)	HA	PV	SV	P(HA,PV)	P(HA,SV)
5	0·15±0·05	0·03±0·01	0·01±0·002	<0·02	<0·02
10	0·21±0·06	0·06±0·01	0·03±0·01	<0·02	<0·02
15	0·27±0·06	0·08±0·01	0·04±0·01	<0·01	<0·01
20	0·29±0·07	0·09±0·01	0·05±0·01	<0·02	<0·01

Values represent mean ± S.E.M. of at least five experiments. Statistical analysis was based on the Student's *t* test. P(HA,PV) represents the probability that the differences between HA and PV are due to chance; P(HA,SV) represents the probability that the differences between HA and SV are due to chance.

by the endoplasmic reticulum, or to defective demethylation by microsomal enzymes.

In the present experiments, the concentration of ^{14}C-edrophonium glucuronide in bile was much greater than the plasma concentration, and the gradient observed ranged from 14·1–21·5. These results suggest that different physiological mechanisms may mediate the transport of the quaternary conjugate at the sinusoidal and the canalicular borders of the liver cell. Thus, while the metabolite may passively diffuse from hepatic cells to portal sinusoids, the increased concentration of ^{14}C-edrophonium glucuronide in bile is perhaps indicative of active transport of the glucuronide from the liver cell to bile. The large gradient observed in the present study may be related to the transport of conjugated bilirubin across the canalicular membrane.

Additional evidence supports this view, that is, that endogenous and exogenous glucuronides may be eliminated from the liver cell to bile by a common mechanism. In the photosensitive mutant Corriedale sheep (Cornelius, Arias & Osborn, 1965 ; Alpert, Mosher, Shanske & Arias, 1969) there is a genetic defect in the canalicular excretion of conjugated bilirubin ; in these animals, biliary elimination of ^{3}H-metanephrine glucuronide, the main metabolite of adrenaline, is only 10% of its excretion in normal sheep (Arias, Bernstein, Toffler & Ben-Ezzer, 1965). In other experimental conditions competition between quaternary amine glucuronides, conjugated bilirubin, sulphobromophthalein and phenolsulphonphthalein can be demonstrated (Calvey, Somani & Wright, 1970 ; Calvey & Back, 1970). It is therefore suggested that the large concentration gradient of edrophonium glucuronide between bile and plasma is dependent on its carrier transport at the canalicular membrane.

Different conditions apply to the elimination of unchanged ^{14}C-edrophonium in bile. During the first hour of these experiments, the concentration of the unchanged drug in plasma and bile was similar (particularly between 30 and 60 min). Thus edrophonium, like some other lipid-insoluble low molecular weight compounds (for instance, urea, erythritol, malonamide, and mannitol) is present in comparable concentrations in bile and plasma (Schanker & Hogben, 1961 ; Forker, 1967, 1968). Although these results are consistent with the passive transport of edrophonium from plasma to bile, it is not clear whether the parenchymal cells of the liver are involved in this process. In general, drugs may be passively transferred from blood to bile in three possible ways. In the first place, they may diffuse from the intrahepatic sinusoids to the biliary canaliculi across parenchymal cells. There is considerable evidence that both the sinusoidal epithelium and the liver cell are exceptionally porous (Kurz, 1961 ; Schanker, 1962) and may not present a barrier to the inward diffusion of most foreign compounds. (In contrast, the permeability of the canalicular membrane is a matter of conjecture.) Secondly, it is possible that low molecular weight drugs may be transferred from perisinusoidal and intercellular spaces into bile across the terminal bars that separate contiguous parenchymal cells (Lanman & Schanker, 1970). Finally, foreign organic compounds may pass directly from blood to bile via the submucosal and subepithelial capillary plexus that surrounds the larger ducts (the peribiliary vascular plexus).

In the present experiments, an attempt was made to determine the anatomical site of transfer of unchanged edrophonium from plasma to bile. In general, the blood supply to parenchymal liver cells is derived from both the portal vein and the hepatic artery. Although the sinusoidal network is primarily perfused by the portal

circulation, intralobular arterioles and capillaries derived from the hepatic artery enter the sinusoids at all levels of the liver lobule, and arterial blood does not exclusively supply the centrilobular zones (Elias & Petty, 1953 ; Elias & Sherrick, 1969). Thus, if parenchymal liver cells in general (or the terminal bars that separate adjacent cells) play an important part in the transfer of ^{14}C-edrophonium from plasma to bile, there is no reason to suppose that the biliary excretion of the unchanged drug after intra-arterial administration will exceed the amount eliminated when the compound is infused into the portal vein or the systemic circulation.

In contrast, most of the anatomical evidence suggests that the subepithelial plexus in the wall of the bile ducts (the peribiliary or periductal vascular plexus) is preferentially supplied by hepatic arterial blood. It is generally accepted that the plexus is not significantly vascularized by the portal vein or its branches in life (Elias & Petty, 1953 ; Elias, 1955 ; Elias & Sherrick, 1969) despite an earlier report to the contrary (Andrews, MaeGraith & Wenyon, 1949). Consequently, the statistically significant increase in the biliary elimination of unchanged ^{14}C-edrophonium after injection into the hepatic artery (Table 5) suggests that the peribiliary vascular plexus may play a major role in the elimination of edrophonium in bile.

Further experimental evidence supporting these conclusions was obtained from studies of the latent period before the appearance of ^{14}C-edrophonium in bile. The total volume of the biliary tree in the rat is roughly 5 μl/g liver (Barber-Riley, 1963), and the normal rate of spontaneous bile flow is about (1·4 μl/g liver)/min. Thus, there is a latent period of at least 3–4 min before drugs excreted at the biliary canaliculus can be detected in the common bile duct. After injection of ^{14}C-edrophonium chloride into the hepatic artery, unchanged drug was always detected in bile within 2–3 minutes. In these experiments, 1·4–2·2 min was required to traverse the dead space (13·5 μl) of the collecting cannula (see **Methods**).

Radioactivity was therefore present in the common bile duct within 1 min of the injection of ^{14}C-edrophonium into the hepatic artery ; clearly, the early appearance of radioactivity in bile indicated that the drug did not gain access to the liver cell or the biliary canaliculus, but must have been directly transferred across the bile duct wall.

These experiments therefore suggest that ^{14}C-edrophonium and its glucuronide conjugate are eliminated at different levels of the biliary tree. Elimination of the unchanged compound is mediated by passive transfer across the periductular epithelium, and is not dependent on the excretion of small amounts of unmetabolized drug by the liver cell via the biliary canaliculus. Since ^{14}C-edrophonium is rapidly removed from the circulation, only small amounts of biliary radioactivity are present as the unchanged drug after 1 hour. Although these findings cannot be uncritically applied to other foreign compounds, they suggest that a fraction of some drugs may be eliminated in bile without gaining access to drug-metabolizing enzymes in liver cells.

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