Biochemical Pharmacology, Vol. 19, pp. 1579–1590. Pergamon Press. 1970. Printed in Great Britain

ROLE OF THE ENTERO-HEPATIC CYCLE OF INDOMETHACIN ON ITS METABOLISM, DISTRIBUTION IN TISSUES AND ITS EXCRETION BY RATS, DOGS AND MONKEYS*

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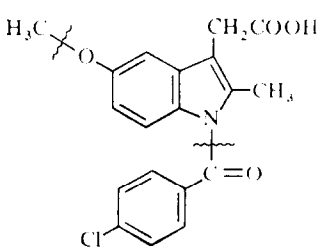
(*Received* 12 *April* 1969; *accepted* 5 *September* 1969)

Abstract—Dogs excrete most of an i.v. dose of ^{14}C-indomethacin unchanged in their feces. Monkeys extensively metabolize the drug to deschlorobenzoylindomethacin and excrete it in urine, and rats, without coprophagy, excrete the major metabolite, desmethylindomethacin, equally in urine and feces. ^{14}C-indomethacin constitutes the major radioactive species in plasma, liver and kidney of rats and monkeys. In both species, a new lipid-soluble metabolite of indomethacin was observed. The half-life of the drug in plasma varied among the three species ranging from minutes in monkeys and dogs to hours in rats.

In rats, plasma clearance of indomethacin by liver, although low, was thirty times the clearance rate by kidney, and the reabsorption of indomethacin from the intestine was extensive. Desmethylindomethacin, the major metabolite, was cleared from plasma equally by liver and kidney and was not reabsorbed from the intestine of rats. Consequently, this metabolite was distributed equally in urine and feces. Indomethacin was extensively and rapidly secreted in bile by dogs, eventually excreted as unchanged drug in their feces, and minimally metabolized to deschlorobenzoylindomethacin, which was excreted in urine. Monkeys were similar to dogs in that the liver was more than ten times as effective as the kidneys in clearing total radioactivity from plasma. However, they differed from dogs in that all drug species were maximally reabsorbed from the intestine. These differences in the plasma clearance of indomethacin and its metabolites by liver and kidney and in the entero-hepatic circulation of these drug species were sufficient to account for the species differences in the distribution and excretion of indomethacin.

INDOMETHACIN, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methyl-indole-3-acetic acid (Fig. 1), has anti-inflammatory activity in the rat[1, 2] and in man,[3–5] and it is purported that this nonsteroidal drug has several toxic side-effects.[3–9] Animals and man differ significantly



INDOMETHACIN (I)

Known metabolites are O-*desmethyl-I*, N-*deschlorobenzoyl-I and acyl glucuronides of all three.*

FIG. 1. Structure of indomethacin and its metabolites.

* This investigation was supported by the National Institute of General Medical Sciences, Contract No. PH-43-66-1139.

in their half-life of indomethacin in plasma, in their metabolism of indomethacin, and in their rates of excretion of drug and metabolites in urine.[10, 11] Knowledge of interspecies variations in drug binding to plasma protein[12–14] and in the metabolism of drug[15, 16] has aided in extrapolating pharmacological and toxicological data from experimental animals to man. In this study, we compare the plasma clearance of indomethacin and its metabolites by kidney and liver, the entero-hepatic circulation of indomethacin and its metabolites, and the physiological disposition of drug and its metabolites in urine, feces and tissues for three animal species: rats, dogs and monkeys.

METHODS

Male, Sprague–Dawley rats were obtained from the Blue Spruce Farm, Inc., Altamont, N. Y. The rats weighed from 140 to 170 g, except those used for the bile duct cannulation, which weighed 350–500 g. Conditioned female Rhesus monkeys, weighing between 2 and 4 kg were purchased from International Animal Exchange, Inc., Ferndale, Mich. They were checked for parasites and tuberculosis prior to use. Female beagle dogs (Roma Kennels, Dunstable, Mass.) weighed between 5 and 7 kg. All animals were dosed i.v. or orally with 2-^{14}C-indomethacin (specific activity was about 5000 or 10,000 dpm/μg respectively) at 5 or 10 mg/kg. (Further details are given in Results.) A stock solution of indomethacin was made by dissolving it in absolute ethanol with heat (less than 50°). It was stored at 4°. Periodic chromatography of this ethanolic solution of indomethacin on Whatman DEAE-23 demonstrated that it was stable under these conditions.[17] Aliquots of this solution were diluted with 0·05 M phosphate buffer (pH 8) to a concentration of 20 or 40 mg/ml within 1 hr of injection.

Monkeys were restrained in Foringer primate control cockpits (Foringer & Co. Inc., Rockville, Md.). Dogs were caged in stainless steel metabolism cages (Fenco Cage Products, Dorchester, Mass.), and rats were housed in individual No. 110 metabolism chambers (Maryland Plastics, Inc., New York, N. Y.) during the drug studies. All animals had access to food and water. Barnes *et al.*[18] have described a technique that we employed with modification for preventing coprophagy in the rats. The tail cups were made from 2-oz, narrow-mouth bottles (Nalgene Co. Inc., Rochester, N. Y.). The neck of the bottle was cut off, a circular hole was made in the bottom of the bottle with a No. 7 cork borer, and this hole was enlarged to a V-shape. The tail cup was covered with a 1·5-in. diameter rubber tubing (Green Rubber Co., Cambridge, Mass.). A common pin through the Elastoplast bandage around the tail of the rat and into the bottom of the tail cup held the cup in place. This latter modification minimized the occurrence of sore tails which was noted by Barnes *et al.*[18]

Blood, collected in tubes containing citrate, was obtained by heart puncture from lightly anesthetized rats (ether), by femoral tap of the monkeys, or by heart puncture of lightly anesthetized monkeys (Pentothal). Urine and feces were collected every 12 hr. After the last blood samples were obtained, the anesthetized animals were exsanguinated. Tissues were taken immediately and frozen in liquid nitrogen, and bile was taken from the gall bladder of the monkeys.

The larger rats selected for the 24-hr bile duct cannulation studies were anesthetized with chloral hydrate, U. S. P. (Fisher Scientific Co., Fairlawn, N. J.) at a dose of 360 mg/kg, and the bile duct was cannulated with Adams polyethylene tubing, P. E. 10 (E. F. Mahady Co., Cambridge, Mass.). These surgically prepared rats were restrained

but had access to food and water at all times. Indomethacin was injected intravenously into the caudal vein 2–3 hr after surgery. In the 2-hr cannulation studies with rats, the smaller rats (150–200 g) were anesthetized with chloral hydrate. In one group of animals the bile duct was cannulated with Adams polyethylene tubing, and the bladder was cannulated with the same tubing after the penis was ligated. In other experiments the bladder was cannulated but the bile duct was ligated by tying it in several places.

Female beagle dogs and Rhesus monkeys were employed in a study of the biliary and urinary excretion of indomethacin. Stage 3 anesthesia was induced by i.v. administration of nembutal (35 mg/kg) and maintained throughout the study by augmental doses of that drug. In the dog, respiration was maintained constant with a Harvard apparatus respirator. The common bile duct was cannulated or ligated, and the gall bladder was isolated by ligation of its stalk. A No. 12 Foley catheter was inserted retrograde through the urethra to remain indwelling in the urinary bladder. Bile and urine samples were collected at intervals through the bile cannula and the Foley catheter. Blood samples were collected by way of a 3-way, indwelling valve in the right jugular vein. Indomethacin was given via this valve.

The quantitative method for determining indomethacin and its major metabolites from biological tissues has been described.[17] Briefly, tissues are extracted with chloroform and anhydrous, acidic methanol and the extract is made biphasic with aqueous acid. Conjugated indomethacin and conjugates of other indomethacin metabolites partition to the aqueous, acid phase while protonated and esterified compounds partition to the organic phase. The ionizable drugs (indomethacin, deschlorobenzoylindomethacin and desmethylindomethacin) in the organic phase are then partitioned to an aqueous, basic phase prior to separation by anion-exchange chromatography (Whatman DEAE-23). The presumed esterified drug (component-X) remains in the basic, organic phase. This method is quantitative with samples of plasma, blood, liver, kidney, bile, urine and feces.[17]

RESULTS

Rats. As shown by Hucker *et al.*[11] rats excreted about equal quantities of indomethacin equivalents in the urine and feces and the half-life of drug in plasma was several hours. In our studies, we have confirmed these observations with rats fed *ad lib.* (Tables 1 and 2). Rats that were deprived of their feces had approximately the same concentration of radioactivity in plasma but the urinary excretion of drug and all metabolites was decreased from about 40 per cent of the i.v. dose to about 25 per cent, and fecal excretion of indomethacin and metabolites was increased from about 20 per cent of the dose to about 30 per cent in 48 hr (Table 2).

The patterns of fecal excretion in these two groups of rats were also different (Table 2). The quantity of indomethacin in the 0–24-hr fecal collection from rats that did not have access to their feces was markedly greater than the quantity found in feces from rats that had access to their feces. Indomethacin is absorbed after an oral dose.[11, 19] Thus, the decreased quantity of indomethacin in the 0–24-hr fecal collection of normal rats after the i.v. dose must have resulted from the absorption of indomethacin ingested during this 24-hr period. A similar analysis suggests a different conclusion for desmethylindomethacin. In normal rats, desmethylindomethacin was excreted principally in the second 24-hr fecal collection; whereas, in rats without access

TABLE 1. PREVENTION OF COPROPHAGY AND ITS EFFECTS ON PLASMA LEVELS OF ^{14}C-INDOMETHACIN IN RATS*

Time after administration of ^{14}C-indomethacin (hr)	Total radioactivity as indomethacin equivalents (μg/ml)†,‡	
	Coprophagy	Coprophagy prevented
0·33	63 ± 5	56 ± 3
1	46 ± 4	39 ± 4
3	35 ± 5	30 ± 4
6	25 ± 3	22 ± 4
24	4 ± 0·1	4 ± 2
48	0·3 ± 0·1	0·3 ± 0·1

* Each animal received 10 mg/kg of ^{14}C-indomethacin (4500 dpm/μg) i.v. All animals had access to food and water.
† Mean ± S. E.
‡ Greater than 85 per cent of total radioactivity was indomethacin; 4–6 per cent was desmethylindomethacin; and 3–4 per cent was deschlorobenzoyl-indomethacin.

TABLE 2. EFFECTS OF COPROPHAGY PREVENTION ON THE EXCRETORY PATTERN OF INDOMETHACIN AND ITS METABOLITES BY RATS*

Prevention of coprophagy	Collection period (hr)	Total radioactivity (per cent of i.v. dose)	Distribution of radioactivity in acidic $CHCl_3$–MeOH extract (μg)			
			Conjugates†	Indomethacin	Deschloro-benzoyl-indomethacin	Desmethyl-indomethacin
			Urine			
No	0–24	34·6 ± 3·5‡	160	20	23	187 ± 22
	24–48	4·6 ± 0·5	30	3	2	27
Yes	0–24	21·5 ± 5·8	140	12	27	118 ± 30
	24–48	3·6 ± 1·8	21	2	5	16
			Feces			
No	0–24	8·6 ± 2·0	0	39 ± 2	2	117 ± 7
	24–48	12·0 ± 4·1	0	18	5	240 ± 5
Yes	0–24	20·9 ± 3·7	0	105 ± 6	11	278 ± 18
	24–48	10·6 ± 1·1	0	19	2	148 ± 6

* There were three animals in each group; each animal received 10 mg/kg of ^{14}C-indomethacin (4500 dpm/μg) i.v. Coprophagy was prevented during the experimental period in one group, and all animals had access to food and water.
† Weight equivalents to indomethacin.
‡ Mean ± S. E. or mean.

to their feces, the major excretion occurred in the first 24 hr. The total excretion of desmethylindomethacin was nearly equivalent (15 per cent difference) for both groups of rats.

A per cent volume of distribution of about 15–20 per cent was calculated from the plasma concentrations of indomethacin in Table 1. This indicates that drug is distributed in the extracellular fluid of tissues. The major fraction in liver and kidney was indomethacin and the concentration ratios of indomethacin in these tissues relative to plasma were about 0·3 which is consistent with the 10–15 per cent volume of distribution. The remaining radioactivity was principally desmethylindomethacin and

component-X (footnote ‡, Table 3). A calculated tissue:plasma ratio for desmethylindomethacin was about 1 for liver and 2–3 kidney at most time periods. Since the concentration of component-X in plasma was not detected, a ratio cannot be obtained.

Half-life of indomethacin in plasma was minutes and the biliary excretion of ^{14}C-labeled indomethacin was a large fraction of the administered dose in several animal species: e.g. guinea pig, dog and monkey, and the rate of biliary secretion of indomethacin equivalents was greater than 40 per cent of i.v. dose in 3 hr.[11] In the rat, total biliary excretion of indomethacin equivalents was as large (Table 4), but the rate

TABLE 3. CONCENTRATION OF INDOMETHACIN IN LIVER AND KIDNEY OF RATS TREATED WITH ^{14}C-INDOMETHACIN*

Time after administration of ^{14}C-indomethacin (hr)	Concentration of indomethacin [μg/g (%)]†,‡	
	Liver	Kidney
0·33	13·5 (67)	10·6 (71)
1	11·5 (69)	13·6 (61)
3	8·7 (59)	9·5 (57)
6	5·4 (61)	5·4 (52)
24	< 0·3 (ca. 50)	< 0·6 (*ca.* 40)

* Specific activity of i.v. injected ^{14}C-indomethacin (10 mg/kg) was 4·750 dpm/μg. Animals had access to food and water, but coprophagy was prevented.

† Average of duplicates.

‡ The remaining percentage was distributed in approximately these ratios—deschlorobenzoylindomethacin : desmethylindomethacin : component-X, 0·5 : 1 :2 for liver and 0·5:5:1 for kidney.

of biliary secretion of indomethacin equivalents was considerably smaller (>10 per cent of i.v. dose in 3 hr, Table 4) than that for other species. (See Hucker *et al.*[11]) The relative percentages of the various drug components in bile from rats were approximately 40 per cent conjugates, 40 per cent indomethacin, 7 per cent deschlorobenzoylindomethacin, 7 per cent desmethylindomethacin and 5 per cent component-X. Plasma clearance of indomethacin by liver was about thirty times the clearance by kidney. Plasma clearance of desmethylindomethacin and deschlorobenzoylindomethacin was statistically equivalent for both tissues and many times greater than the plasma clearance of indomethacin.

In the 2-hr cannulation studies (Table 4), a small percentage of the i.v. dose of indomethacin was cleared by both kidney and liver and the greater clearance was through the liver. The plasma clearance of total radioactivity by these animals corresponded to the plasma clearance for indomethacin in the 24-hr bile cannulation studies which suggests that indomethacin was the principal drug component being cleared in the 2-hr cannulation studies. In these bile- and urine-cannulated rats the plasma levels of indomethacin equivalents were similar to those of normal rats (Table 1). When the bile duct was ligated, the plasma clearance of total radioactivity, total per cent of i.v. dose and urine volume increased slightly, but the plasma levels of indomethacin equivalents did not vary significantly from those in Table 1.

Monkeys. Like the guinea pig, the monkey had a short half-life of indomethacin in

plasma, excreted mostly deschlorobenzoylindomethacin in the urine[10] and excreted a large fraction (48 per) cent of the administered dose in the bile.[11] In the guinea pig, high tissue:plasma ratios were obtained, which suggests that indomethacin equivalents may be concentrated in various tissues.[11] Using the plasma concentration for monkey,[11] a per cent volume of distribution of about 200 can be determined, suggesting a similar localization of drug in tissue and/or excretion.

TABLE 4. BILIARY AND URINARY EXCRETION OF INDOMETHACIN AND ITS METABOLITES BY RATS

Determinations, mean ± S. E.		Urine	Bile
24-hr Bile cannulation, urine excretion*			
Per cent of i.v. dose in 24 hr		24·1 ± 7·8	65·9 ± 6·3
Volume—ml		9·2 ± 0·2	18·0 ± 8·5
Plasma clearance based on:			
(1) total dpm	ml/min†	0·0247 ± 0·0152	0·0750 ± 0·0390
(2) indomethacin	ml/min†	0·0010 ± 0·0002	0·0325 ± 0·0125
(3) deschlorobenzoylindomethacin	ml/min†	0·0330 ± 0·0165	0·0813 ± 0·0318
(4) desmethylindomethacin	ml/min†	0·1193 ± 0·0522	0·2584 ± 0·1054
2-hr Bile and urine cannulation‡			
Per cent of i.v. dose in 2 hr		0·27 ± 0·20	4·15 ± 1·00
Volume—ml		1·2 ± 0·40	2·5 ± 0·8
Plasma clearance based on:			
(1) total dpm—ml/min†		0·0012 ± 0·0010	0·0167 ± 0·0071
2-hr Urine cannulation, bile ligation§			
Per cent of i.v. dose in 2 hr		1·39 ± 0·10	
Volume—ml		1·3 ± 0·6	
Plasma clearance based on:			
(1) total dpm—ml/min†		0·0044 ± 0·0017	

* Four rats (350–500 g) with cannulated bile ducts were given single i.v. doses of radioactive indomethacin at 10 mg/kg (ca. 10,000 dpm/μg). All rats were restrained during the experimental period and had access to food and water. Bile was collected every hour for 6 hr and thereafter every 3 hr to 24 hr and urine was collected every 3 hr for 24 hr.

† Plasma clearances were calculated using the mean concentration of drug in plasma and the total drug excreted in bile or urine for many collection periods. (See footnotes * and ‡.)

‡ Four rats (150–200 g) with cannulated bile duct and bladder were given a single i.v. dose of ^{14}C-indomethacin at 10 mg/kg (ca. 10,000 dpm/μg). All rats were anesthetized with chloral hydrate during the experiment. Bile and urine were collected at 15, 30, 45, 60, 90 and 120 min after the i.v. dose of drug.

§ Same as ‡; bladder was cannulated, but bile duct was ligated.

The concentration of total radioactivity in most tissues (lung, heart, muscle, brain and fat) was low and tissue:plasma ratios of about 0·5–1 prevailed 0·5, 1, 2 and 4 hr after the administration of ^{14}C-indomethacin. In the liver and kidney, the concentration of indomethacin was high initially and decreased rapidly (Table 5). The percentage and concentration of indomethacin in both tissues (Table 5) were generally smaller than those seen for the rat (Table 3). Component-X represented the second most concentrated fraction in both liver and kidney of monkey (footnote §, Table 5). A high tissue:plasma ratio for indomethacin is apparent for liver and kidney, and might indicate a concentration of indomethacin by these tissues. However, one has to consider the rate of biliary excretion and the magnitude of the entero-hepatic circulation of indomethacin that might decrease plasma concentrations of indomethacin and metabolites rapidly to affect a high tissue:plasma ratio.

TABLE 5. CONCENTRATION OF INDOMETHACIN IN PLASMA, LIVER AND KIDNEY OF MONKEY*

Time after administration of ^{14}C-indomethacin (hr)	Indomethacin concn (μg/g) (Per cent of extracted radioactivity)		
	Plasma†	Liver‡	Kidney‡
0·33	2·9 ± 1·6 (85)§	16·4 ± 6·6 (57)	14·5 ± 3·6 (72)
1	0·9 ± 0·7 (85)	10·8 ± 1·1 (59)	3·2 ± 0·1 (78)
2	0·5 ± 0·3	3·4 ± 0·1 (53)	2·6 ± 1·1 (53)
3	0·6 ± 0·1		
4	0·6 ± 0·2	1·4 ± 0·8 (63)	0·6 ± 0·5 (44)
5	0·4 ± 0·3		
6	0·5 ± 0·1 (85)	1·5 ± 1·0 (35)	1·7 ± 0·6 (58)
24	0·1 ± 0·06	0·8 ± 0·05 (37)	0·5 ± 0·4 (35)

* ^{14}C-indomethacin (8700 dpm/μg) was injected i.v. at 5 mg/kg of body weight.
† Mean ± S. E.; the number of determinations were 9 at 0·33 hr, 5–7 at 1, 2 and 3 hr, 3–4 at 4, 5 and 6 hr, and 2 at 24 hr.
‡ Mean of duplicates ± S. E.
§ The remaining percentage was distributed between deschlorobenzoylindomethacin and component-X in these ratios: 2:1 for plasma; 1:6–10 for liver; and 1:2–3 for kidney.

TABLE 6. ^{14}C-INDOMETHACIN EQUIVALENTS IN BILE OF MONKEYS AT AUTOPSY*

Time after administration of ^{14}C-indomethacin (hr)	^{14}C-indomethacin equivalents in bile†,‡	
	(μg/ml)	(Per cent of i.v. dose)
0·5	7250; 61,700	10·3;§ 58·5
1	38,700; 12,000	18·2; 8·7
2	9430; 13,100	4·1; 8·1
4	18,000	16·1
6	36,200	20·4
24	19,800; 25,300	18·0; 20·4

* Radioactive indomethacin (8700 dpm/μg) was injected at 5 mg/kg of body weight. Bile was collected during autopsy of the animal, but no bile was collected from the duplicate monkey at 4 and 6 hr.
† Individual determination.
‡ Using the extraction and chromatographic method (Yesair and Coutinho),[17] the radioactivity was distributed about 15–20 per cent conjugates, 15–20 per cent component-X, 60–70 per cent indomethacin, < 5 per cent deschlorobenzoylindomethacin, and < 1 per cent desmethylindomethacin.
§ This animal at 0·5 hr corresponds to the same animal with superscript † in Table 7.

A large percentage of the administered radioactivity was found in the bile obtained from the gall bladder about 10–15 min after sacrifice of monkeys (Table 6). Most (60–70 per cent) of the radioactivity was indomethacin, and the remainder was mostly conjugates (presumably of indomethacin) and component-X. There was a 5-fold variation in percentage of i.v. dose in bile at 0·5 hr, but the variation at other time periods was not so great. As seen in Table 7, there was also a 3- to 4-fold variation in drug concentration in duodenal tissue and content between the two animals at 0·5 hr. The animal with the higher levels in duodenal tissue and content (footnote †, Table 7) had a smaller percentage of the i.v. dose in its bile (footnote §, Table 6). The concentration of radioactivity in washed intestine and its contents was exceedingly high (Table 7) and in general the ratio of radioactivity in content to that in tissue was about

3:5. The fecal excretion of radioactivity by the monkey was about 3 to 4 per cent of the i.v. dose per 24 hr for 120 hr.[11] In our studies, we find less than 10 per cent excreted in the feces in 72 hr. Most (>95 per cent) of this radioactivity was in the acidic chloroform:methanol extract and chromatographed as deschlorobenzoylindomethacin (60 per cent) and indomethacin (40 per cent). In the fecal extract, less than 1 per cent of the total radioactivity was desmethylindomethacin. The excretion of indomethacin

TABLE 7. RADIOACTIVITY IN INTESTINAL TISSUE AND CONTENT OF MONKEYS TREATED INTRAVENOUSLY WITH ^{14}C-INDOMETHACIN*

Time after i.v. dose of ^{14}C-indomethacin (hr)	^{14}C-indomethacin equivalents (μg/g)					
	0·5		4		24	
Intestinal tissue						
Duodenum	675†	155	40	15	10	5
Jejunum	45	205	85	50	30	10
Ileum	40	35	340	35	20	40
Cecum	25	20	1040	255	130	190
Colon	10	20	25	2	5	35
Intestinal content‡						
Duodenum	2200	760	100	25	60	5
Jejunum	135	515	315	85	100	110
Ileum	60	35	1680	105	60	305
Cecum	§	§	3640	660	2160	204

* Radioactive indomethacin (8700 dpm/μg) was injected i.v. at mg/kg. Determinations were made on portions of the intestinal tissues and contents from two monkeys at each time period.
† This animal at 0·5 hr corresponds to the same animal with superscript § in Table 6.
‡ Colon had been stripped of contents at time of autopsy.
§ No intestinal content available.

TABLE 8. EXCRETION OF INDOMETHACIN AND ITS METABOLITES IN URINE OF MONKEYS AFTER ORAL AND I.V. ADMINISTRATION OF ^{14}C-INDOMETHACIN*

Treatment	Collection period (hr)	Total radioactivity (Per cent of dose)‡	Distribution of radioactivity in acidic $CHCl_3$–MeOH extract (μg)†		
			Conjugates	Indomethacin	Deschloro-benzoyl-indomethacin
Oral§	0– 3	1·4 ± 0·2	85	120	15
	3–24	10·4 ± 0·2	440	150	500
	24–48	8·1 ± 0·5	180	60	160
	48–72	4·3 ± 1·2	392	10	85
Intravenous‖	0– 3	6·0	150	280	170
	3–24	14·0	350	360	540
	24–48	22·6	800	250	995
	48–72	10·1	330	50	430

* ^{14}C-indomethacin (10–11,000 dpm/μg) was given orally or i.v. at a dose of 5 mg/kg.
† These three components represent greater than 90 per cent of the extractable radioactivity in the urine, and the remainder was distributed equally between component-X and desmethylindomethacin.
‡ By 72 hr less than 10 per cent of administered radioactivity was excreted in feces and was distributed in the feces accordingly; conjugates:indomethacin:deschlorobenzoylindomethacin:desmethylindomethacin and component-X, 23:29:14:23:11 per cent.
§ Urine was collected from two animals and samples for total radioactivity (mean ± S. E. for duplicate determinations). The distribution of radioactivity was done on samples which were pooled from two animals.
‖ Represents a single animal. Similar distributions of radioactivity were obtained on additional monkeys which were studies for 0–2, 2–6 and 6–24 hr.

and its metabolites by kidney is given in Table 8. The predominant radioactive compounds were deschlorobenzoylindomethacin and conjugates (presumably of deschlorobenzoylindomethacin).[10] The excretion of indomethacin relative to other radioactive compounds in the urine was highest during the first 3 hr, then decreased.

The plasma clearances of indomethacin and its metabolites by kidney and liver of monkeys are given in Table 9. The major excretion of radioactivity (mostly indomethacin, Table 6) was in the bile (ca. 52 per cent) of monkeys with cannulated bile

TABLE 9. BILIARY AND URINARY EXCRETION OF INDOMETHACIN AND ITS METABOLITES BY MONKEYS*

Biological samples	Bile and bladder Cannulated monkeys†	Bile ligated and bladder Cannulated monkeys†
Plasma		
μg/ml at 15 min	18·1 ± 7·7	38·9 ± 8·2
μg/ml at 30 min	9·3 ± 5·5	26·3 ± 6·7
μg/ml at 45 min	5·3 ± 3·8	20·1 ± 6·4
μg/ml at 60 min	4·3 ± 2·2	15·1 ± 5·7
μg/ml at 90 min	2·1 ± 1·5	11·2 ± 5·4
μg/ml at 120 min	1·4 ± 1·0	8·8 ± 5·3
Urine		
Per cent i.v. dose in 2 hr	4·1 ± 0·1	11·0 ± 4·0
Volume (ml) in 2 hr	9·0 ± 1·4	31·1 ± 4·9
Plasma clearance—ml/min‡	1·18 ± 0·70	0·81 ± 0·56
Bile		
Per cent i.v. dose in 2 hr	51·7 ± 13·6	
Volume (ml) in 2 hr	1·4 ± 0·6	
Plasma clearance—ml/min‡	14·1 ± 5·0	

* Monkeys were anesthetized with Nembutal, common bile duct was cannulated or ligated, and a catheter remained indwelling in the urinary bladder. Each animal (four total) received 5 mg/kg of ^{14}C-indomethacin (10,800 dpm/μg). Plasma was taken from the jugular vein at stated time period. Total bile and urine was collected at 15-min intervals for 60 min and at 30-min intervals for the next hour.

† There were two animals in each group. The data are mean ± S. E. of duplicate determinations, except plasma clearance. Plasma clearances were based on nine determinations.

‡ Plasma clearance was calculated using the mean concentration of drug in plasma and total quantity of drug excreted in bile or urine for each of the collection periods between 15 and 120 min. (See footnote *.)

ducts. The plasma clearance by liver was about fourteen times the clearance by kidneys. In these animals, plasma concentrations of indomethacin decreased rapidly although not quite as rapidly as in the normal, nonanesthetized monkey (Table 5). When the bile duct and gall bladder were ligated, much higher plasma levels of indomethacin were observed and the percentage of administered dose in the urine also increased. It is important to note, however, that this 3-fold increase in urinary output of indomethacin equivalents was associated with a 3-fold increase in urine volume rather than an increased clearance rate.

Dogs. Hucker *et al.*[11] have reported that the dog excretes at least 80 per cent of the administered radioactivity in the feces, excretes a large fraction of dose in the bile and absorbs a significant percentage (ca. 50) of the biliary radioactivity from the intestine.

TABLE 10. EXCRETION OF INDOMETHACIN AND ITS METABOLITES IN URINE AND FECES OF DOGS*

Collection period (hr)	Total radioactivity (Per cent of i.v. dose)†	Distribution of radioactivity in†,‡ acidic $CHCl_3$–MeOH extract (%)		
		Conjugates	Indomethacin	Deschlorobenzcyl-indomethacin
		Urine		
0–24	4·5 ± 1·7	43·4 ± 2·7	3·4 ± 1·6	50·5 ± 2·9
24–48	3·1 ± 0·5	38·8 ± 3·6	5·5 ± 2·0	54·3 ± 3·4
48–72	0·3 ± 0·1			
		Feces		
0–24	63·6 ± 15·9	2·7 ± 0·3	88·9 ± 1·6	5·2 ± 1·4
24–48	2·1 ± 1·8	5·2 ± 1·0	87·8 ± 4·5	3·6 ± 2·5
48–72	10·6 ± 9·8	4·0 ± 0·8	92·6 ± 2·8	1·6 ± 1·5

* ^{14}C-indomethacin (5300 dpm/μg) was injected i.v. at 5 mg/kg of body weight and three dogs were used.
† Mean ± S. E.
‡ Greater than 95 per cent of the radioactivity was extracted by the acidic $CHCl_3$–MeOH extract. Desmethylindomethacin and component-X were negligible (< 2 per cent each).

In our studies, most of the administered radioactivity from ^{14}C-indomethacin also appeared in the feces (Table 10). The major fecal component was indomethacin. Although deschlorobenzoylindomethacin was the predominant radioactive species in urine, the amount was relatively small. The concentration of desmethylindomethacin was insignificant in both urine and feces.

The plasma clearances of indomethacin equivalents by kidney and liver of a dog were approximately 1 and 10 ml/min respectively. Drug concentrations in plasma decreased rapidly ($T_{1/2}$ = 10–20 min). Most of the radioactivity (>65 per cent) appeared in the bile within 1 hr and less than 10 per cent of the administered dose was cleared by the kidney in 4 hr.

DISCUSSION

Pharmacological responses to drugs by animal species can be comparable when drug concentrations in plasma are equivalent.[20, 21] However, many factors will affect drug concentration in plasma.[21] In these studies, we have evaluated species differences in the metabolism of indomethacin, in plasma clearances of drug and its metabolites, and in the extent of the entero-hepatic circulation of drug and metabolites.

The half-life of indomethacin in plasma ranged from hours in rats to minutes in dogs and monkeys. Indomethacin was the major compound in plasma, liver and kidney of both rats and monkeys, and in plasma of dogs. Biliary excretion of indomethacin and its conjugates was extensive and rapid in dogs and monkeys, but slow in rats. Most of the i.v. dose of ^{14}C-indomethacin appeared unchanged in feces of dogs. Monkeys extensively metabolized indomethacin to deschlorobenzoylindomethacin which was excreted in urine. A major metabolite, desmethylindomethacin, was excreted by rats equally in urine and feces, whereas deschlorobenzoylindomethacin appeared primarily in urine.

Rats metabolize indomethacin to desmethylindomethacin.[10] There was an extensive but slow excretion of indomethacin and its conjugates in bile by rats. Most of this excreted drug can be expected to be reabsorbed from the intestine.[11, 19, 22] Conjugated

components in bile are probably not absorbed *per se*, but may first be hydrolyzed in the intestine.[23] Because there is an entero-hepatic circulation of indomethacin, it can be extensively metabolized, although slowly, to desmethylindomethacin, deschlorobenzoylindomethacin and their conjugates. Since both unconjugated metabolites are cleared from the plasma by liver and kidney equally well, equivalent excretion of both metabolites into urine and feces would be expected. Although this appears true for desmethylindomethacin, deschlorobenzoylindomethacin must be reabsorbed from the intestine since this metabolite was only in the urine. Desmethylindomethacin, which was cleared equally well by both the kidney and liver, appeared in both feces and urine and thus it is presumed that this metabolite was not readily reabsorbed from the intestine.

The half-life of indomethacin in plasma of monkeys was considerably smaller than that seen in rats. This rapid fall in plasma concentration of indomethacin was probably due to the rapid clearance of indomethacin by the liver into bile since relatively little indomethacin was excreted into urine. When the bile duct was ligated, the concentration of drug in plasma of these monkeys increased significantly and the half-life of drug in plasma increased three to five times above normal. Plasma clearance of drug by kidney did not significantly change from normal in these bile-duct-ligated monkeys, even though there was a small increase in urinary excretion of radioactivity.

In the monkey, these data support the conclusion that there is an extensive entero-hepatic circulation of indomethacin and its metabolites: an extensive excretion of indomethacin in bile, a movement of this radioactivity down the intestine with time, an absorption of radioactivity along the intestine which may depend on the concentration of radioactivity in the contents, a small amount (>10 per cent of i.v. dose in 72 hr) of radioactivity in the feces, a slow excretion of radioactivity in urine, and small concentrations of radioactivity in plasma and tissue. Because of this entero-hepatic cycle for indomethacin in monkey, indomethacin can be extensively metabolized, even at a slow rate to deschlorobenzoylindomethacin which is excreted in the urine.

Dog excreted about 7 per cent of the administered dose of indomethacin in the urine, principally as deschlorobenzoylindomethacin. The reabsorption of biliary excreted indomethacin is about 50 per cent.[11] Thus, by the time that 80 per cent of the administered dose appears in the feces as indomethacin, it has entered this entero-hepatic cycle many times. This cycling allows for the metabolism of indomethacin, but little (7 per cent) was metabolized to deschlorobenzoylindomethacin by the dog. The minimal extent of this metabolism, therefore, suggests that dogs metabolize indomethacin at a slow rate.

In both the rat and monkey, we have observed an unidentified radioactive compound in the liver, kidney and bile. It partitioned to the acidic, chloroform phase of extracts and remained in the organic phase after 2 extractions with aqueous base. These partitioning characteristics suggest that the carboxyl group of indomethacin and/or of its metabolites cannot be ionized. This uncharacterized metabolite(s) was more concentrated in tissues from monkeys than from rats. Species differences in concentration of lipophilic metabolites of thalidomide in liver have been observed and have been correlated with toxicity of this drug.[24, 25] Similarly, the activity of indomethacin in different species may correlate with the difference in concentration of the lipophilic, non-ionizable metabolite(s) of indomethacin.

Acknowledgement—We thank K. Meany, M. Stein, J. Wall and P. Denine for their expert assistance in these studies.

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