THE FATE OF PHENOL, o-PHENYL PHENOL AND DISOPHENOL IN RATS

by

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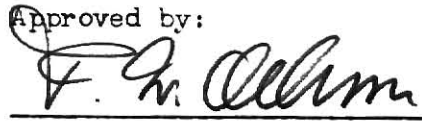

Major Professor

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Without the gracious hand of God on me a dream of life could have evaporated overnight, I acknowledge the works of God with great appreciation for they are just too marvellous in my eyes.

Lastly but perhaps of greater importance, my deep appreciation goes to my wife, Liz, my son Ayo and my daughter Bukie who patiently bore my long absence from our home and sustained me by being a source of continuous encouragement and joy. My family deeply appreciates the scholarship award by the Federal Government of Nigeria which made this study possible. To all these and many other friends too numerous to mention I dedicate this thesis, with sincere and grateful thanks.

FATE OF PHENOL IN RATS

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Fate of Phenol in Rats. GBODI, T. A. and OEHME, F. W. (1977). Toxicol. Appl. Pharmacol. 00, 000 - 000. Disappearance of UL-^{14}C phenol (P) from the blood of unanaesthetized or pentobarbital anaesthetized rats with venous, arterial and biliary cannula, and its excretion into bile, was evaluated for 5 hr after iv administration of 25 mg P/kg. The bile contained 10.73 ± 0.45 % and 5.01 ± 0 % of the dose in anaesthetized and unanaesthetized rats, respectively. Maximum biliary excretion of P occurred during the first 1/2 hr. Enzyme induction due to pentobarbital appeared to occur in the anaesthetized rats. The major biliary metabolite was phenyl glucuronide (PG). The decline in the apparent P concentration in the bile of unanaesthetized rats paralleled that in the blood, providing a constant bileblood ratio. Blood kinetic data of both groups of rats were different. The majority of injected radioactivity appeared in urine in the first 6 hr (81.69% ± 2.64) and was associated with metabolites identified as PG and PS; PS predominated. Limited enterohepatic circulation was demonstrated. Two hours after P administration, highest levels of radioactivity were found (in decreasing order) in liver, kidney, lungs, testis, heart muscle, and spleen.

INTRODUCTION

Abou-el-Makarem et al (1967) showed that compounds with a molecular weight of less than 325 ± 50 are poorly excreted in bile. Phenol (P) is one of these compounds with only 4% of the administered dose in rats so excreted. They reported that 54% of the P in bile was phenyl glucuronide (PG) with three other unidentified metabolites. Capel et al (1972) studied the renal excretion of ^{14}C-P in several species and reported 95% of the administered dose excreted in 24 hr in urine as four metabolites: PG, phenyl sulfate (PS), quinol sulfate (QS), and quinol glucuronide (QG).

This study is concerned with metabolism and mode of excretion of P, its tissue distribution, the effect of pentobarbital on P metabolism, and the enterohepatic circulation of P and its metabolites.

METHODS

Animals

Charles River Dunkin-Hartley short hair male rats weighing 250-350 g were used throughout. The rats were housed individually in suspended rodent cages and fed Purina Rat Chow and tap water ad libitum.

Chemicals

Phenol[a], benzoyl peroxide[a], 2,6-dichloroquinone chloroimide[a], naphtharesorcinol[a], trichloroacetic acid[a], ^{14}C-P[b] (specific activity 58 mCi/mM, 611 uCi/mg, 98% radiochemically pure, β-glucuronidase[a] (prepared from bovine liver, specific activity 989800 Fishman Units/g), and β-glucuronidase with sulfate activity[a] (prepared from helix pomatia,

specific activity 380,000 Fishman Units/g) were all purchased commercially.

Determination of Blood Levels

Rats were anesthetized with sodium pentobarbital (60 mg/kg ip). Femoral veins and arteries of the rats were cannulated with PE-50 polyethylene tubing[c] attached to a heparinized syringe. The P was administered iv at the rate of 25 mg/kg. The representative animal received 5 µCi of activity with 6.26 mg chemical P in 1 ml saline. Blood was collected before injection and thereafter at $\frac{1}{2}$ hr interval for 5 hr. Triplicate samples of 50 µl whole blood were solubilized with 1 ml NCS tissue solubilizer in counting vials immediately after collection. Samples were left for two days and then bleached with 150 µl 20% benzoyl peroxide in toluene. Ten ml of toluene counting solution (5 g PPO and 0.1 g dimethyl-POPOP/1 toluene) was added to each vial. All samples were counted in a Unilux II liquid scintillation counter at an efficiency of 73-90%. Kinetic data was calculated using standard equations. The apparent volume of distribution (V_d) = meandose/C_o, where C_o is the extrapolated mean blood radioactivity at time zero. The specific volume of distribution (V_d') = V_d/mean weight of the animals. The kinetic constant (Kd) = $0.693/T\frac{1}{2}$.

Determination of Urinary Excretion and Metabolites

Rats were anesthetized with ether, the femoral veins were cannulated, and radioactive P administered as described earlier. The incisions were closed, the animals allowed to recover from anesthesia, and they were then placed in suspended steel metabolism cages with free access to water

and food. Urine samples were collected in conical flasks to which 2 ml of saturated mercuric chloride had been added to prevent bacterial decomposition of metabolites. Urine samples were collected at 6, 12, 24, 48, and 72 hr and measured. One hundred µl of urine were evaluated in duplicate by adding the samples directly to 10 ml Bioflour[b] and counting with an efficiency of 81-83% in the liquid scintillation counter.

Fifty to 100 µl of the 6 and 12 hr urine samples were placed on 5 cm x 56.5 cm strips of Whatman 1 chromatograph paper. The strips were hung in an airtight discending chromatograph tank systems with the appropriate developing solvent system placed in the bottom of the tank for 3-4 hr to equilibrate (system A = 1-propanol: ammonia hydroxide 7:3 ; system B = 1-butanol: ammonia hydroxide : distilled water 10:1:1). The trough was then filled with the solvent system and the paper developed for 17-19 hr. The developed strips were removed, and dried and viewed under 254 nm ultra violet light. Strips were sprayed with specific reacting chemical mixtures and dried. For the detection of glucuronide and sulfate conjugate of P, naphtharesorsinol, Gibbs reagent and hydrochloric acid were used as described by Capel et al (1972). Other strips were scanned using a Parkard radio-chromatogram scanner model 7201. The radioactivity under each peak was quantitated by mechanical intergration. For enzyme hydrolysis, 200 µl of urine was incubated with 300 µl of β - glucuronidase or with β-glucuronidase with sulfatase activity. (9 mg/ml in 0.1 M acetate buffer pH 4.5) Products were then chromatographed and compared with similar strips of unhydrolyzed urine to identify unknown metabolites.

Determination of Biliary Excretion and Metabolites.

For bile duct cannulation, rats were anesthetized ip with sodium pentobarbital. A small midline abdominal incision was made, and the common bile duct was exposed at its junction with the duodenum. A small opening was made on the bile duct about 1.5 cm from the duodenal junction with a microdissecting scissor. A PE-10 polyethylene tubing[c] was inserted 3 cm into the duct and sutured in position. The abdominal incision was then closed with the end of tubing in a graduated tube for bile collection. Rectal temperatures of the anesthetized rats were maintained at 37 C with a heat lamp connected to an automatic temperature regulator[f] to prevent hypothemic alteration in hepatic function (Roberts et al, 1967). Femoral veins and arteries were cannulated as described earlier.

Bile and blood was collected for 15 min for baseline evaluation prior to administration of P. Bile and blood were then collected simultaneously every 30 min for 5 hr. Sodium pentobarbital was administered periodically to maintain anesthesia. Blood samples were handled and evaluated as described earlier, and 100 µl bile aliquots were counted in 10 ml of Bioflour with an efficiency of 87-90%. Biliary metabolites were identified and quantitated in $\frac{1}{2}$ and 1 hr bile samples using the identical technics as for urinary metabolites.

Investigation of Differences in Biliary Excretion Between Pentobarbital Anesthetized and Unanesthetized Rats

The bile ducts and femoral arteries and veins were cannulated exactly as described above except that ether was used instead of sodium pentobarbital to anesthetize the rats during surgery. The rats were

placed in Econo-restraining cages[g] following recovery, and bile and blood were collected as described earlier.

Determination of Enterohepatic Circulation

The ^{14}C-P was administered iv to anesthetized bile donor rats and the bile collected for 2 hr via biliary cannula. The radioactivity in the bile was quantitated. The bile was then injected into the duodenum of recipient rats with cannulated bile ducts whose blood and bile were being collected for 2 hr. At the end of 2 additional hr the recipient rats were sacrificed with sodium pentobarbital, and the radioactivity in blood, bile, liver and kidneys was quantitated.

Determination of Tissue Residues

Phenol was administered to rats that were sacrificed after two hr, and the liver, kidneys, heart, lungs, thymus, thyroid, testis, abdominal wall fat, brain, and portion of the quardraceps muscle were removed for quantitation of radioactivity. Approximately 50 mg tissue samples were placed in counting vials and treated with 2 ml NCS tissue solubilizer for five days at 40 C. Two hundred µl 20% benzoyl peroxide in toluene were added to each vial to bleach the mixture prior to liquid scintillation counting in toluene counting solution.

RESULTS

Phenol Excretion and Distribution

After iv injection of P, activity could be detected in bile within 5 min. Fig. 1 and 2 provide the apparent mean activity (± SE) of P and

its metabolites in bile and blood for the pentobarbital anesthetized and unanesthetized rats during the 5 hr period. In the pentobarbital anesthetized rats Vd was calculated as 0.24 l, Vd' was 0.85 l/kg, and Kd was 0.60/hr. For the unanesthetized rats the Vd was 1.2 l, the V_d' was 4.14 l/kg and Kd was 0.53/hr.

Semi-logarithmic plots of biliary radioactivity against time, revealed that the line was a first order process (Fig.1 and 2). The biliary elimination half life for pentobarbital anesthetized rats was 0.71 hr or 43 min, while for unanesthetized rats it was 1.30 hr or 78 min.

Table 1 shows the recovery of radioactivity in bile as percent of total dose every half hour for the pentobarbital anesthetized and unanesthetized rats. About 11% of the dose was recovered in the anesthetized rats. Only 5% of the total dose was recovered in the bile collected for 5 hr from the unanesthetized rats for 5 hr. Highest excretion occured within the first $\frac{1}{2}$ hr after the injection of phenol, and biliary excretion of phenol was almost completed by the end of the first 1 hr.

Color and Enzymatic Detection of Metabolites of Phenol in Urine and Bile

The color reactions were not found to be specific. The naphthare-sorcinol test for detection of PG and QG produced a blue color that was too faint to be specific and was not limited to areas where metabolites were detected by scanning or enzyme hydrolysis. The Gibbs reagent and hydrochloric acid tests were likewise nonspecific.

In urine and bile treated with β-glucuronidase, the Rf 0.11 peak disappeared with a corresponding increase in the P peak (Rf 0.92). This showed that it was a glucuronide metabolite. Both the Rf 0.11 and Rf 0.52

peaks disappeared in the samples treated with glucuronidase with sulfatase activity, with a corresponding increase in P peak, showed that the Rf 0.52 peak was a sulfate metabolite.

Urinary Excretion and Metabolites of Phenol

Table 2 shows that about 82% of the injected radioactivity was excreted in urine within the first 6 hr. By 12 hr 89% of the dose was excreted in the urine, with 90% of the total administered dose recovered in 48 hr. Only traces of activity were found in all urine samples at the end of 48 hr. Two major metabolites were found (Table 3) and in some urine samples phenol was also detected, possibly as a result of limited bacteria decomposition of metabolites during the collection period.

Biliary Metabolites

The radiochromatogram scans of $\frac{1}{2}$ hr and 1 hr bile samples (Table 4) showed four peaks for most rats, none identified as P. Some rats had an additional peak of Rf 0.81 and 0.61 in solvent systems A and B respectively. Bile developed in solvent system B showed only four peaks. Glucuronidase-treated bile developed in solvent system A gave two peaks; the peaks at Rf 0.17, 0.51 and 0.68 disappeared with a corresponding appearance of a phenol peak at Rf 0.96. The β-glucuronidase-treated bile developed in solvent system B showed only two peaks on scanning (Rf 0.00 and 0.61), with the peak at Rf 0.00 reduced in height and area and peaks at Rf 0.16 and 0.30 having disappeared from pretreated samples. This showed that three of the five metabolites (Rf 0.17, 0.51 and 0.68 in solvent system A) were glucuronide conjugates.

The β-glucuronidase with sulfatase activity-treated bile gave similar results as the β-glucuronidase-treated bile, and showed that no sulfate conjugate of P was excreted in bile. Since the commercial enzymes were dissolved in acetate buffer, bile samples were also treated only with acetate buffer; these had all the same peaks found in untreated bile samples. This showed that the buffer had no effect on the bile samples.

Two of the metabolites, denoted B and D in Tables 4 and 5, and the metabolite identified as PG, were hydrolysed by β-glucuronidase. The glucuronide with Rf 0.51 (identified as PG) accounted for 61-65% of the metabolites in the $\frac{1}{2}$ and 1 hr bile samples. Metabolites denoted by A and D were unaffected by β-glucuronidase and β-glucuronidase with sulfatase activity. Metabolites A, B, C and D accounted for approximately 40% of the total metabolites excreted in the $\frac{1}{2}$ and 1 hr bile samples. The bile metabolite results from anesthetized rats are comparable to those from unanesthetized rats in Table 4 and 5.

Enterohepatic Circulation

About 2% of the activity in bile collected from donor rats appeared in the bile of recipient rats. Trace amounts of activity was detected in the liver and kidneys of recipient rats.

Tissue Distribution

Fig. 3 shows that two hours after the injection of P most of the activity had been excreted. When compared to the total dose given no significant accumulation of activity could be detected in any of the organs. The highest concentration of P was found in the kidneys, followed

by decreasing concentrations in blood, lungs and spleen.

DISCUSSION AND CONCLUSIONS

Since Vd was less than 1 l (0.24 l) and Vd' was 0.85 l/kg in anesthetized rats, there was no significant tissue binding of phenol at that dose level. However, a significant difference was noticed in the kinetic data from the unanesthetized rats. The Vd for unanesthetized rats was 1.21 l and Vd' was 4.146 l/kg. These figures indicate extensive tissue distribution of P in unanesthetized rats. The kinetic constants of the two groups of rats were also different; 0.60/hr for anesthetized and 0.53/hr for unanesthetized rats. This indicates a faster biotransformation and excretion of P in anesthetized rats. This difference may be due to the well-documented enzyme induction effect of the barbiturates, of which pentobarbital is a member. If true, the availability of greater amount of metabolizing enzymes in pentobarbital-treated rats could effect a rapid conjugation of P into more polar, easily excretable, and less protein bound metabolites. This effect has been noted to occur in vitro almost instantaneously (Kuriyama et al, 1969). It is well documented that where as some compounds, like bilirubin, steroids and bromosulphthalein (BSP), are extensively and strongly bound to plasma albumin, their metabolic transformation products are less protein bound (Smith, 1973). Baker and Bradley (1966) showed that BSP is strongly bound to plasma proteins but the form in bile, the gluthathione conjugate, has 100 times less affinity for binding to plasma proteins than does BSP itself. Thus it is possible that the treatment of

rats with pentobarbital could have also had an indirect effect on the distribution and protein binding affinity, and hence increased the rate of P elimination.

Renal excretion of P was almost completed by the end of the first 12 hr. This result is close to that of Capel et al (1972), who used female rats and found that about 95% of the oral administered dose was excreted in 24 hr. The two major urinary metabolites observed had the same Rf values (0.11 and 0.52 in solvent system A) for PG and PS as found by Capel et al (1972).

Capel et al (1972) also detected ΨS and ΨG which we failed to detect. Our results support the suggestions of Garton and Williams (1949), Porteous and Williams (1949), and Williams (1959) that the metabolites formed from P are dependent on the speed of conjugation as well as the enzymatic pathways present. They found that iv injection of P gave only two metabolites, PG and PS, where as oral administration gave other oxidation conjugated products also. They speculated that iv injection of P made it immediately available for conjugation and hence little opportunity was provided for formation of oxidation products.

It is also known that metabolism of chemicals differ in different strains of animal species. Mitoma et al (1967) observed differences in hexobarbital sleeping time and microsomal hexobarbital oxidase activity in various strains of rats. Our metabolite studies indicated that about 42% of P excreted in 6 hr urine sample was PG and 57% was PS; 28% of PG and 71% of PS were produced in 12 hr urine samples. Our results from the 6 hr samples agree with that of Capel et al (1972) who found 54% PS and 42% PG in 24 hr urine samples from rats.

By the end of the first hr biliary excretion of P was almost

completed. Abou-el-Makarem et al (1967) using female wistar albino rats recovered 4.60% of the P dose in 24 hr.

It is interesting to compare the results of P biliary excretion in the unanesthetized and anesthetized rats; only 5% of the dose was recovered in 5 hr, i.e. less than half the amount recovered from pentobarbital anesthetized rats. One apparent reason for the difference in biliary excretion between the two groups of rats is enzyme induction, since the only variable was pentobarbital anesthesia. Several groups of compounds, especially the barbiturates, are known to have enzyme induction effects on microsomal enzyme systems of animals. Conney (1967) demonstrated the effect of a single injection of phenobarbital on microsomal enzyme systems. Kuriyama et al (1969) showed that a single administration of phenobarbital induced a prompt increase in the rate of total microsomal protein and NADPH-cytochrome C-reductase synthesis in vitro. Thus it is possible that the difference observed in pentobarbital treated and untreated rats was due to enzyme induction of the microsomal enzyme system.

Abou-el-Makarem et al (1967), using female wistar albino rats, recovered 54% PG from the metabolites of P in bile; three other unidentified metabolites were also detected. We detected a fifth unidentified metabolite in some of our rat's bile.

The result of the enterohepatic circulation studies indicated that 2% of the P and its metabolites entered the enterohepatic circulation. The detection of higher activity in the kidneys than in other tissues tends to confirm the importance of the remal system in the elimination of P in rats.

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The authors thank Steve Galitzer for help in conducting this study.

Fig. 1. Radioactivity (mean ± SE) in bile and blood of anesthetized rats after iv administration of ^{14}C-phenol (25 mg/kg). For the biliary data, n = 6 rats; for blood, n = 9 rats. The correlation coefficients (r) for both curves = 0.99.

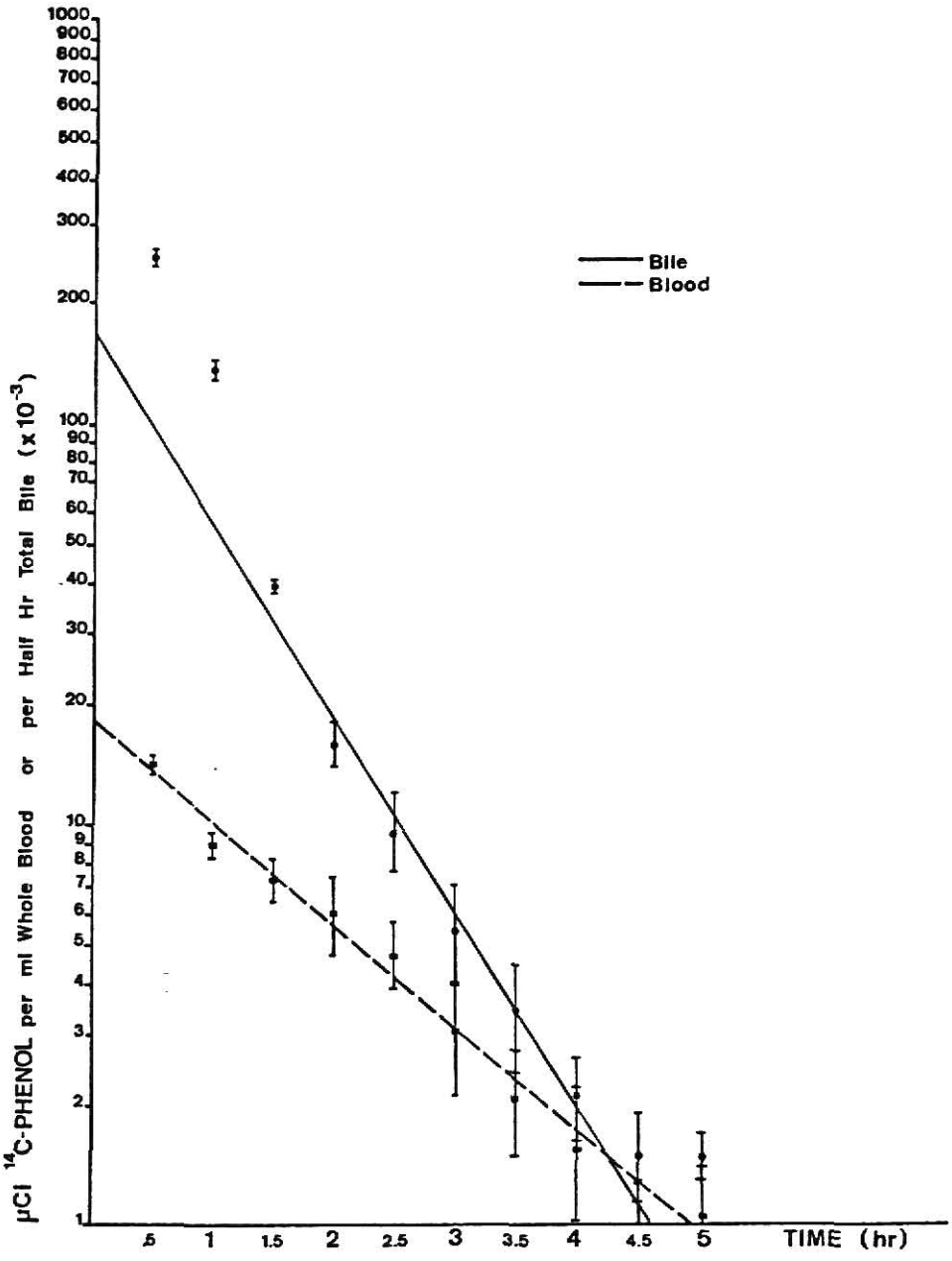
Bile
Blood
μCi ¹⁴C-PHENOL per ml Whole Blood or per Half Hr Total Bile (x10⁻³)
1000
900
800
700
600
500
400
300
200
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
.5
1
1.5
2
2.5
3
3.5
4
4.5
5
TIME (hr)

Fig. 2. Radioactivity (mean ± SE) in bile and blood of unanesthetized rats after iv administration of ^{14}C-phenol (25 mg/kg). For the biliary and blood data, n = 4 rats. The slopes of the two least square regression lines for the declining phase were not statistically different (P 0.05). The correlation coefficient (r) for biliary line = 0.96 and for blood line = 0.99.

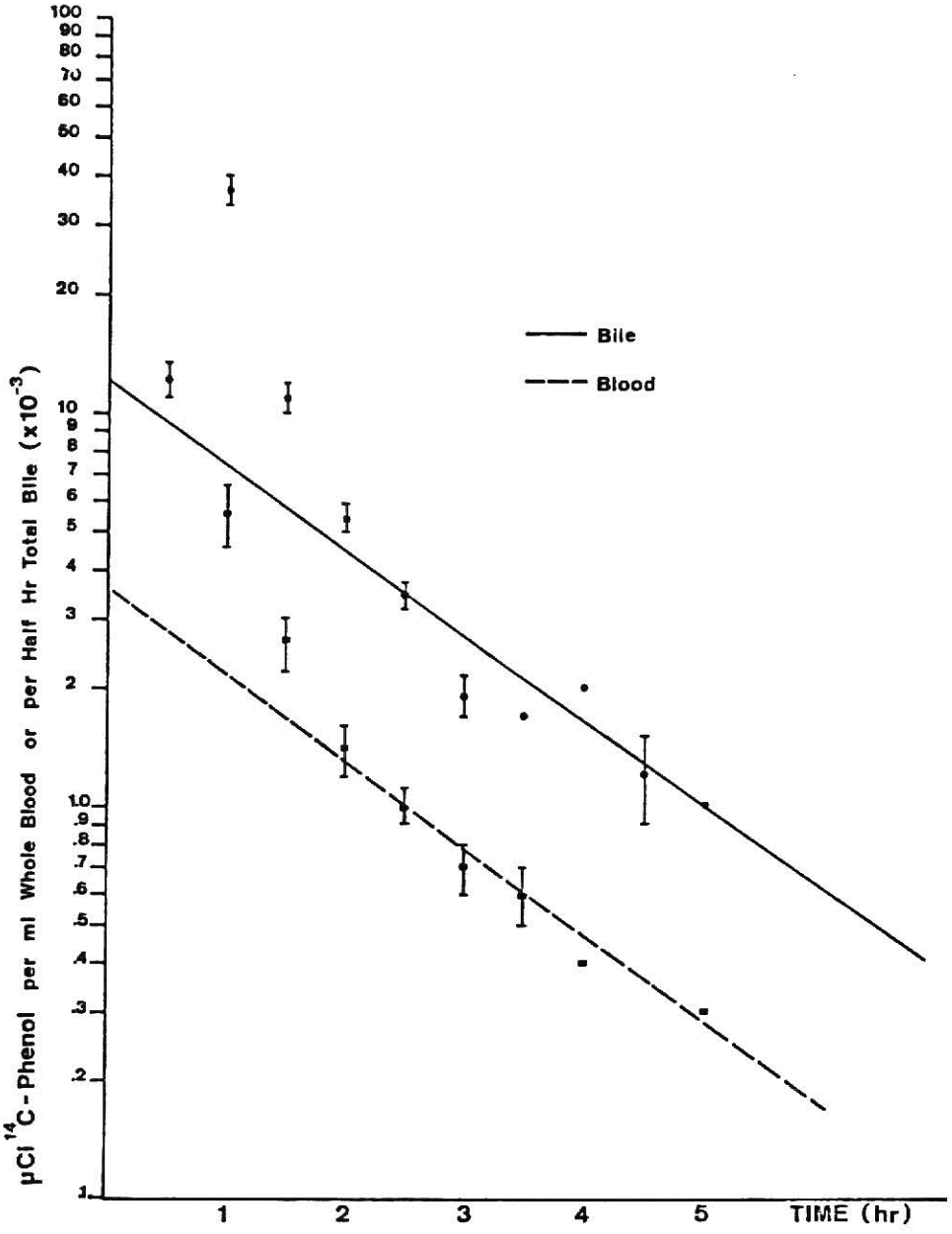
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1.0
.9
.8
.7
.6
.5
.4
.3
.2
1
1
2
3
4
5
TIME (hr)
Bile
Blood
μCi 14C-Phenol per ml Whole Blood or per Half Hr Total Bile (x10-3)

Fig. 3. Concentration of phenol radioactivity in various tissues from rats 2 hr after receiving 25 mg phenol/kg by iv administration.

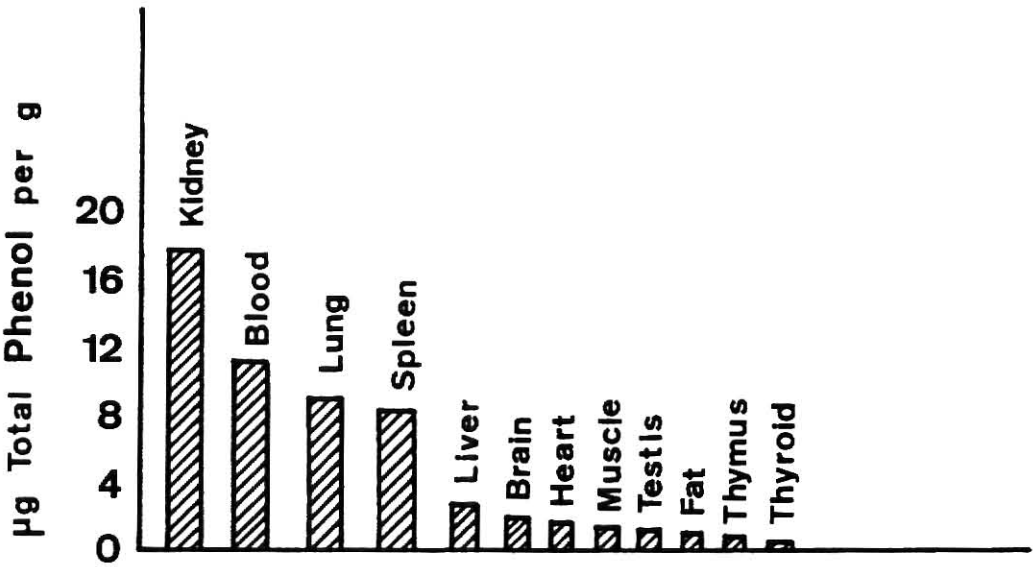
µg Total Phenol per g
0
4
8
12
16
20
Kidney
Blood
Lung
Spleen
Liver
Brain
Heart
Muscle
Testis
Fat
Thymus
Thyroid

TABLE 1

DISTRIBUTION OF RADIOACTIVITY IN BILE OF PENTOBARBITAL ANESTHETIZED AND UNANESTHETIZED RATS FOLLOWING INTRAVENOUS ADMINISTRATION OF PHENOL

	ANESTHETIZED RATS		UNANESTHETIZED RATS	
TIME AFTER DOSING (HR)	MEAN[a] % OF PHENOL DOSE	RANGE	MEAN[b] % OF PHENOL DOSE	RANGE
1/2	5.82 ± 0.23[c]	4.89 - 6.48	3.52 ± 0.57	2.24 - 4.99
1	3.09 ± 0.18	2.67 - 3.40	0.86 ± 0.08	0.67 - 1.04
1½	0.89 ± 0.05	0.72 - 1.09	0.24 ± 0.02	0.19 - 0.33
2	0.36 ± 0.04	0.28 - 0.57	0.12 ± 0.00	0.10 - 0.14
2½	0.22 ± 0.05	0.07 - 0.49	0.07 ± 0.00	0.05 - 0.10
3	0.12 ± 0.03	0.05 - 0.31	0.04 ± 0.00	0.29 - 0.05
3½	0.07 ± 0.02	0.03 - 0.19	0.03 ± 0.00	0.03 - 0.04
4	0.05 ± 0.01	0.02 - 0.10	0.04 ± 0.00	0.03 - 0.27
4½	0.03 ± .00	0.02 - 0.05	0.02 ± 0.00	0.02 - 0.03
5	0.03 ± .00	0.02 - 0.05	0.02 ± 0.00	0.02 - 0.02
% TOTAL DOSE	10.73 ± 0.45	8.92 -12.26	5.01 ± 0.00	3.64 - 6.71

[a] Six male rats

[b] Four male rats

[c] Standard Error of Mean

TABLE 2

MEAN PERCENT OF TOTAL PHENOL DOSE (25 mg/kg) EXCRETED IN URINE OF ELEVEN RATS AT VARIOUS TIME INTERVALS

	% of dose excreted in urine				
TIME	6 hr	12 hr	24 hr	48 hr	Total % dose
$\bar{X} \pm SE$	81.69 ± 2.64	6.85 ± 1.93	1.92 ± 0.37	0.83 ± 0.24	91.16 ± 2.25
Range	64.57 - 94.38	1.32 - 23.64	1.00 - 5.45	0.09 - 2.82	73.72 - 100.69

TABLE 3

MEAN PERCENT OF ^{14}C-PHENOL EXCRETED IN URINE AS PHENOL AND METABOLITES

METABOLITES	Rf_A	6 hr Urine Sample		12 hr Urine Sample	
		% Urinary radioactivity recovered as	Range	% Urinary radioactivity recovered as	Range
Phenyl Glucuronide	0.11	42.12 ± 1.27	36.37-49.39	28.45 ± 3.30	13.74-40.67
Phenyl Sulfate	0.52	57.49 ± 1.04	3.08- 0.36	70.84 ± 3.34	59.32-86.25
Phenol	0.92	0.36	0.3.61	--	--

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

[a] Each value represents the mean ± SE of 11 rats.

TABLE 4

MEAN PERCENT ^{14}C-PHENOL EXCRETED AS VARIOUS METABOLITES OF PHENOL IN 1/2 HR AND 1 HR BILE OF ANESTHETIZED RATS

METABOLITES	Rf_A	Rf_B	1/2 hr[a]		1 hr[b]	
			Mean %	Range	Mean %	Range
A	0.00	0.00	13.33 ± 1.44[c]	10.58 - 17.29	27.63 ± 0.97	24.44 - 32.40
B(Glucuronide)	0.17	0.00	16.78 ± 0.97	14.19 - 18.78		
Phenyl Glucuronide	0.51	0.16	61.43 ± 2.79	47.41 - 74.18	65.20 ± 1.95	57.62 - 74.18
C(Glucuronide)	0.68	0.30	5.83 ± 1.44	1.04 - 10.72	5.22 ± 0.96	0 - 7.00
D	0.81	0.61	3.84 ± 0.53	0 - 5.42	3.77 ± 0.81	0 - 5.03

[a] N = 8 rats

[b] N = 7 rats

c = ± SE

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

Rf_B = Rf value of metabolites in 1-butanol: ammonia hydroxide: distilled water (10:1:1)

TABLE 5

MEAN PERCENT ^{14}C-PHENOL EXCRETED AS VARIOUS METABOLITES OF PHENOL IN 1/2 HR AND 1 HR BILE OF UNANESTHETIZED RATS.

METABOLITES	Rf_A	Rf_B	1/2 hr[a]		1 hr[b]	
			Mean %	Range	Mean %	Range
A	0.00	0.00	12.41 ± 1.46	9.63 - 23.05	13.05 ± 1.15	11.42 - 23.54
B(Glucuronide)	0.17	0.00	11.04 ± 1.00	9.67 - 13.50	14.34 ± 2.06	11.42 - 17.27
Phenyl Glucuronide	0.51	0.16	62.45 ± 1.53	57.82 - 65.16	57.20 ± 4.04	47.32 - 62.85
C(Glucuronide)	0.68	0.30	9.98 ± 0.90	8.27 - 12.96	9.08 ± 1.27	6.83 - 12.08
D	0.81	0.61	4.19 ± 0.39	3.49 - 5.40	7.59 ± 0.67	5.95 - 8.63

[a] N = 4 rats

[b] N = 3 rats

c = ± SE

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

Rf_B = Rf value of metabolites in 1-butanol: ammonia hydroxide: distilled water (10:1:1)

[a] Sigma Chemical Company, St. Louis, Missouri.

[b] New England Nuclear, Boston, Massachusetts.

[c] Clay Adams, Parsippany N.J.

[d] Amersham/Searle, Arlington Heights, Illinois.

[e] Nuclear-Chicago Corporation, Des Plaines, Illinois

[f] Model 71A, Yellow Springs Instrument Co., Yellow Springs, Ohio.

[g] Laboratory Animal Care Products, Maryland Plastic Inc., New York, N.Y.

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FATE OF o-PHENYL PHENOL IN RATS

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Fate of o-Phenyl phenol in Rats. GBODI, T. A. and OEHME, F. W. (1977). Toxicol. Appl. Pharmacol. 00, 000 - 000. The disappearance of ^{14}C - o-phenyl phenol (OPP) from the blood of pentobarbital anesthetized and unanesthetized male rats, provided with venous, arterial and biliary cannula, and its excretion into bile was evaluated for 5 hr after iv administration of 40.8 mg OPP/kg. Blood kinetic data from both groups of rats was significantly different. The majority of the injected radioactivity appeared in urine during the first 6 hr (73.42 ± 3.31 % of the dose) and was associated with four glucuronide metabolites. The unchanged OPP accounted for (71.82 ± 1.70 %) of the urine radioactivity. The bile contained 20.10 ± 1.23 % or 15.96 ± 1.23 % of the dose is anesthetized or unanesthetized rats respectively. Maximum biliary excretion occured during the first 1/2 hr. An enzyme induction effect due to pentobarbital appeared to occur in the anesthetized rats. The major biliary metabolite was the glucuronide conjugate (80.19 ± 1.82% of biliary activity). Prominent enterohepatic circulation was demonstrated. The highest level of radioactivity 2 hr after administration was found in the kidneys.

INTRODUCTION

The sodium salt of o-phenyl phenol (OPP) has found wide use as a household germicidal and in disinfectant sprays (Deichman and Keplinger 1963); thus human and animal exposure is a reality. The molecular weight of OPP is 170.2 which is lower than the minimum threshold weight of 325 ± 50 required for appreciable biliary excretion in rats (Abou-el-Makarem et al, 1967). However, OPP can acquire both this threshold molecular weight and the polarity required for appreciable biliary excretion by undergoing biotransformation in the animal body. The introduction of the phenyl group into a simple benzene or aromatic ring can enhance biliary excretion; 37% of 4-hydroxy biphenyl was excreted in rat bile as the glucuronic acid (Smith 1973). Dodgson et al (1948) and Kamil et al (1951) showed that both OPP and p-phenyl phenol were highly conjugated with glucuronic acid and excreted in the urine of rabbits. The studies of Oehme and Smith (1972) in cats and dogs showed that the urinary metabolites of OPP included unchanged OPP; its glucuronide and sulfate conjugates, and phenol (P) derived from cleavage of the phenyl-phenol bond followed by ring hydroxylation. The P metabolites resulted from both OPP-ring moieties.

This current study investigates the metabolism and excretion pattern of OPP, the effect of pentobarbital on OPP metabolism, OPP biliary excretion, the enterohepatic circulation of OPP and its metabolites, and OPP tissue distribution in rats.

METHODS

Animals

Charles River Dunkin-Hartley short hair male rats weighing 250-350 gm were used. The animals were handled previously as described in studies with phenol (Gbodi and Oehme, 1977).

Chemicals

Radioactive ^{14}C - o-phenyl-phenol (specific activity 6.7 μCi/mM on the phenyl moiety) was purchased commercially[a]. All the other chemicals were secured and prepared as previously described (Gbodi and Oehme, 1977). A dose of 40.8 mg chemical OPP/kg was utilized; the representative animal received 10.2 mg chemical OPP and 5.56 μCi radioactive OPP dissolved in 1 ml of polyethylene glycol: distilled water (92:100, w:v) administered iv.

Investigated Biological Parameters

Evaluations were performed of blood levels, urinary excretion, effect of pentobarbital on OPP metabolism, biliary excretion, enterohepatic circulation, and tissue residue of OPP using methods described previously (Gbodi and Oehme, 1977). The biological half-life ($t_{\frac{1}{2}}$) was related to the kinetic constant (Kd) according to the equation $t_{\frac{1}{2}} = 0.693/Kd$. Urinary and biliary metabolites were identified utilizing β-glucuronidase and β-glucuronidase with sulfatase activity hydrolysis (Gbodi and Oehme, 1977).

RESULTS

Blood Disappearance and Biliary Excretion of OPP

Blood and biliary OPP decay line are shown in Fig. 1 and 2. The blood data in pentobarbital anesthetized rats resulted in an apparent volume of distribution of (Vd) of 0.30 l, a specific volume of distribution (Vd') of 0.92 l/kg and a kinetic constant (Kd) of 0.40/hr. The blood data from unanesthetized rats showed a Vd of 0.434 l, a Vd' of 1.31 l/kg, and a Kd of 0.38/hr.

Activity could be detected in bile within 5 min after OPP administration. Biliary excretion of OPP was a first order process. The $t_{\frac{1}{2}}$ for anesthetized rats was 0.66 hr (39 min) while for unanesthetized rats it was 0.75 hr (45 mins).

Table 1 shows that 20.10 ± 1.23% and 15.96 ± 1.23% of the administered OPP dose was recovered in bile collection from anesthetized and unanesthetized rats, respectively in 5 hr. In the first ½ hr and 1 hr, 14.09 ± 1.36% was recovered in anesthetized rats while 10.59 ± 0.94% and 3.44 ± 0.55% was found in bile from unanesthetized rats.

Urinary Excretion

Table 2 shows the radioactivity recovered in urine. Almost 74% of the dose was excreted in the urine during the first 6 hr, and by the end of 24 hr the urinary excretion of OPP was almost completed. Approximately 89% of the dose was recovered at the end of 72 hr.

Urinary Metabolites

Chromatographic separation and quantitation of 6 hr and 12 hr urinary metabolites of OPP are recorded in Table 3. There was no significant

difference in results between the two sample periods. The major urinary metabolite was unchanged OPP, with four other metabolites detected. Urine treated with β-glucuronidase lost metabolites A, B, C and D with a subsequent increase in OPP concentration. When β-glucuronidase with sulfatase activity was used to treat urine, the results were identical. The majority of the radioactivity was excreted in the urine as unchanged OPP (71.82 ± 1.70%). Metabolite C was the second major metabolite, with 14.83 ± 0.92% of the urinary radioactivity within 6 hr.

Biliary Metabolites

Bile samples gave one major peak (C) and four others (A,B,D and OPP), as shown in Table 4. Unchanged OPP was detected in bile of all rats. Samples treated with β-glucuronidase produced only one peak (OPP), which increased substantially in the hydrolyzed specimens. All four metabolites were therefore glucuronide conjugates.

Enterohepatic Circulation

Of the 0.95 µCi of bile activity injected into the duodenum of recipient rats, 0.10% appeared in 100 µl urine, 0.02%in the kidneys, 0.56% in the liver, and 1 0.60% was found in 1 ml of blood. The significant degree to which OPP undergoes enterohepatic circulation is indicated by the fact that 1.022% of the radioactivity was found in the bile during the 2 hr collection period.

Tissue Residues

The tissue radioactivity 2 hr after iv administration of OPP is given in Fig. 3. The highest level was found in the kidneys, followed

by lesser concentration in liver, fat, lungs, muscle, heart, testis and brain.

DISCUSSION AND CONCLUSIONS

Fig. 1 and 2 show the relationship between blood and biliary radioactivity after iv administration of OPP. As the blood radioactivity (OPP and metabolites) declined, the biliary excretion of OPP also had a corresponding fall. However, biliary radioactivity declined faster than that of the blood in both anesthetized and unanesthetized rats. The blood kinetic data indicated lack of extensive tissue distribution of OPP in both groups.

The slight difference in Kd values (0.40/hr in anesthetized and 0.38/hr in unanesthetized rats) indicates that blood clearance of OPP was faster when pentobarbital anesthesia was used.

This could be due to the difference in OPP biliary excretion between the two groups; that of the anesthetized rats was higher. The biotransformation enzyme systems for OPP, as in the case of P (Gbodi and Oehme, 1977), may have been promptly induced by the pentobarbital with the greater capability developing for glucuronide formation and biliary excretion. This view is further enhanced by the work of Garth et al (1976) who showed that the administration of a single oral dose of some alcohols to rats produced prompt selective induction of some components of the hepatic microsomal enzyme systems.

The major glucuronide biliary metabolite of OPP (C) had the same Rf value as the major urinary glucuronide metabolite (C). They are probably the same compound. This metabolite constituted about 80% of the radioactivity

associated with bile and 14% of the urine radioactivity. This metabolite is most likely the glucuronide conjugate of OPP, and the other three minor glucuronide conjugates in urine and bile are likely various glucuronide conjugates of the oxidation products of OPP.

Milburn et al (1967) administered p-phenyl phenol (4-hydroxy biphenyl) ip to rabbits and found that 31% of the dose was excreted in bile in 24 hrs. About 57% of this was associated with 4-glucuronosido biphenyl and 43% was 4-glucuronosido-4-hydroxy biphenyl. They also found traces of unchanged 4-hydroxy biphenyl and an unidentified glucuronide conjugate. They demonstrated a higher biliary excretion of 4-hydroxy biphenyl than we did using 1-hydroxy biphenyl (OPP). However factors apart from molecular weight and polarity can play a remarkable role in biliary excretion. The change in relative positions of certain groups in a molecule may alter the extent of that compound's biliary excretion significantly. Iga et al (1970, 1971) showed that the stuff of an SO_3 group from one position in the molecule to another caused a 4-8 fold change in the extent of biliary excretion of a number of sulphonated dyes in rats.

The findings of high radioactivity in the kidneys two hours after OPP administration confirmed the importance of the kidneys in the excretion of small molecular weight phenolic compounds. The liver only had a fifth of the activity found in the kidneys.

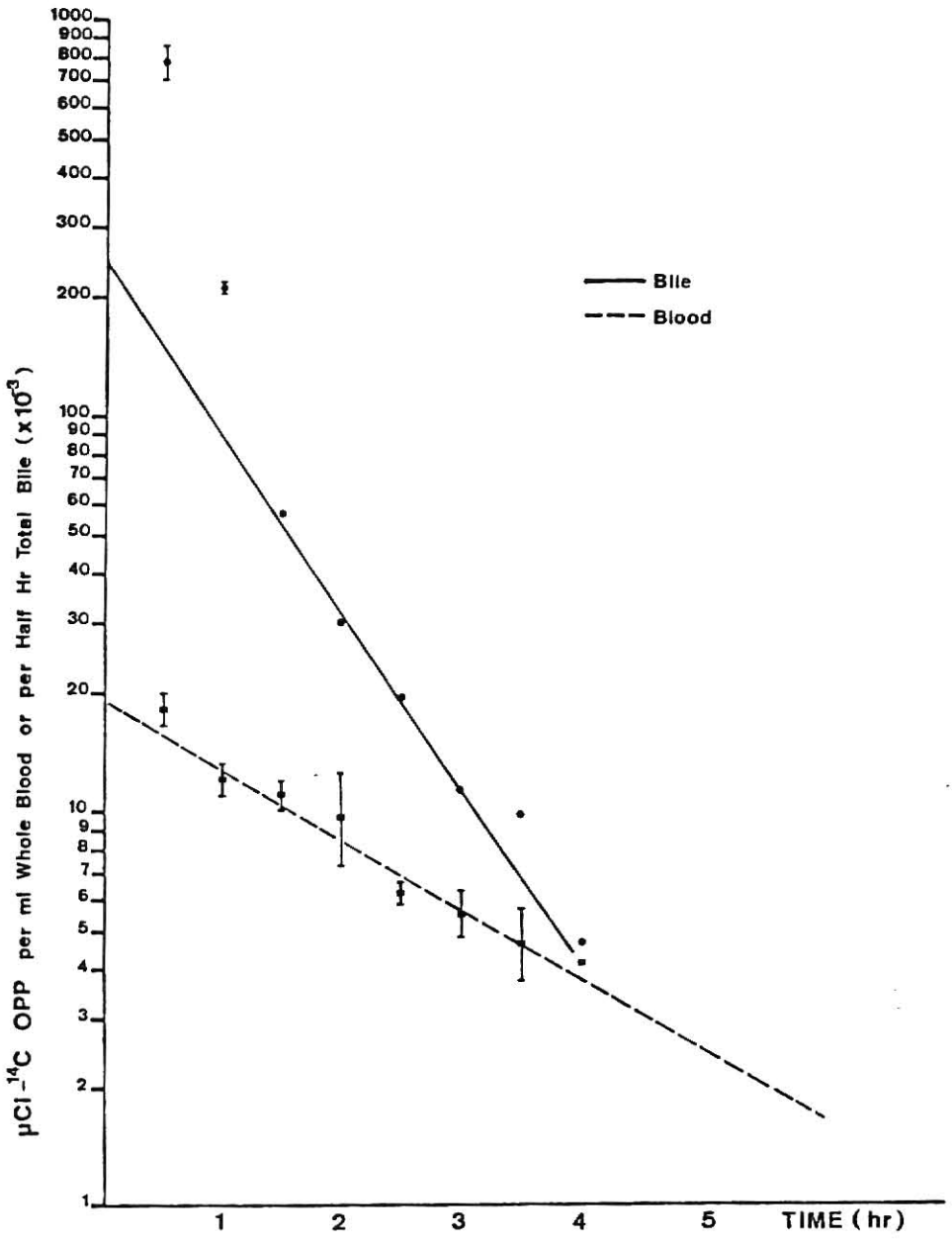
The differences in the pattern of excretion between OPP and P (Gbodi and Oehme, 1977) can be explained due to the introduction of a phenyl ring into P and the change in molecular weight and polarity that resulted. The mean blood levels of OPP were higher than that of P over the entire 5 hr period and the Kd for OPP-treated rats was smaller than

that of the P-treated rats, indicating the slower rate of OPP elimination. However biliary excretion studies showed a difference between the OPP and P-treated rats with greater biliary excretion in anesthetized and in unanesthetized rats receiving OPP.

Four metabolites were found in the urine of OPP-treated rats compared with two found in P-treated rats. Whereas the sulfate conjugate was a major urinary metabolite in P-treated rats, no sulfate conjugate was apparent in OPP-treated rats. Unchanged OPP was the major urinary constituent of OPP-treated rats, but unchanged P was a minor urinary constituent from P-treated rats. The apparent lack of an OPP sulfate conjugate was interesting since Oehme and Smith (1972) found a sulfate metabolite of OPP in cats and dogs. Species differences in biotransformation pathway and quantitative capabilities may partially explain this variability. The difference in number of urinary metabolites of OPP and P is understandable, since OPP presents more sites for oxidation and conjugation reactions. While significant amounts of OPP was found in the bile of OPP-treated rats, no P was excreted in the bile of P-treated rats.

The enterohepatic circulation and tissue distribution studies were essentially similar in the rats receiving OPP and in the previously reported P-treated rats (Gbodi and Oehme, 1977).

Fig. 1. Radioactivity in bile and blood of pentobarbital anesthetized rats after iv administration of 40.8 mg ^{14}C-OPP/kg. Each point represents the mean ± SE of three animals. The slopes (k) and correlation coefficients (r) were -1.399 and -0.99 for the biliary least square regression line and -0.396 and -0.98 for the blood values.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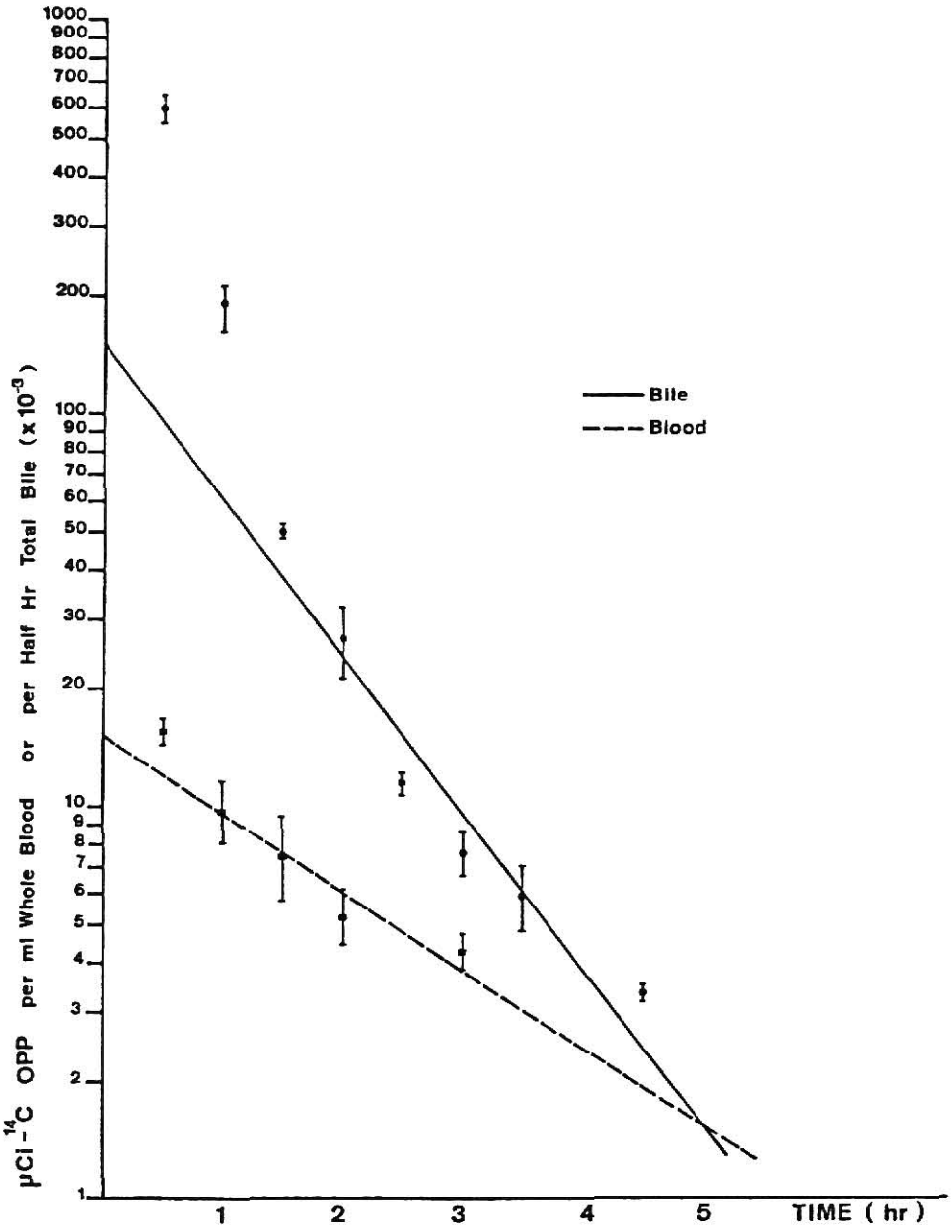
9
8
7
6
5
4
3
2
1
μCi ^{14}C OPP per ml Whole Blood or per Half Hr Total Bile ($x10^{-3}$)
Bile
Blood
1
2
3
4
5
TIME (hr)

Fig. 2. Radioactivity in bile and blood of unanesthetized rats after iv administration of 40.8 mg ^{14}C-OPP/kg. Each point represents the mean ± SE of three animals. The slopes (k) and correlation coefficients (r) were -0.92 and -0.96 for the biliary least square regression line and -0.38 and -0.96 for the blood values.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900
800
700
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200
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
μCi - 14C OPP per ml Whole Blood or per Half Hr Total Bile (x10-3)
Bile
Blood
1
2
3
4
5
TIME (hr)

Fig. 3. Concentrations of OPP radioactivity in various tissues 2 hr after the iv administration of 40.8 mg/kg.

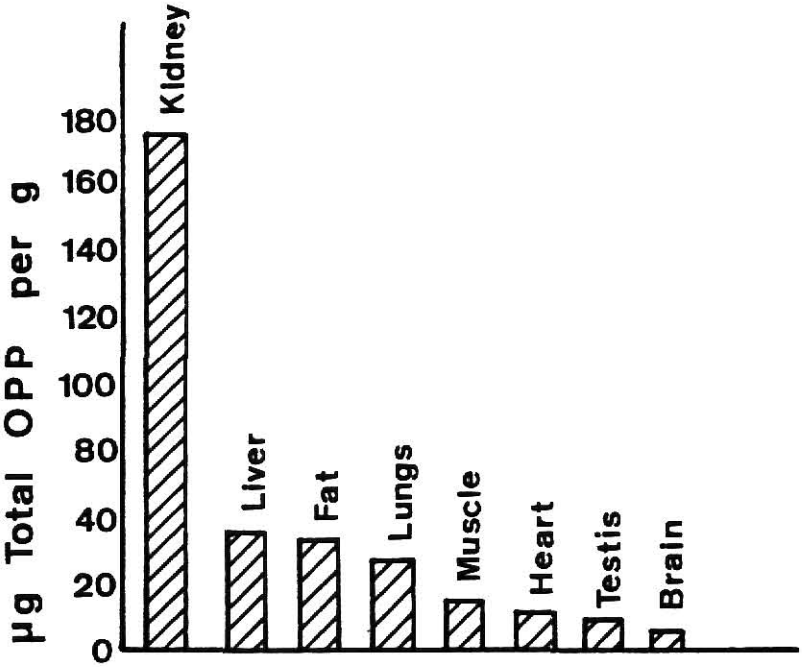
µg Total OPP per g
0
20
40
80
100
120
140
160
180
Kidney
Liver
Fat
Lungs
Muscle
Heart
Testis
Brain

TABLE 1

o-PHENYL PHENOL RADIOACTIVITY IN BILE OF PENTOBARBITAL ANESTHETIZED AND UNANESTHETIZED MALE RATS AFTER INTRAVENOUS ADMINISTRATION OF 40.8 mg OPP/kg

	PENTOBARBITAL ANESTHETIZED		UNANESTHETIZED	
TIME (Hr)	% of Dose ± SE	Range	% of Dose ± SE	Range
1/2	14.09 ± 1.36 [a]	10.79 - 16.69	10.59 ± 0.94 [a]	9.35 - 12.89
1	3.79 ± 0.19	3.40 - 4.22	3.44 ± 0.55	2.12 - 4.36
1½	1.01 ± 0.11	0.75 - 1.19	0.90 ± 0.04	0.80 - 0.95
2	0.54 ± 0.05	0.41 - 0.63	0.47 ± 0.09	0.41 - 0.70
2½	0.34 ± 0.02	0.32 - 0.38	0.20 ± 0.01	0.17 - 0.22
3	0.20 ± 0.01	0.18 - 0.22	0.14 ± 0.02	0.10 - 0.17
3½	0.18 ± 0.02	0.15 - 0.21	0.11 ± 0.02	0.07 - 0.14
4	0.08 ± 0	0.07 - 0.09	0.14 ± 0.05	0.07 - 0.21
4½	0.10 ± 0.02	0.07 - 0.13	0.06 ± 0	0.05 - 0.06
5	0.08 ± 0.03	0.04 - 0.12	0.08 ± 0.02	0.06 - 0.11
Total	20.10 ± 1.23	17.57 - 22.78	15.96 ± 1.23	14.21 - 18.99

[a] Mean values for 3 rats.

TABLE 2

o-PHENYL PHENOL RADIOACTIVITY IN URINE OF MALE RATS AFTER INTRAVENOUS ADMINISTRATION OF 40.8 mg OPP/kg

Time (hr)	% Dose ± SE	Range
6	73.42[a] ± 3.31	66.02 - 85.75
12	11.51 ± 1.09	7.86 - 14.71
24	2.62 ± 0.21	1.76 - 3.05
48	0.97 ± 0.13	0.71 - 1.52
72	0.40 ± 0.06	0.23 - 0.65
Total Recovery	88.94 ± 3.20	81.77 -102.22

[a] Mean values for 5 rats

TABLE 3

o-PHENYL PHENOL METABOLITES IN URINE OF MALE RATS

AFTER INTRAVENOUS ADMINISTRATION OF 40.8 mg OPP/kg

Metabolites	Rf_A	6 HR URINE		12 HR URINE	
		% of Dose ± SE	Range	% of Dose ± SE	Range
A(Glucuronide)	0.47	1.89[a] ± 0.31	1.43 - 2.68	3.41 ± 0.59	2.30 - 5.75
B(Glucuronide)	0.55	4.64 ± 0.41	3.17 - 5.89	5.72 ± 0.51	4.11 - 6.71
C(Glucuronide)	0.64	14.43 ± 0.92	11.44 - 17.54	12.44 ± 1.21	9.59 - 17.26
D(Glucuronide)	0.78	7.19 ± 0.38	6.04 - 8.06	5.39 ± 0.44	4.11 - 6.84
OPP	0.90	71.82 ± 1.70	66.58 - 77.90	73.02 ± 2.15	63.56 - 76.77

[a] Mean values for 5 rats

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

TABLE 4

o-PHENYL PHENOL METABOLITES IN BILE OF PENTOBARBITAL ANESTHETIZED AND UNANESTHETIZED MALE RATS AFTER INTRAVENOUS ADMINISTRATION OF 40.8 mg OPP/kg

Metabolites	Rf_A	1/2 HR BILE		1 HR BILE	
		% of Dose ± SE	Range	% of Dose ± SE	Range
ANESTHETIZED RATS					
A(Glucuronide)	0.37	1.20[a] ± 0.12	1.05 - 1.49	1.83[a] ± 0.15	1.48 - 2.14
B(Glucuronide)	0.49	13.85 ± 1.98	11.27 - 18.71	12.80 ± 2.01	10.03 - 17.72
C(Glucuronide)	0.64	80.19 ± 1.82	75.97 - 83.56	77.14 ± 1.85	72.93 - 80.70
D(Glucuronide)	0.86	3.39 ± 0.69	2.54 - 5.11	6.62 ± 0.07	5.65 - 8.53
OPP	0.92	1.33 ± 0.06	1.23 - 1.49	1.57 ± 0.07	1.48 - 1.75
UNANESTHETIZED RATS					
A(Glucuronide)	0.37	1.75[a] ± 0.43	0.91 - 2.72	2.11[a] ± 0.25	1.72 - 2.72
B(Glucuronide)	0.49	10.81 ± 0.79	9.82 - 12.75	10.11 ± 0.24	9.82 - 10.70
C(Glucuronide)	0.64	79.06 ± 2.23	73.63 - 82.63	77.81 ± 1.79	73.63 - 81.08
D(Glucuronide)	0.86	5.64 ± 1.78	3.08 - 10.00	6.27 ± 1.53	4.09 - 10.00
OPP	0.92	2.71 ± 0.45	1.96 - 3.81	3.67 ± 0.16	3.27 - 3.93

[a] Mean values for 3 rats

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

[a] Mallinckrodt Co., St. Louis, Mo.

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FATE OF 2,6-DIIODO-4-NITROPHENOL IN RATS

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Fate of 2,6-Diiodo-4-nitrophenol in Rats. GBODI, T. A. and OEHME, F. W. (1977). Toxicol. Appl. Pharmacol. 00, 000-000. Disappearance of ^{14}C-2,6-diiodo-4-nitrophenol (DNP) from the blood of unanesthetized or pentobarbital anesthetized rats with venous, arterial and biliary cannula, and its excretion into bile, was evaluated for 5 hr after iv administration of 9.6 mg DNP/kg. Blood kinetic data did not fit a first order process. Bile contained 3.00 ± 0.58% and 3.29 ± 0.13% of the dose, respectively, from the anesthetized or unanesthetized rats; maximum biliary excretion occured during the first 1/2 hr. No enzyme induction effect appeared to occur in the pentobarbital anaesthetized rats. Higher blood concentrations of DNP were found in anesthetized rats. Higher biliary concentrations of DNP and metabolites were found in the anesthetized rats until the third hour, when the biliary concentration of DNP and metabolites coincided with that of unanesthetized rats; thereafter the biliary concentration in unanesthetized rats was higher. The major biliary metabolites were glucuronide conjugates and unchanged DNP. Renal excretion of DNP was protracted and only 46.95 ± 4.43% of the dose was excreted in 4 days. Maximum excretion occurred during the second day (16.13 ± 1.67%). Five metabolites were detected in urine; glucuronide conjugates predominated. No enterohepatic circulation was demonstrated. Two hours after DNP administration, highest levels of radioactivity were found, in muscle and fat.

INTRODUCTION

2,6-Diiodo-4-nitrophenol (DNP), also known as disophenol, is an effective systemic anthelmintic for the treatment of hookworm infestation in dogs and cats. The distribution and biotransformation of disophenol have not been documented (Jones, 1965). Kaiser (1964) compared the toxicity of DNP and 2,4-dinitrophenol and concluded that the two compounds produced similar toxicologic signs in laboratory animals, but following repeated administration DNP appeared the more toxic compound because of its accumulation in plasma.

The molecular weight of DNP is 391, falling well within the threshold limit of 325 ± 50 required for extensive biliary excretion in rats (Millburn et al, 1967). The molecular size hypothesis for biliary excretion was supported by studies that showed that the biliary excretion of simple aromatic compounds increased if they were substituted so as to increase their molecular size. Williams et al (1965) showed that iodination of 4-aminohippuric acid (Mol. Wt. 194) to give the 3-iodo-derivative (Mol. Wt. 320) increased its biliary excretion from 2% to 25%, and the introduction of two iodine atoms into anthranilic acid (Mol. Wt. 137) to form five 3,5-diiodo anthranilic acid (Mol. Wt. 389) enhanced the biliary excretion of this acid from 5% to 35%.

From 82 to 92% of p-nitrophenol was excreted in rabbits urine as glucuronide conjugate in less than one day, and its reduction product and conjugated aminophenols were excreted during two days (Williams 1959). Biliary excretion of nitrophenols have not been documented. It is of interest to determine the effect on its excretory pattern of introducing

two iodine atoms into the p-nitrophenol molecule.

This study was conducted to evaluate the pattern of DNP excretion, the effect of pentobarbital anesthesia on DNP metabolism, DNP enterohepatic circulation, and its tissue distribution.

METHODS

Animals

Charles River Dunkin-Hartley short hair male rats weighing 250-350 g were used. The animals were treated as previously described (Gbodi and Oehme, 1977 a, b).

Chemicals

UL-^{14}C-DNP (specific activity 6.7 μCi/mM) was purchased commercially[a]. Other chemicals were purchased as previously reported (Gbodi and Oehme, 1977 a, b) and used in the same manner for this study. Each animal received an iv administration of a 1 ml mixture of polyethylene glycol and water (92:100, w:v) containing 2.4 mg chemical DNP and 4.7 μCi of radiochemically pure DNP. A dose of approximately 9.6 mg chemical DNP by body weight resulted.

Measurements of Parameters

The determination of blood levels, urinary and biliary excretion, enzyme induction, enterohepatic circulation, and tissue residues were performed as previously described (Gbodi and Oehme, 1977 a, b). Biliary and urinary metabolites were identified using the enzymatic hydrolysis reported earlier (Gbodi and Oehme, 1977 a, b).

RESULTS

Blood and Bile Parameters

Fig. 1 depicts the apparent mean concentration of DNP in blood and bile of unanesthetized and anesthetized rats at various time intervals following iv administration of 9.6 mg DNP/kg. A fall in blood concentration was noticed in both groups for the first few hours, followed by an increase and then decrease in blood concentration. Higher blood concentrations were found in the blood of unanesthetized rats than in anesthetized rats.

Maximum biliary excretion was found in both groups of rats during the first $\frac{1}{2}$ hr; DNP and its metabolites were detected in bile in the first five minutes. Following the rapid drop in biliary DNP concentration, marked differences between the two groups was noticed. Greater biliary excretion was noticed in the anesthetized rats for the first 3 hr when the biliary DNP excretion of both groups coincided. For the last 2 hr, the excretion rate of DNP in the bile of unanesthetized rats exceeded that found in anesthetized rats. Both groups eliminated almost the same total amount of DNP, in bile during the 5 hr period (3.00 ± 0.58 for anesthetized rats and 3.29 ± 0.13 for unanesthetized rats)(Table 1).

Urinary Studies

Table 2 shows the urinary excretion of DNP over a period of 96 hrs. The lowest excretion was seen in the 6 hr samples (3.89 ± 0.78% dose) from all rats. Then a gradual increase occurred until maximum excretion was reached at 48 hr (16.12 ± 1.46% dose).

Table 5 shows the results of radiochromatogram scans of 12 hr urine

samples. Of the five peaks seen, the one at Rf 0.92 was identified as unchanged DNP. Metabolites B and C were identified as glucuronide conjugates, but specific identification was not completed.

Metabolites in Bile

Tables 4 and 5 show the results of radiochromatogram scans of 1/2 hr and 1 hr bile samples from the anesthetized and unanesthetized rats. Five peaks were present in both samples. No major quantitative differences in metabolites were found between the two groups of rats. The metabolite with Rf 0.90 was identified as unchanged DNP. Metabolites B and C were identified as different glucuronide conjugates. When the bile was hydrolysed using β-glucuronidase, there was a marked increase in the D and DNP peaks with concurrent disappearance of the B and C peaks. Thus metabolites B and C are glucuronide conjugates of DNP and metabolite D. About 26% of the bile activity was unchanged DNP.

Enterohepatic Circulation

The mean activity of bile injected into the duodenum of recepient rats was 0.32 μCi. Only traces of activity was found at two hours in liver, and none was present in kidneys, blood and bile.

Tissue Residues

Fig. 2 depicts the tissue concentrations of DNP two hours after iv administration. Highest levels were found in the muscle and fat, with blood, lungs, liver, kidneys, heart, spleen, thymus, testis and brain having decreasing amounts.

DISCUSSION AND CONCLUSIONS

The blood concentration of DNP over the 5 hr experimental period was almost constant in both anesthetized and unanesthetized rats (Fig.1). However, higher blood concentrations of DNP were seen in unanesthetized rats than in anesthetized rats for the entire experimental period. There was no significant difference in biliary excretion of DNP in both groups of rats. The marked difference was in the pattern of excretion of DNP with time; and initially higher biliary excretion in anesthetized rats was noticed, which was later compensated for after the third hour in unanesthetized rats. Almost the same amount of DNP was excreted in both groups.

Pentobarbital treatment did not have as marked an enzyme induction effect as expected, but the change in pattern of biliary excretion and the lower blood concentration of DNP in the anesthetized rats might have resulted from the pentobarbital. The enzyme systems responsible for DNP metabolism may not be inducable by pentobarbital, since the enzyme induction effects of certain chemicals are specific to only certain groups of enzymes (Gelboin, 1972). The biliary data (Fig. 1, Table 1) indicates that biliary excretion of DNP was slow.

Peak renal excretion of DNP occurred between 24-48 hr after administration of the DNP (Table 4). Thereafter, excretion continued to decrease. Thus even renal excretion of DNP was slow.

The nature of the urinary metabolites A and D are unknown. These metabolites were apparently similar to those found in bile based upon their Rf values in solvent system A, but more of the urinary glucuronide conjugates B and C were produced with resulting less unchanged DNP

(6.81 ± 1.49% in urine and 26.17 ± 0.90% in bile).

The metabolism of DNP have not been documented (Kaiser, 1967) but from the known biotransformation patterns of compounds with similar structures and aromatic side groups, it is possible to speculate on the possible biotransformation products. It is known that in rabbits, man and dogs, the nitro groups undergo reduction to amino groups and then conjugated with glucuronic acid (Robinson et al, 1951; Guerbet et al, 1932; Georgescu, 1932). Also well documented are deiodination and o-methylation of compounds having phenyl iodine atoms. Flock et al (1962) showed that one of the biotransformation products of thyroxine (3,5,3',5'-tetraiodothyronine) was the o-glucuronide conjugate of 3,3',5'-triiodothyronine. Tomita et al (1964) demonstrated in vitro enzymatic o-methylation of iodinated phenols and thyroid hormones using homogenized liver supernatants. Since, DNP has a phenyl nitro group, two phenyl iodine atoms, and a hydroxyl group, the reduced nitro, deiodinated and o-methylated conjugated and unconjugated biotransformation products are good probable metabolites of DNP.

The enterohepatic circulation of DNP was minimal and possibly insignificant. The DNP was excreted in the bile in a small and protracted pattern, and it may be possible that the extent of enterohepatic circulation was not fully evaluated in 5 hr. On the other hand, the administered conjugates might have been stable to microbial action and eliminated in feces (Smith et al, 1966). Unpublished work in our laboratory indicates that some DNP is eliminated in the feces of dogs. Long term experimental studies must be conducted to fully evaluate the enterohepatic circulation of DNP.

Tissue residue studies (Fig. 2) indicated that DNP accumulated in muscle, fat and blood. Additional ongoing work in our laboratory indicates that DNP is 85% bound to plasma protein. Such preferential binding of DNP to other tissue could play a remarkable role in its metabolism and protracted elimination.

The effects on pattern of excretion in the same animal species of alterating a compound's structure and molecular weight can clearly be seen when one compares the metabolism and excretion of phenol (P) (Gbodi and Oehme, 1977a) and o-phenyl phenol (OPP) (Gbodi and Oehme, 1977b) with DNP. The blood levels of P were the lowest of the three compounds. Blood concentrations of DNP were the highest despite the fact that lesser amounts of DNP were administered. While the disappearances of P and OPP from blood was clearly a first order process, that of DNP could not be fitted to first order kinetics.

A transient drop in DNP blood concentration was followed by a gradual increase. It is known that DNP accumulates in the plasma in dogs (Kaiser, 1964); this agrees with our findings of high DNP blood levels in rats. Whereas the Kd for OPP and P in rats were 0.59 hr and 0.40 hr, that of DNP could not be estimated because an almost constant blood concentration was maintained.

Marked differences were seen in the renal and biliary excretion of DNP and that of P and OPP. The biliary excretion of P and OPP were almost completed by the third hour after iv administration, but that of DNP was sustained at a lower constant rate over the entire 5 hr period. Eleven and 20% of the P and OPP, respectively, were excreted in the bile of rats during five hours, but only 3% of the DNP was excreted in the

same period. A large amount of unchanged DNP (26%) was found in the bile of rats compared to the small amount of OPP found (3.5%) and the total absence of P observed.

Pentobarbital anesthesia did not have any effect on the metabolism of DNP. Renal excretion of both P and OPP were almost completed in 24 hrs (90% of the P dose and 87% of the OPP dose). Only 15% of DNP was eliminated in the urine in that same period, and only 46% of the dose was recovered in four days.

Intense enterohepatic circulation was involved in the biotransformation of P and OPP, but none was seen with DNP. This can be explained by considering possible differences in conjugate stability and the reabsorption potential of the respective aglycone in the intestine (Smith and Williams, 1966).

Tissue distribution studies 2 hr after iv administration of DNP showed that significant amounts were associated with the muscle, fat and blood. Similar studies with OPP and P showed significant amounts associated with kidneys and liver. This was associated with their rapid excretion in urine and bile. It appears that DNP may be sequested in muscle and fat tissues, and thus retains significant and prolonged blood levels.

Fig. 1. Blood and biliary concentration of *DNP* at various time intervals following iv administration. Each point represents the mean and SE of three animals.

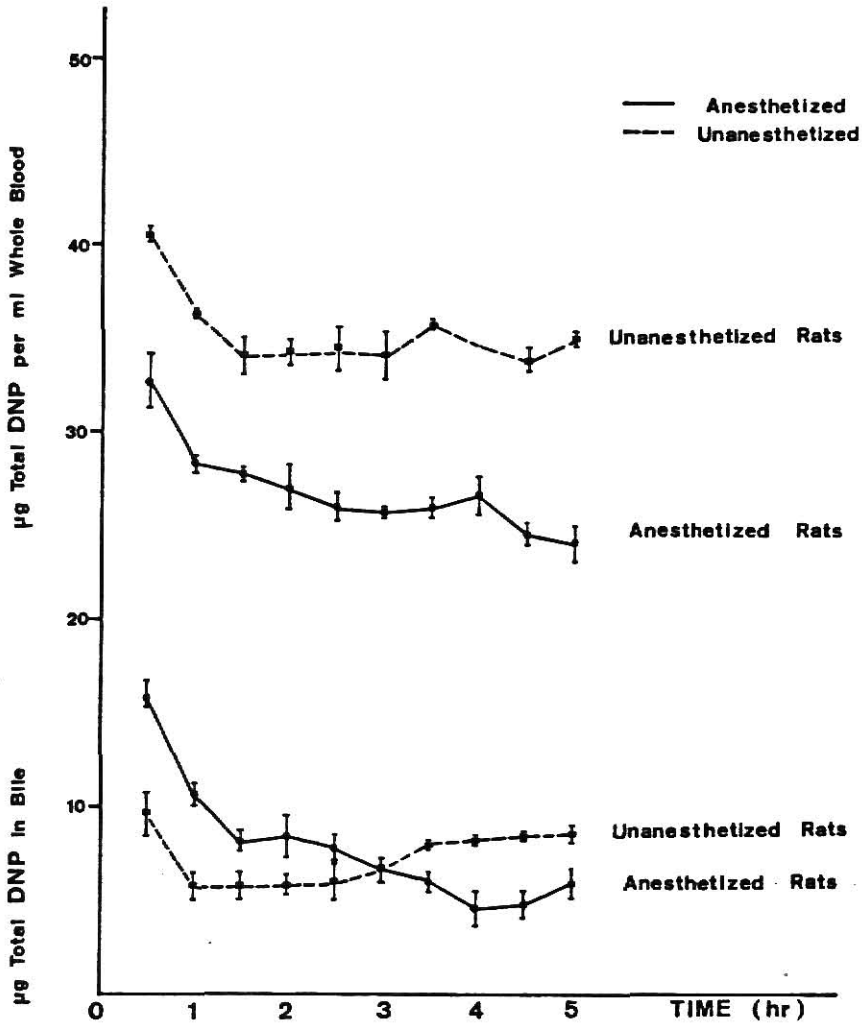
Anesthetized
Unanesthetized
µg Total DNP per ml Whole Blood
µg Total DNP in Bile
Unanesthetized Rats
Anesthetized Rats
Unanesthetized Rats
Anesthetized Rats
50
40
30
20
10
0
1
2
3
4
5
TIME (hr)

Fig. 2. Concentration of DNP in various tissues of rats 2 hr after receiving 9.6 mg DNP/kg iv administration.

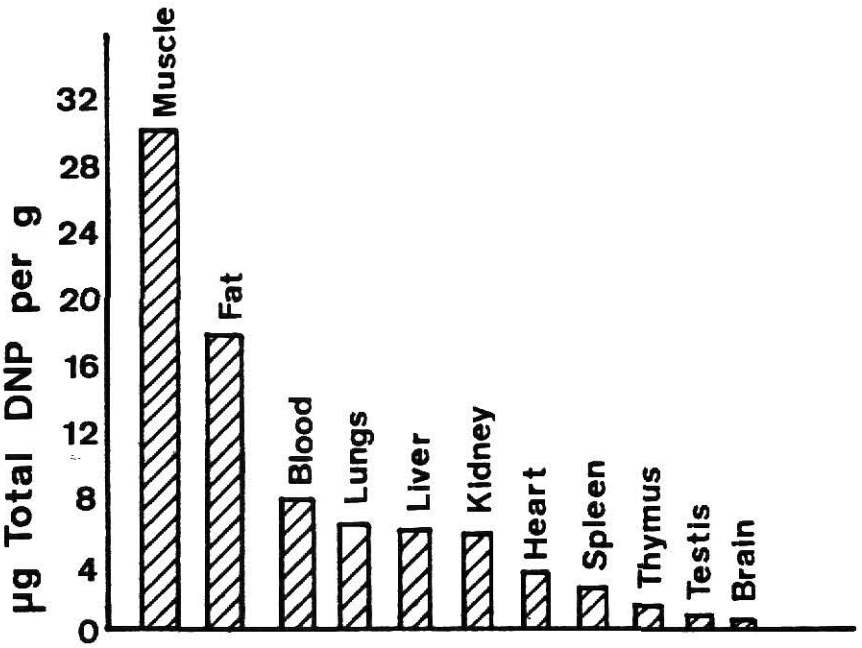
µg Total DNP per g
0
4
8
12
16
20
24
28
32
Muscle
Fat
Blood
Lungs
Liver
Kidney
Heart
Spleen
Thymus
Testis
Brain

TABLE 1

BILIARY EXCRETION OF DNP AT VARIOUS TIME INTERVALS AFTER IV ADMINISTRATION OF 2.4mg DNP TO ANAESTHETIZED AND UNANAESTHETIZED RATS

	PENTOBARBITAL ANESTHETIZED RATS		UNANESTHETIZED RATS	
TIME (HR)	µg Excreted	Range	µg Excreted	Range
1/2	16.15[a] ± 0.76	14.71 - 17.99	9.60 ± 1.02	7.15 - 11.45
1	10.63 ± 0.51	9.76 - 11.90	5.72 ± 0.76	5.57 - 8.99
1½	8.07 ± 0.41	7.36 - 9.14	5.72 ± 0.76	4.60 - 7.61
2	8.38 ± 1.33	6.49 - 11.70	5.87 ± 0.51	4.70 - 7.00
2½	7.71 ± 0.71	5.98 - 8.79	5.81 ± 1.02	3.37 - 7.25
3	6.69 ± 0.25	6.28 - 7.36	6.69 ± 0.51	5.41 - 7.61
3½	5.82 ± 0.51	4.90 - 7.05	8.07 ± 0.20	7.77 - 8.38
4	4.60 ± 0.97	2.81 - 7.00	8.23 ± 0.35	7.71 - 8.79
4½	4.85 ± 0.82	3.47 - 6.85	8.48 ± 0.15	8.28 - 8.74
5	6.08 ± 0.92	4.81 - 8.38	8.48 ± 0.56	7.66 - 9.30
Total % Dose	3.00 ± 0.58	3.02 - 3.83	3.29 ± 0.13	3.10 - 3.47

[a] Each value represents mean of three animals ± SE.

TABLE 2

PERCENT DOSE ^{14}C-DNP EXCRETED IN URINE AT VARIOUS TIME INTERVALS

Time (Hr.)	Radioactivity in Urine (% Dose)	Range
6	3.89[a] ± 0.78	2.09 - 6.11
12	5.92 ± 1.26	3.94 - 10.24
24	6.88 ± 1.67	4.49 - 12.65
48	16.13 ± 1.46	11.94 - 15.91
72	9.23 ± 0.89	7.09 - 11.63
96	4.89 ± 0.20	4.23 - 5.32
Total Recovery	46.95 ± 4.43	37.87 - 59.32

[a] Mean values for four animals ± SE.

TABLE 3

PERCENT ^{14}C-DNP AND METABOLITES EXCRETED IN 12 HOUR URINE SAMPLES

METABOLITES	Rf_A	% ^{14}C-DNP EXCRETED AS		RANGE
A	0.41	9.08	0.84[a]	8.54 - 11.09
B(Glucuronide)	0.52	44.32	1.02	41.48 - 46.29
C(Glucuronide)	0.62	21.85	1.95	17.40 - 25.57
D	0.77	19.44	1.27	16.79 - 22.18
DNP	0.92	6.81	1.49	3.22 - 9.21

[a] Mean of 3 rats ± SE

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

TABLE 4

PERCENT ^{14}C-DNP EXCRETED AS VARIOUS METABOLITES OF DNP AND DNP IN 1/2 HOUR AND 1 HOUR BILE SAMPLES OF ANESTHETIZED RATS

METABOLITES	Rf_A	1/2 hr Sample		1 hr Sample	
		% Excreted as	Range	% Excreted as	Range
A	0.43	10.94 ± 0.87[a]	9.49 - 13.81	7.42 ± 0.81	6.28 - 8.57
B(Glucuronide)	0.52	33.42 ± 1.54	30.16 - 37.85	38.47 ± 1.75	36.00 - 40.95
C(Glucuronide)	0.62	14.73 ± 0.66	13.04 - 16.73	14.45 ± 3.11	10.05 - 18.85
D	0.72	18.23 ± 1.37	15.21 - 18.99	14.39 ± 0.48	13.71 - 15.07
DNP	0.90	22.63 ± 1.47	18.42 - 26.08	25.24 ± 1.69	22.85 - 27.63

[a] Mean values for 3 rats ± SE

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

TABLE 5

PERCENT ^{14}C-DNP EXCRETED AS VARIOUS METABOLITES OF DNP AND DNP IN 1/2 HOUR AND 1 HOUR BILE SAMPLES OF UNANESTHETIZED RATS

		1/2 hr Sample		1 hr Sample	
METABOLITES	Rf_A	% Excreted as	Range	% Excreted as	Range
A	0.43	9.09 ± 0.78[a]	7.40 - 10.71	4.10 ± 0.40[b]	3.57 - 5.09
B(Glucuronide)	0.52	32.97 ± 2.32	27.52 - 37.14	43.35 ± 3.66	34.37 - 48.12
C(Glucuronide)	0.62	15.16 ± 2.25	10.00 - 19.44	14.24 ± 1.73	12.05 - 18.50
D	0.72	16.26 ± 1.78	13.88 - 20.64	11.94 ± 1.88	9.14 - 16.51
DNP	0.90	26.17 ± 0.90	24.07 - 27.85	26.32 ± 3.66	33.48 - 27.44

[a] Mean values for 3 rats ± SE

[b] Mean values for 4 rats ± SE

Rf_A = Rf value of metabolites in 1-propanol: ammonia hydroxide (7:3)

[a] Mallinckrodt Co., St. Louis, Missouri.

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A P P E N D I C E S

A P P E N D I X I

Introduction to Phenols

I. INTRODUCTION TO PHENOLS

Phenols, are hydroxy derivatives of aromatic hydrocarbons and have found great use in many industrial processes. They are the starting materials for many products such as plastics, dye stuffs, antiseptics and therapeutic formulations. A large number of phenols have been identified as constituents of active principles in many natural products. The organs and urine of animals normally contains some phenols and their conjugates which are derived from the diet and the bacterial decomposition of food residues in the gastrointestinal tract (Baumann et al, 1876; Nuttal et al, 1896; Deichmann et al, 1942; Curzon et al, 1964).

The fate of both endogenous and exogenous phenols have been known in part as early as 1876, when Baumann found that indigo-forming substances and phenols in urine contained bound sulfate and concluded that conjugated sulfuric acids were compounds of urinary phenols, with sulfuric acid. He was also able to isolate potassium phenyl sulfate (PS) from human urine and in 1879, he and Preusse described the oxidation of phenol to catechol and quinol in dogs dosed orally with phenol (P).

In 1890 Kulz observed the presence of phenyl glucuronide (PG) in the urine of rabbits injected with P. Thus by the end of 1900, both the two major metabolites of P, PG and PS, and oxidation of the aromatic ring were known. More recently Parke (1968) showed that phenol was mainly metabolised by conjugation with glucuronic acid and sulfate moiety, by further hydroxylation of the aromatic ring, and by oxidation of substituent alkyl side chains to give phenolic acids, or in the case of nitrophenols the nitro group on the phenol ring could be reduced to form the amine group before conjugation (Parke, 1968). Some factors are

well known to modulate the excretory patterns of xenobiotics in animals. They are broadly divided into two, the physico-chemical characteristics of the xenobiotics and the biological constitution of the animals concerned. The physico-chemical characteristics of the xenobiotics include the molecular weight, polarity, and structural features. The biological constitution of the animals include species, sex and genetic factors. (Smith, 1973). Basically a number of generalizations have been made with regards to the molecular weight effects on excretory pattern of xenobiotics. Brauer (1959), Sperber (1963), Williams et al (1965) and Millburn et al (1967) all proposed that substances of low molecular weights (i.e. less than 300) after metabolism were poorly excreted ($< 10\%$) into the bile, thus renal excretion was predominant with them. This hypothesis have been substantiated by the above mentioned workers and others. More recently the work of Hirom et al (1976) have shown the complementary nature of biliary and renal excretion pathways for xenobiotics. Using 30 aromatic compounds with molecular weight of 100-850 and largely excreted unchanged. They concluded from their results that these compounds fell into three groups as regards their pattern of elimination which were related by molecular weight: group 1, with molecular weight less than 350, major route of elimination being the urine and even when urinary excretion was prevented by ligating the renal pedicles, the biliary excretion remained low; group 2, with molecular weight of 450-850 which were excreted predominantly in bile, and even when the bile duct was obstructed, only small amounts of these compounds were found in urine; and group 3, with molecular weight of 350-450, which were eliminated extensively in both urine and bile and when any of these routes was blocked excretion by the other increased. This strongly emphasized the interrelationship of urine and bile as

excretory routes for organic compounds. Most phenolic compounds fall into the first and last groups, mentioned above.

The presence of strong polar group in a molecule appears to be a requirement for extensive biliary excretion (Smith, 1973). It is known that the suppression of the conversion of a compound to its polar metabolic conjugate may be accompanied by reduced biliary excretion, i.e. methylation of the carboxyl groups of bilirubin markedly reduced its biliary excretion in rats (Jivsa et al, 1968). Another indirect evidence about need of strong polar group for extensive biliary excretion is given by many polar substances which are known to be excreted in the bile unchanged provided they satisfy the appropriate requirements of molecular weight, e.g. succinyl sulphathiazole, molecular weight = 355 with polar group - COOH, and 54% of its dose excreted in rat (Smith, 1973).

It is known that structural factor also play a major role in biliary excretion of xenobiotics. The introduction of a group or the change in relative position of certain groups in a molecule may alter the extent of biliary excretion out of all proportion to any effect that the change may have on molecular size or polarity (Smith, 1973). Iga et al (1970, 1971) found that some sulfonated dyes in which a single shift in SO_3^- group from one position to another in the molecule caused a 4 to 8 fold change in the extent of biliary excretion in rats. Hirom et al (1972) studied the role of structural factor in the biliary excretion of some sulfonamides, in the rat, and they concluded that the structural factor might influence biliary excretion in one or more of three ways, namely, by changing lipid solubility, the shape of the molecule or the relative intramolecular relationship of the polar and non-polar parts of the molecule.

It has also been well documented that sex differences play a major role in both metabolism and elimination pattern of drugs by rat in particular. Adult male rats are said to metabolize many foreign compounds at higher rates than their female counterparts due to differential degree of microsomal hepatic enzymes induction by the same compound (Kato et al, 1965). This difference is said to be as a result of sex hormones since the difference could be abolished by castration and the administration of androgens to female rats increased the activities of the microsomal enzymes to the level of the males. (Parke, 1968). Miller et al, (1974) demonstrated that the extent of biliary elimination of 2,6-dimethoxyphenol in rat, showed some sex difference.

It is also well known that there are great differences in the metabolic fate of drugs in different animal species. These differences have been associated with deficiencies or defective in some enzyme systems, improper formation of active intermediate, absence of conjugates, peculiar to certain species and unavailable conjugating agents (Williams, 1974) in some species and that may be the reason why some xenobiotic show differential toxicity in some species. These species differences is of great importance since screening of drugs for safety of potentially toxic substances are carried out on laboratory animals such as mice, rats, rabbits, guinea pigs, dogs etc. and the data obtained with these animals are extrapolated to man. The idea is to get an animal in which the absorption, distribution, excretion and the rate and pattern of metabolism of a compound are similar to those in man. It is apparent that such animal does not exist and therefore what one can seek is species that comes close to man in these respects. Any defect in metabolism of xenobiotics in any

species can put that species at great disadvantage in some cases. For example it has been known for a long time that the cat has a defective glucuronide-synthesizing mechanism. In most mammals conjugation with glucuronic acid is one of the most effective and versatile of the detoxication mechanisms and this defect in the cat seems to put it at a disadvantage. Low glucuronide synthesis in the cat was first shown by Hartiala (1955) through studies in vitro with organ slices and confirmed in vivo by Robinson and Williams (1958) with several xenobiotics. The defect was shown to be due to the lack of the transferring enzyme, UDP-glucuronyl transferase, with o-aminophenol as substrate, but not to lack of the active intermediate, UDP-glucuronic acid, which occurred in cats liver at expected concentrations, or to the occurrence of a natural inhibitor (Williams, 1974; Dutton and Greig, 1957). The work of Capel et al (1972) and Capel (1973) showed that very little glucuronide of most phenols were formed in cat. Of recent Miller et al (1976) have shown that the nature of the urinary metabolic products of phenols in cats is dependent upon the dose administered. Since the sulphate enzyme conjugating enzyme systems are saturable, at high doses phenols are oxidised into the corresponding quinols which is an obligatory step leading to poisoning in the cat. The pig appears to have the opposite defect to that of cats in that it conjugates most phenols with glucuronic acid and very little with sulfate (Capel et al, 1972; Capel, 1973; Oehme, 1970). Spencer (1960) and McEvoy and Carrol (1971) suggested that the defect could be as a result of defect in various sulphotransferases and their acceptors.

The inability of the dog to acetylate aromatic amines and hydrazines,

like many other species including man is well known (Williams, 1967). The dog is said to lack some N-acetyltransferases, and Leibman and Anaclerio (1962) obtained evidence that dog liver contains an inhibitor of arylamine trans-acetilase also.

In addition to species differences in metabolism, it is now well established that the activities of the drug-metabolizing enzymes differ among certain strains of the same species. Mitoma et al (1967) observed differences in hexobarbital sleeping time and microsomal hexobarbital oxidase activity in various strains of rats.

Also well documented is the induction of several microsomal enzymes by certain xenobiotics. Kuriyama et al (1969) examined the effect of phenobarbital on the biosynthesis and degradation of total microsomal proteins and NADPH cytochrome "C" reductase and cytochrome b_5 using ^{14}C-leucine and ^{14}C-guanidine labelled arginine. They showed that a single administration of phenobarbital induced a prompt increase in the rate of total microsomal protein and NADPH - cytochrome "C" reductase synthesis but did not affect the rate of cytochrome b_5 synthesis. Thus phenobarbital treatment of rats can increase biliary excretion of some compounds as shown in the case of sulfobromophthalein (BSP) and bilirubin as shown by Roberts and Plaa (1967) and Klaasen and Plaa (1968). Furthermore, Jay (1955) has shown that there are strain differences in the inducibility of the hepatic drug metabolizing enzymes.

An important effect of biliary excretion of conjugated compounds is the establishment of an enterohepatic circulation which could have a marked effect on the persistence of xenobiotics in the body. Keberle et al (1962) demonstrated the enterohepatic circulation of gluthethimide in

rats and Smith and Williams (1966) established the enterohepatic circulation of some glucuronide conjugates. The efficiency of this enterohepatic circulation is dependent upon the stability of the glucuronide in the intestinal tract. Conjugates stable in the intestinal environment undergo limited hydrolysis and reabsorption, and are excreted unchanged in the feces. Glucuronides compounds are known to be effectively reabsorbed and recirculated (Smith and Williams, 1966). Garton and Williams (1949) indicated that PG possesses suitable properties for enterohepatic circulation.

The effect of protein binding properties of certain xenobiotics on their excretion pattern is still not very clear. It is generally felt that protein binding could have a negative effect on xenobiotics primarily excreted in urine because its excretion could be delayed by prevention of glomerular filtration, tubular secretion, and modifying of plasma-red cell, plasma-extracellular, and extracellular-intracellular transport, and intracellular distribution (Davidson, 1972). In the case of biliary excretion, it appears as if there is no direct relationship between plasma binding and biliary excretion of four sulphonamide derivatives in the rat and concluded that there was no direct relationship between biliary excretion and protein binding.

It is in the light of some of the above mentioned factors affecting pattern of excretion of xenobiotics that a study of three commonly used phenolic compounds, P, o-phenyl phenol and disophenol was undertaken.

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A P P E N D I X II

Phenol

Litrature Review

Individual Animal Data

II. PHENOL

LITRATURE REVIEW

Chemistry.

The generic designation of phenol (P) is hydroxybenezene. It has a molecular weight of 94.11, melting point of 42.5 - 43°C. It has a sweetish pungent taste. It is soluble to the entent of 8.2 parts/100 parts of water at 15°C. Its pKa at 25°C is 10. It is very soluble in alcohol, chloroform, ether and glycerol, also in aquous alkali hydroxides. LD_{50} orally in rats is 530 mg/Kg.

Uses.

Phenol is used as general disinfectant (either in solution or mixed with slaked lime etc.) for toilets, stables floors, drains and in laundry work. It is used for the manufacture of artificial rasins, for many medical and industrial organic compounds and dyes as a reagent in chemical analysis. It is also used as topical anaesthetic for pruritic lesion (1% lotion or ointment); and used for its cautic effect in animal bites, as a cauterizing agent, and employed in ear-mange in dogs.

Contraindications and Toxicity.

Its use is contraindicated in meat animals and in animals with heart weakness. It could cause residue and environmental health problems (Bevenue and Beckman, 1967). It is strongly recommended not to be used in cats because of its toxic effects on this species (Clarke and Clarke, 1967; Jones, 1965). Even, a small ingestion of small amount by human being may cause nausea, vomiting, circulatory collapse, tachypnea, paralysis, convulsions, coma, greenish or smoky colored urine, necrosis of

the mouth and G-I tract, icterious, death from respiratory failure and sometimes from cardiac arrest. The average fatal dose is 15g but death from 1.5g have been reported. Fatal poisoning may also occur by skin absorption and renal and hepatic damage may occur from industrial exposure (Merck Index

Metabolism.

A lot have been documented on the metabolic fate of P in several animals. Some of the earliest works include that of Baumann (1876) and Baumann and Herter (1877) who found that P is not excreted in free form in dogs but in conjugation with sulfuric acid as ethereal sulfate. Kulz (1883) isolated from the urine of P-fed rabbits an organic P compound which he (1890) identified as phenol glucuronate. Mayer and Neuberg (1900) found that phenol glucuronate is a normal constituent of human urine and it was synthesized by Neuberg (1905) and found identical with the natural product.

In 1943 Deichmann revived the interest in phenol metabolism when he orally administered P to rabbits and found that 59-88% of the dose was excreted in the urine during the first 24 hr. Of the recovered P, 20% was present as the free P, 25% as phenyl sulfate (PS) and about 12.5% as phenyl glucuronide (PG) and the rest unidentified. Ten to 40% of the P unaccounted for was said to be oxidised to CO_2. He also found traces of free and conjugated P in the feces and breath and after 24 hr, 2 to 5% of the dose was still present in the body.

In studies on rats and rabbits, Deichmann (1944) found that following oral administration of P as survival time increased larger proportions

of the P were conjugated, that the urinary excretion of P increased with time, and that rats were found to be capable of more rapid excretion than rabbits.

Parke and Williams (1953) administered ^{14}C-labelled P, orally to two rabbits, and recovered 89.5% of the chemical dose and 96.5% of the injected radioactivity in a 48 hr urine sample. They found 43.5% and 46% of the urinary excretion to be PG and PS respectively and 10% was quinol and less than 1% was catechol. No free P was found.

More recently, Abou-el-Makarem et al (1967) showed that the biliary excretion of P is limited to about 5% of the administered dose in 24 hr period. Their result showed that 54% of P eliminated in the bile was in the form of PG and other three unidentified metabolites. The work of Oehme (1969) elucidated more on species variation in metabolism of phenol in dogs, cats, pigs and goats. Capel et al (1972), using female Wistar rats, showed that 91-100% of phenol dose was excreted by the rats in 24 hr in urine. Of this, 54% was PS, 1% was quinol sulfate (QS), 42% was PG, and 2% was quinol glucuronide (QG).

Interspecies Variation in the Metabolism of Phenol.

The classical work of Oehme (1969) and Capel et al (1972) showed vividly the nature of interspecies variation in phenol metabolism. Oehme (1969) reported that dogs excreted P in equal proportions of free P, PS and PG, cats excreted P as PS, pigs excreted it mainly as P and PG, while goats excreted P largely as PS and a smaller portion as free P. He further concluded that all the animals eliminated the metabolites in

the urine at rates that increased with dosage. Where as PG excretion rates progressively increased with dose, that of PS tended to reach a maximum above 50 mg/kg. Cats had the least excretion of PG, and he concluded that cats were more prone to toxicity by P and that cats had aquantitative deficiency in the ability to form PG.

Capel et al (1972) found that in man, 90% of an oral dose of 0.01 mg/kg was excreted in 24 hr, mainly as PS (71% of 24 hr excretion) and PG (16%) with very little amounts of QS and QG. Four metabolites, the sulfates and glucuronides of phenol and quinol in various proportions, were found in the urine of the rodents, the rat, mouse, jerboa, gerbil, hamster, lemming and guinea-pig after an oral dose of 25 mgP/kg The rat seemed to be the fastest excretor of P among the rodents and all animals they considered. Three metabolites were excreted by some species (squirrel, monkey and capuchin monkey) namely, P, QG and PS. Ferret, dog, hedgehog and rabbit excreted P, QS and PG. Two metabolites were excreted by the rhesus monkey, fruit bat and hen (PS and PG). One metabolite (PG) only was excreted by the pig. The cat again appeared to form no PG and the pig no PS, but detailed examination showed that the cat did excrete small amounts of the glucuronide and the pig excreted small amounts of the sulfate. Out of the seventeen animals considered including man, the rat appeared to be the fastest metabolizer of P. The rat excreted 95% of a 25 mg/kg dose of P in 24 hr and four metabolites, PS (54%), PG (42%), QS (1%) and QG (2%), were the urinary metabolites.

Tissue Distribution

Deichmann (1944) found that the tissues of rabbits and rats contained insignificant traces of free or conjugated P and following oral administration of P, the compound penetrated tissues in a matter of minutes and conjugation began immediately.

With regards to the tissue distribution of P, Barac (1937) and Deichmann (1944) and others emphasized that P is rapidly absorbed and distributed through out the different organs when given orally. Little has been documented on distribution of P and its metabolites in the rat after iv administration. The influence of route of administration on P distribution is illustrated by the findings of Bischoff (1883) and Deichmann (1944a) who found that shortly after oral administration of P to man and rabbits very large amounts of P in the liver, where as after its intravenous injection into dogs the liver contained much less in reference to other organs and relatively little after subcutaneous injection to rabbits (Smith, 1933). Intravenous injection of P in dogs resulted in a uniform distribution of phenol in body fluids and tissues (Pelkan and Whipple, 1922). They found that the concentration of free P disappeared rapidly from the blood while the proportion of conjugated phenols were reached in one hour and thereafter slowly decline as excretion occured.

The work of Gehme (1969) showed the species differences in distribution and metabolism of P. He found that the plasma disappearance of injected P was most rapid in goats, slower in pigs and dogs and slowest in cats. The time required for most of the injected P to be in the conjugated form in the plasma was proportional to the rate of plasma

disappearance and inversely related to the dose given. The apparent specific volume of distribution for free P in dogs, pigs and goats indicated tissue binding of phenol, but none in the cat.

Biliary Excretion of Phenol

Abou-el-Makarem et al (1967) showed that only 4.6% of 50 mg/kg dose of P was excreted in the bile of rats and 54% of the metabolites in bile was PG and three other unidentified metabolites. Large numbers of chemicals excreted in bile to great extent are excreted as their glucuronide conjugates. An important effect of biliary excretion of glucuronides is the establishment of an enterohepatic circulation (Smith and Williams, 1966). The efficiency of this enterohepatic cicrulation is said to be dependent upon the stability of the glucuronide in the intestinal tract since glucuronides stable in the intestinal tract will only undergo limited hydrolysis and reabsorption and therefore excreted unchanged in the feces (Smith and Williams, 1966). Considerable evidence seems to imply that this deconjugation reaction is catalyzed by β-glucuronidase of bacterial origin (Schelmē, 1973). Little or nothing have been done on the possibility of enterohepatic circulation studies in rats with regards to phenol. It is however, known that the two major metabolites of P, PS and PG, are rapidly excreted in the urine unchanged. After feeding PS to rabbits, Rhode (1923) was able to account for 80 to 95% of the dose in the urine as unchanged ethreal sulfate. When PG was given to rabbits, almost the total dose was recovered as unchanged PG in a 24 hr urine collection (Nakano, 1937; Garton and Williams 1949). Oehme (1969) also injected PS and PG into pigs, dogs cats, and

goats and he also recovered almost the total dose as unchanged compounds in 24 hr urine collection.

Effects of Route of Administration of Phenol on Metabolic Products.

Porteous and Williams (1949) reported that rabbit urine contained only a trace of free P and essentially all the orally administered P was excreted as conjugates. Upon injection of the P to rabbits, 4 to 5% of the P was excreted in the free form and that P excretion was completed within 24 hr of its administration. On the average, 70% of the P recovered from the rabbits treated orally was present in the urine as PG, 14% was PS, and about 10% was proposed as being oxidation products, e.g. quinol and catechol conjugates, but in contrast to earlier report of Deichmann (1944) only traces of free phenol were found in the urine.

Site of Conjugations

With the discovery of PS, Baumann (1876) investigated the site of its formation. He obtained 19% more PS from the liver than from any other tissue and concluded that little, if any, conjugation of P took place in the kidneys. Baumann and Herta, (1877) Baumann, (1878) Kochs (1879, 1880) working with ground liver, kidney, pancreas, muscle in an attempt to show the formation of PS in those tissues, concluded that moderate amounts of conjugation occured in each tissue.

Using a series of perfusion experiments, Embden and Glaessner (1902) showed that PS formation occured almost exclusively in the liver, but minor amounts were found in the lungs and kidneys. Pelkam and Whipple (1922) performing series of injections and oral administrations of P to dogs, concluded confidently that the synthesis of PS and PG occured only

in the parenchyma of the liver. More recently, Powell et al (1974) using ^{14}C-phenol, showed by autoradiographic work and perfusion of isolated rat gut preparations concluded that the liver has no ability to concentrate either P or its metabolites, which suggested that ^{14}C-phenol either failed to enter the liver cells or alternatively have a transient cellular existence due to rapid turnover in the liver. They also strongly supported the view that orally administered P was not transported as such from the intestinal lumen but was in conjugated form. They suggested that the role of the liver was minimal in detoxication of orally ingested P, but that when the digestive tract was by-passed by iv administration the liver may assume major importance in conjugation of P with sulfate and glucuronide.

Mechanism of Biosynthesis of Phenyl Sulfate

Several studies have shown that PS synthesis occurs in liver, kidney, and intestinal mucosal cells (Sollmann, 1957; Roy, 1960; Parke, 1968). The reaction occurs widely and is found in most mammals including man, hen, amphibians, insects and in the molluscs (Williams, 1964).

The biochemical sequence of PS biosynthesis was reviewed by Gregory (1962). It consists of the enzymatic conversion of phenols to monoesters of sulfuric acid and can take place in vitro from P and sulfate ions in the presence of ATP, magnesium ions, and the soluble fraction of mammalian liver cells. Two separate steps are necessary: (1) the activation of ionic sulfate, and (2) the transferral of the sulfate from its active form to P.

In the first step, sulfate is activated by the addition of two units of ATP, the first addition being catalyzed by ATP-sulfate adenyl

transferase and resulting in adenosine-5'-phosphosulfate (APS). The APS then reacts with a second ATP under the influence of ATP-adenylyl-sulfate 3'-phosphotransferase to yield 3'-phosphoadenosine 5'-phospho-sulfate (PAPS) which is the active sulfate donor. PAPS is formed in liver, colonic mucosa and mast cell tumors and is present also in chick, embryo, hen, oviduct, snail mucous gland, yeast, algae, and molds.

The second step of PS biosynthesis, that is, the transfer of the sulfate to P, requires a relatively non-specific phenol sulfokinase which is found in the soluble fraction of mammalian liver, kidney and intestinal mucosa, and in granulation tissue from the guinea pig (Williams, 1964; Parke, 1968). In the presence of sulfokinase, PAPS donates the sulfate to P with the resulting formation of PS. Other phenols, as well as alcohols and steroids, may also act as acceptors of the activated sulfate. Exogenous inorganic sulfate may be utilized in the formation of these sulfate conjugates. (Laidlow and Young, 1948).

Mechanism of Biosynthesis of Phenyl Glucuronide

Although the conjugation of glucuronide acid with P was known since 1890 (Kulz), it was not until the early 1950's that the enzymatic mechanism of the process was elucidated. Hartiala (1955) studied the potential synthesis of glucuronide in a wide variety of tissue slices. He found that in addition to liver and kidney, which were well known to be capable of synthetizing PG, specimens from the alimentary canal also gave positive results.

With the discovery by Dutton and Storey (1953) and Storey and Dutton (1955) of a heat-stable cofactor necessary for the biosynthesis of glucuronides, the mechanism of glucuronic acid conjugation was clarified.

Dutton (1966) has made an extensive review of glucuronide biosynthesis.

Conjugation of P with glucuronic acid is also a two-step process involving (1) the biosynthesis of the heat-stable cofactor, uridine diphosphate glucuronic acid (UDPGA), and (2) the transfer of the glucuronyl moiety from UDPGA to the compound to be conjugated. The first step consists of the formation of uridine diphosphate glucose (UDPG) by the uridyl transferase-catalyzed reaction of glucose-1-phosphate and UTP. These then follow a NAD-linked oxidation catalyzed by UDPGA. The UDPG dehydrogenase enzyme and UDPGA are found in the particle free soluble fraction of the tissue homogenates of liver, kidney, digestive tract, skin, milk, placenta, uterus and tumors from a variety of mammals and birds (Dutton, 1966; Parke, 1968).

The last step involves the transfer of the glucuronyl residue from UDPGA to the P. This step is catalyzed by UDP-glucuronyl transferase, which is located in the microsomal fraction of tissue homogenates (Dutton, 1956). Glucuronyl transferase activity has been found in mammalian liver, kidney, digestive tract, skin and selected other tissues. It is absent in heart, skeletal muscle, blood, and tumor tissue (Dutton, 1966).

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TOTAL HALF HOUR BILE RADIOACTIVITY IN µCi

PHENOL DOSE: 25 mg/kg (Average sp. act. admin./Animal= 4.407 µCi ^{14}C -Phenol)

HR. AFTER ADMIN.	PENTOBARBITAL ANAESTHETIZED RAT NO.					
	1	2	3	4	5	6
1/2	6.3591	5.5950	5.3739	6.4855	6.2458	4.8951
1	3.4029	3.7086	2.3556	3.1710	3.27.24	2.6773
1½	0.9373	0.7692	0.9211	1.0950	0.9498	0.7238
2	0.3653	0.2474	0.4787	0.2540	0.5736	0.2840
2½	0.1888	0.1413	0.2983	0.0761	0.4951	0.1252
3	0.0933	0.0613	0.1482	0.0523	0.3177	0.0741
3½	0.0554	0.0363	0.0793	0.0501	0.1935	0.0540
4	0.0410	0.0298	0.0479	0.0354	0.1086	0.0315
4½	0.0252	0.0233	0.0493	0.0242	0.0591	0.0283
5	0.0275	0.0247	0.0515	0.0237	0.0472	0.0288
TOTAL %	11.4958	10.6369	9.8038	11.2672	12.2628	8.9221

RADIOACTIVITY IN µCi/ml WHOLE BLOOD

HR. AFTER ADMIN.	1	2	3	4	5	6	7
1/2	.3026	.3420	.3046	.2696	.2752	.3625	.3658
1	.1827	.2004	.2199	.2254	.1383	.2901	.1520
1½	.1561	.1213	.2502	.0835	.1423	.3174	.0833
2	.0846	.0669	.2130	-	.1282	.3087	.0537
2½	.0487	.0434	.1458	-	.1050	.2441	.0307
3	.0272	.0392	.0584	-	.0926	.2048	.0212
3½	.0225	.0292	.0287	-	.0770	.1251	.0222
4	.0056	.0083	.0417	-	.0531	.1095	.0142
4½	.0053	.0058	.0461	-	.0495	.0609	.0162
5	.0047	.0033	-	-	.0424	.0649	.0134
BODY WT. IN g	258.40	264.00	246.6	287.80	297.80	300.00	315.00

(PHENOL) PENTOBARBITAL ANAESTHETIZED RATS

Percent Biliary Radioactivity Associated with Metabolites

Rat No.	Metabolites	A	B	Phenyl Glucuronide	C	D
	R_f value in D	0.00	0.17	.51	.68	.81
	R_f value in B	0.00	0.00	.16	.30	.61
		1/2 HOUR BILE SAMPLES				
1	1/2 hr.	17.29	14.19	61.01	6.03	-
2	1/2 hr.	39.21	A + B	74.18	1.09	-
3	1/2 hr.	30.57	A + B	65.63	3.79	-
4	1/2 hr.	27.31	A + B	69.33	1.048	-
5	1/2 hr.	12.66	16.53	47.41	10.72	5.42
6	1/2 hr.	12.79	18.78	55.45	7.15	4.02
7	1/2 hr.	10.58	17.64	56.47	8.23	3.52
8	1/2 hr.	26.75	A + B	62.16	8.64	2.43
		1 HOUR BILE SAMPLES				
1	1 hr.	24.72	A + B	74.18	1.09	-
2	1 hr.	32.40	A + B	67.60	-	-
3	1 hr.	29.62	A + B	69.33	1.048	-
4	1 hr.	28.31	A + B	64.42	5.44	1.81
5	1 hr.	-	-	-	-	-
6	1 hr.	27.05	A + B	61.64	6.58	-
7	1 hr.	26.89	A + B	61.62	7.00	4.48
8	1 hr.	8.32	16.12	57.62	6.04	5.03

INDIVIDUAL ANIMAL DATA

TOTAL HALF HOUR BILE RADIOACTIVITY IN μCi

PHENOL DOSE: 25 mg/kg (Average sp. act. admin/Animal = 4.407 μCi ^{14}C-Phenol)

HR. AFTER ADMIN.	UNANAESTHETIZED RAT NO. 1	2	3	4
1/2	4.9934	2.9780	3.8892	2.2472
1	1.0180	1.0407	.6750	.7339
1½	.3381	.2007	.1991	.2484
2	.1486	.1010	.1137	.1348
2½	.0584	.1053	.0628	.0827
3	.0463	.0291	.0468	.0530
3½	.0408	.0363	.0420	.0405
4	.0276	.0331	.0320	.0399
4½	.0207	.0210	.0284	.0245
5	.0213	.0245	.0201	.0246
TOTAL %	6.7132	4.5697	5.1091	3.6428

RADIOACTIVITY IN μCi/ml WHOLE BLOOD

HR. AFTER ADMIN.	1	2	3	4
1/2	-	.3255	.1899	.3280
1	-	.1187	.0810	.1863
1½	-	.0655	.0363	.0793
2	-	.0395	.0218	.0398
2½	-	.0295	.0157	.0275
3	-	.0237	.0119	.0199
3½	-	.0225	.0100	.0138
4	-	-	.0077	.0121
4½	-	.0152	.0078	.0101
5	-	.0134	.0073	.0090
BODY WT. IN g	318.0	286.7	289.5	273.10

(PHENCL) UNANAESTHETIZED RATS

Percent Biliary Radioactivity Associated with Metabolites

Rat No.	Metabolites	A	B	Phenyl glucuro.	C	D
	R_{f_A}	0.00	0.17	.51	.68	.81
	R_{f_B}	0.00	0.00	.16	.30	.61
		1/2 HOUR BILE SAMPLES				
1	1/2 hr.	23.05(A+B)	-	65.16	8.27	3.50
2	1/2 hr.	9.63	13.50	61.56	9.90	5.40
3	1/2 hr.	11.86	9.67	65.27	8.79	4.39
4	1/2 hr.	15.75	9.97	57.82	12.96	3.49
		1 HOUR BILE SAMPLES				
1	1 hr.	23.54(A+B)	-	61.43	6.83	8.19
2	1 hr.	11.42	11.42	62.85	8.33	5.95
3	1 hr.	-	-	-	-	-
4	1 hr.	14.68	17.27	47.32	12.08	8.63

A+B = both metabolites A and B have the same R_f value in solvent system B.

PHENOL

Percent Radioactivity Recovered

in Urine

PHENOL DOSE:25 mg/kg (4.407 uCi/Animal)

Rat #	6 hrs.	12 hrs.	24 hrs.	48 hrs.	% of Dose Recovered
1	84.67	1.50	1.43	.0956	87.6956
2	86.63	1.3262	1.2763	-	89.2325
3	88.88	3.24	0.7689	-	92.8929
4	64.578	4.60	1.74	2.8290	73.7390
5	91.50	5.1517	2.0980	0.5547	99.3044
6	94.38	4.780	1.002	0.5316	100.6936
7	69.71	23.64	2.9788	0.5494	96.8782
8	76.39	3.5250	1.6802	0.9821	82.5773
9	80.6500	12.3800	1.2667	0.3692	94.6659
10	84.88	2.8536	1.4689	0.9154	90.1179
11	76.41	12.40	5.4590	0.7031	94.9721

PHENOL

Percent Urinary Radioactivity Associated with Metabolites

6 HR. URINE SAMPLES

Rat No.	Metabolites	Phenyl Glucuronide	Phenyl Sulfate	Phenol
	R_f value in A	.17	.68	.96
	R_f value in B	0.00	.30	.91
1	6 hr.	35.07	61.24	3.61
2	6 hr.	47.80	57.19	-
3	6 hr.	43.83	53.51	2.57
4	6 hr.	39.58	57.83	-
5	6 hr.	36.37	63.38	-
6	6 hr.	49.39	50.60	-
7	6 hr.	45.58	54.41	-
8	6 hr.	41.59	58.40	-
9	6 hr.	43.06	56.94	-
10	6 hr.	41.09	58.90	-
11	6 hr.	39.93	60.06	-

PHENOL

Percent Urinary Radioactivity Associated with Metabolites

12 HR. URINE SAMPLES

Rat No.	Metabolites	Phenyl Glucuronide	Phenyl Sulfate	Phenol
	R_f value in D	0.17	.68	.96
	R_f value in B	0.00	.30	.91
1	12 hr.	35.68	60.25	4.07
2	12 hr.	-	-	-
3	12 hr.	-	-	-
4	12 hr.	33.95	66.04	-
5	12 hr.	40.67	59.32	-
6	12 hr.	19.71	80.28	-
7	12 hr.	37.78	62.21	-
8	12 hr.	15.75	82.05	-
9	12 hr.	36.84	63.15	-
10	12 hr.	21.97	78.05	-
11	12 hr.	13.74	86.25	-

TISSUES RADIOACTIVITY TWO HOURS AFTER ADMINISTRATION

PHENOL DOSE: 25 mg/kg (Average Specific activity admin./Animal = 4.407 μCi ^{14}C-Phenol)

	Rat I μCi/g	Rat II μCi/g	Rat I μCi/organ	Rat II μCi/organ
Liver	.0017	.0025	.0244	.0353
Kidney	.0082	.0204	.0176	.0573
Lung	.0044	.0065	.0067	.0108
Heart	.0011	.0016	.0011	.0344
Testis	.0010	.0015	.0029	.0892
Spleen	.0014	.0115	.0010	.1257
Brain	-	.0015	-	.0001
Thymus	-	.0009	-	.0005
Muscle	.0014	.0012	-	.0012
Fat	.0009	.0015	-	.0015
Blood	-	.0089	-	-
Thyroid	-	.0002	-	.0002
Body Wt. in g.	347.5	-	350.0	-

A P P E N D I C E S III

o-Phenyl phenol

Litrature Review

Individual Animal Data

IV. o-PHENYL PHENOL

LITRATURE REVIEW

Chemistry.

o-phenyl phenol (OPP) is also known as dowicide, o-hydroxydiphenyl or 2-hydroxydiphenyl. It has a molecular weight of 170.2. It is practically insoluble in water but it is very soluble in fixed alkali, hydroxide solutions, and in most organic solvents. The sodium salt is highly soluble in water.

Uses.

It is used in germicide and fungicide preparations and in rubber industry.

Metabolic Fate of OPP and its isomers.

Early unquantitated work of Dodgson et al (1948) showed that rabbit fed p-phenyl phenol (PPP) suspended in water (as a model experiment for stilbesterol metabolism) excreted PPP as p-β-D-glucuronosido biphenyl in urine. Unquantitated work of Kamil et al (1957) showed that rabbits fed OPP orally excreted it in urine as o-phenyl phencl glucuronide and a glycone. More recent and unquantitated work of Oehme and Smith (1972) showed that dogs and cats excreted orally fed ^{14}C-OPP in the form of the aglycone and as glucuronide and sulfate conjugates. Phenyl glucuronide derived from cleavage of the phenyl phenol bond followed by ring hydroxylation was also excreted.

Milburn et al (1967) injected PPP (4-hydroxy biphenyl) dissolved in propane-1-2-diol ip to rabbits and found that 37% of the dose was excreted in bile in 24 hrs. Of this about 57% was in the 4-glucuronosido biphenyl and about 43% was 4-glucuronoside 4-hydroxy biphenyl. They also found

traces of unchanged 4-hydroxy biphenyl and unidentified small amounts of another glucuronide.

Interspecies Variation in Metabolism of OPP.

Interspecies variation in metabolism of OPP have not been documented, but that of the parent compound, biphenyl, have been investigated. Aromatic hydroxylation appears to occur in the 4-position in all species tested, but in some species it is also hydroxylated in the 2-position. Preparations of liver from guinea pigs, adult rats, adult rabbits, foxes, hens and trout produced only 4-hydroxy biphenyl in vitro, but those from frogs, mice, cats, copypus, young rats, young rabbits and especially hamsters produced 2-hydroxy biphenyl as well (Creaven et al, 1965). Thus there are species differences in the orientation of hydroxylation when the compound offers more than one position for hydroxylation. From metabolism of OPP one would expect species variations which signify the variation in enzymatic systems in the different species. These variations might be responsible for the different toxicity of OPP to different species of animals.

Toxicity of OPP.

MacIntosh (1945) showed that the feeding of OPP at levels of 2, 20, and 200 mg/kg per day to rats neither impeded their growth nor caused ill health effects in general. Later studies showed that rats could not tolerate 2% OPP in their diets for an extended period of time. Those on the 2% showed marked retarded growth, dilation of kidney tubules, and slight OPP kidney tissue residues. But dogs fed OPP at 0.02, 0.2 and 0.5 g/kg per day for one year produced no recognisable adverse effect (Hodge et al, 1952).

The recent studies of Oehme and Smith (1972) showed that dogs readily survived doses of 1 and 3 g/kg of OPP/kg, but cats succumbed to the low dose in 15 hr and the higher dose within 6 hours. Dogs were able to excrete OPP in urine readily and completely. Tissue residue studies in dogs and cats were highest in lung, liver, kidney, spleen, bile, and feces. Three times more OPP was required to produce signs in canines than felines (Oehme and Smith 1972). Greenblatt and Koch-Weser (1975) showed that multiple exposures may lead to increased body burden.

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o-PHENYL PHENOL (OPP)

OPP DOSE: 40.8 mg/kg (Specific Activity admin./Animal = 5.5632 µCi)

TOTAL HALF HR. BILE RADIOACTIVITY IN µCi

HR. AFTER ADMIN.	PENTOBARBITAL ANAESTHETIZED RATS NO. 1	2	3
1/2	.9285	.6116	.8113
1	.1891	.2094	.2349
1½	.0610	.0661	.0420
2	.0353	.0325	.0229
2½	.0212	.0180	-
3	.0124	.0100	-
3½	.0084	.0115	-
4	.0050	.0040	-
4½	.0040	.0074	-
5	.0024	.0070	-
TOTAL %	22.78	17.57	19.97

RADIOACTIVITY IN µCi/ml WHOLE BLOOD

HR. AFTER ADMIN.	1	2	3
1/2	.0159	.0225	.0165
1	.0097	.0150	.0118
1½	.0095	.0105	.0132
2	.0071	.0063	.0165
2½	.0056	.0068	-
3	.0050	.0061	-
3½	.0037	.0057	-
4	.0029	.0054	-
4½	-	-	-
5	-	.0052	-
BODY WT. IN G	347.20	344.00	303.5

OPP IN PENTOBARBITAL ANAESTHETIZED RATS

Percent Biliary Radioactivity Associated with Metabolites

1/2 HR. BILE SAMPLES

	Metabolites	A	B	C	D	OPP
Rat No.	Rf_A	.37	.49	0.64	.86	.92
1		1.49	18.71	75.97	2.54	1.27
2		1.05	11.27	81.05	5.11	1.49
3		1.06	11.57	83.56	2.54	1.23

1 HR. BILE SAMPLES

	Metabolites	A	B	C	D	OPP
Rat No.	Rf_A	.37	.49	0.64	.86	.92
1		2.14	17.72	72.93	5.69	1.50
2		1.88	10.03	77.79	8.53	1.75
3		1.48	10.66	80.70	5.65	1.48

o-PHENYL PHENOL (OPP)

OPP DOSE: 40.8 mg/kg (Specific Activity admin./Animal = 5.5632 μCi)

TOTAL HALF HR. BILE RADIOACTIVITY IN μCi

HR. AFTER ADMIN.	UNANAESTHETIZED RATS NO. 1	2	3
1/2	.7170	.5301	.5202
1	.2424	.1179	.2147
1½	.0445	.0529	.0532
2	.0229	.0389	.0174
2½	.0097	.0119	.0123
3	.0056	.0096	-
3½	.0041	.0078	-
4	.0038	.0120	-
4½	.0031	.0036	-
5	.0033	.0059	-
TOTAL %	18.9890	14.2112	14.70

RADIOACTIVITY IN μCi/ml WHOLE BLOOD

HR. AFTER ADMIN.	1	2	3
1/2	.0174	.0122	.0167
1	.0136	.0073	.0065
1½	.0113	.0042	.0072
2	.0094	.0023	.0040
2½	.0094	.0020	.0028
3	.0098	.0015	.0018
3½	-	.0010	.0016
4	-	.0008	.0010
4½	-	.0009	.0009
5	-	.0011	.0008
BODY WT. IN G	321.3	357.20	316.60

OPP UNANAESTHETIZED RATS

Percent Biliary Radioactivity Associated

with Metabolites

1/2 HR. BILE SAMPLES

	Metabolites	A	B	C	D	OPP
Rat No.	R_{f_A}	.37	.49	0.64	0.86	.92
1		0.91	12.75	81.29	3.08	1.96
2		1.63	9.86	82.28	3.85	2.37
3		2.72	9.82	73.63	10.00	3.81

1 HR. BILE SAMPLES

	Metabolites	A	B	C	D	CPP
Rat No.	R_{f_A}	.37	.49	0.64	0.86	.92
1		1.89	10.70	78.74	4.72	3.93
2		1.72	9.82	81.08	4.09	3.27
3		2.72	9.82	73.63	10.00	3.81

% DOSE RADIOACTIVITY RECOVERED IN URINE

OPP DOSE: 40.8 mg/kg (Average specific activity admin./Animal = 5.5632 μCi)

Rat No.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	Total % Excreted	BODY WT. IN G.
1	85.7509	13.7092	1.7613	0.7669	0.2347	102.2230	349.2
2	66.0202	10.7916	3.0518	1.5257	0.3821	81.7714	343.5
3	68.0243	14.7114	2.4408	0.8894	0.6526	86.7185	330.5
4	78.0162	7.8631	2.9714	0.7127	0.3294	89.8928	350.0
5	69.3046	10.5019	2.8753	0.9741	0.4406	84.0965	347.0

OPP

Percent Urinary Radioactivity

Associated with Metabolites

Rat No.	Metabolite R_f value A	A 0.47	B .55	C .64	D .78	OPP .90
		6 HR. URINE SAMPLES				
1		1.59	4.24	14.63	6.36	73.17
2		1.92	4.80	13.10	8.05	72.11
3		1.83	5.89	15.44	7.46	69.37
4		2.68	5.12	17.54	8.06	66.58
5		1.43	3.17	11.44	6.04	77.90
		12 HR. URINE SAMPLE				
1		2.30	6.71	9.59	4.60	76.77
2		2.38	6.66	10.95	5.23	74.76
3		5.75	6.57	17.26	6.84	63.56
4		4.02	4.55	11.00	6.16	74.26
5		2.59	4.11	13.42	4.11	75.75

TISSUES RADIOACTIVITY TWO HOURS AFTER ADMINISTRATION

OPP DOSE: 40.8 mg/kg (Average Specific Activity/Animal = 5.5632 μCi)

RAT 1

Organ	μCi/g Tissue	μCi/Total Organ
Liver	.0196	0.3074
Kidneys	.0952	0.3170
Testis	.0038	.0112
Brain	.0021	.0035
Heart	.0056	.0051
Lungs	.0140	.0218
Thymus	-	.0002
Muscle	.0071	-
Fat	.0167	-
Thyroid	-	.0005

A P P E N D I X IV

2,6-Diiodo-4-nitrophenol

Litrature Review

Individual Animal Data

III. 2, 6-DIIODO-4-NITROPHENOL

LITRATURE REVIEW

Chemistry.

The genetic designation of disophenol is 2, 6-diiodo-4-nitrophenol (DNP). It has a molecular weight of 391 and only sparingly soluble in water. Addition of sodium hydroxide increases its solubility in aqueous formulations. Solvents, such as a 1:1 water and polyethyleneglycol, 0.1N sodium hydroxide and distilled water, or water-polymethylene, are used for parenteral administration of DNP (Jones, 1965).

Uses.

Disophenol is an effective systemic anthelminthic used for treatment of hook wor, infestation in dogs and cats.

Metabolism and Toxicity of DNP.

Disophenol is an effective systemic anthelminthic used for treatment of hook worm infestation in dogs and cats. The distribution, biotransformation and mechanism of action of DNP have not been documented (Jones, 1965). The clinical signs of DNP toxicosis resemble those produced by 2, 4-dinitrophenol (Kaiser et al, 1964; Tainter et al, 1933; Thienes et al, 1972), and the mechanism by which the latter produces toxicosis may apply to DNP. Kaiser (1964) made a comparative toxicology studies of DNP and 2, 4-dinitrophenol in dogs and rodents and concluded that qualitatively the two compounds produced similar toxicologic signs in laboratory animals, but quantitatively there was a pronounced distinction between the two compounds. Acutely, on a dry weight basis, DNP was much less toxic to mice and rats than was dinitrophenol. On a molecular weight

basis, the toxicity of the two compounds was nearly similar. However, following repeated administration, DNP appeared to be the more toxic compound. This difference was in part explained by DNP accumulation in plasma whereas under similar conditions accumulation in plasma was not evident with dinitrophenol.

Disophenol is rapidly absorbed in dogs by oral and parenteral routes (Jones, 1965; Kaiser, 1964). Following oral administration, both DNP and 2, 4-dinitrophenol are rapidly absorbed and reach detectable plasma concentrations in 30 minutes. A constant increase in plasma concentration was observed with DNP. Increase in plasma concentration together with prolonged blood levels indicate the tendency of DNP to accumulate in the body tissues. When dogs received a daily oral dose of 5 mg DNP or 2, 4-dinitrophenol/kg for 13 days, plasma concentrations of 102 and 1 ug/ml respectively were observed on the 13th day. Urinary excretion of DNP during the first 24 hours was neglegible, while 177 ug of dinitrophenol was recovered in a 24 hr urine sample. Tissue binding, movement to extravascular sites, or other mechanisms were suggested (Kaiser et al, 1964).

Like 2, 4-dinitrophenol, DNP was said to possess a cataractogenic potential and young pupies were said to be more susceptible (Martin et al, 1972). The age variation among dogs, rabbits and ducks in susceptibility to DNP cataractogenic effect was suggested to be related to an immature drug-metabolizing enzyme system as well as to an intraocular deficiency in the blood-aqueous barrier (Gehring et al, 1969; Kinsey et al, 1945). Another factor might be an age-related difference in lens metabolic activity (Martin et al, 1972).

Biotransformation of 2, 4-dinitrophenol and possible similarity with disophenol.

Various workers have reported upon the metabolites of 2, 4-dinitrophenol. In man and dog, its metabolites appear to be 2-amino-4-nitro, 4-amino-2-nitro and 2, 4-diamino-phenols, their glucuronic acid conjugates, and unchanged dinitrophenol (Guerbet and Mayer, 1932; Georgescu 1932). Following subcutaneous injections of 20 mg/kg of 2, 4-dinitrophenol, 2-amino-4-nitrophenol was the only reduction product; but when 2, 4-dinitrophenol was incubated with rat liver homogenates the major reduction product was 4-amino-2-nitrophenol with small amounts of the 2-aminoderivative. It can be speculated that one of the metabolites of DNP could be the conjugated reduction product. The biotransformation and fate of disophenol in the animal body are yet to be elucidated.

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DISOPHENOL (DNP)

DNP DOSE: 9.6 mg/kg (Average specific activity admin./Animal = 4.6939 μCi)

TOTAL HALF HR. BILE RADIOACTIVITY IN μCi

HR. AFTER ADMIN.	PENTOBARBITAL ANAESTHETIZED RAT NO. 1	2	3
1/2	.0288	.0352	.0309
1	.0233	.0191	.0202
1½	.0151	.0144	.0179
2	.0127	.0136	.0229
2½	.0172	.0117	.0165
3	.0127	.0123	.0144
3½	.0108	.0096	.0138
4	.0055	.0080	.0137
4½	.0068	.0084	.0134
5	.0101	.0094	.0164
TOTAL % IN BILE	3.0465	3.0188	3.8369

RADIOACTIVITY IN μCi/ML WHOLE BLOOD

HR. AFTER ADMIN.	1	2	3
1/2	.0687	.0558	.0676
1	.0594	.0475	.0593
1½	.0596	.0487	.0547
2	.0537	.0483	.0557
2½	.0513	.0464	.0546
3	.0521	.0442	.0549
3½	.0514	.0443	.0564
4	.0599	.0445	.0522
4½	.0499	.0434	.0515
5	.0475	.0425	.0512
BODY WT. IN g	337.30	250.00	280.00

DISOPHENOL (DNP) IN PENTOBARBITAL ANAESTHETIZED RATS

Percent Biliary Radioactivity Associated

with Metabolites

1/2 HR. BILE SAMPLES

	Metabolites	A	B	C	D	DNP
Rat No.	Rf. value in A	.43	.52	.62	.72	.90
1	1/2 hr.	9.60	37.85	14.69	16.38	21.46
2	1/2	10.86	34.78	13.04	15.21	26.08
3	1/2	13.81	30.92	14.47	22.36	18.42
4	1/2	9.49	30.16	16.75	18.99	24.58

1 HR. BILE SAMPLES

	Metabolites	A	B	C	D	DNP
Rat No.	Rf. A	.43	.52	.62	.72	.90
1	1 hr.	8.57	36.00	18.85	13.71	22.85
2	1 hr.	6.28	40.95	10.05	15.07	27.63

DISOPHENOL (DNP)

DNP DOSE: 9.6 mg/kg (Average specific activity admin./Animal = 4.6939 µCi)

TOTAL HALF HR. BILE RADIOACTIVITY IN µCi

HR. AFTER ADMIN.	UNANAESTHETIZED RATS NO. 1	2	3
1/2	.0140	.0201	.0224
1	.0109	.0176	.0116
1½	.0090	.0149	.0098
2	.0092	.0137	.0118
2½	.0066	.0142	.0139
3	.0106	.0138	.0149
3½	-	.0164	.0152
4	-	.0172	.0151
4½	-	.0171	.0162
5	-	.0182	.0150
TOTAL % DOSE IN BILE	-	3.4768	3.1085

RADIOACTIVITY IN µCi/ML BLOOD

HR. AFTER ADMIN.	1	2	3
1/2	.0762	.0812	.0784
1	.0687	.0724	.0726
1½	.0599	.0698	.0669
2	.0609	.0698	.0701
2½	.0563	.0710	.0702
3	.0565	.0696	.0684
3½	-	-	.0691
4	-	-	.0682
4½	-	-	.0686
5	-	-	.0674
BODY WT. IN g	296.10	304.0	296.6

DISOPHENOL (DNP) UNANAESTHETIZED RATS

Percent Biliary Radioactivity Associated

with Metabolites.

1/2 HR. BILE SAMPLES

	Metabolite	A	B	C	D	DNP
Rat No.	Rf_A	.43	.52	.62	.72	.90
1		10.71	37.14	10.00	14.28	27.85
2		9.17	27.52	16.05	20.64	26.60
3		7.40	34.25	19.44	13.88	24.07

1 HR. BILE SAMPLES

	Metabolite	A	B	C	D	DNP
Rat:	Rf_A	.43	.52	.62	.72	.90
1		3.57	34.37	12.05	16.51	33.48
2		5.09	48.12	18.50	10.18	18.04
3		3.65	47.56	12.19	9.14	27.44

% DOSE RADIOACTIVITY RECOVERED IN URINE

DNP DOSE 9.6 mg/kg (4.6939 μCi)

Rat No.	6 hrs.	12 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.	Total % in 4 days	BODY WT. IN G.
1	2.8378	5.1206	5.1029	11.9435	8.0260	4.8458	37.8766	290.00
2	2.0949	3.9419	4.4996	16.42.96	7.0922	5.1498	39.2080	256.00
3	4.5477	4.3956	5.2714	20.2231	11.6315	5.3257	51.3950	263.80
4	6.1171	10.2466	12.6491	15.9162	10.1594	4.2371	59.3255	-

DISOPHENOL (DNP)

Percent Urinary Radioactivity
Associated with Metabolites

Rat No.	Metabolite	A	B	C	D	DNP
	Rf_A	0.41	0.52	0.62	0.77	0.92
	6 HR. URINE SAMPLE					
1		8.54	40.20	20.10	10.05	21.10
	12 HR. URINE SAMPLE					
1		11.09	44.71	17.40	22.18	9.21
2		7.63	41.98	25.57	16.79	8.01
3		8.54	46.29	22.58	19.35	3.22
	48 HR. URINE SAMPLE					
3		16.14	43.22	12.98	18.35	9.09

TISSUES RADIOACTIVITY TWO HOURS AFTER ADMINISTRATION

DNP DOSE: 9.6 mg/kg Average Specific Activity/Animal 4.6939 μCi

Organs	μCi/g	μCi/Organ	μCi/g	μCi/Organ
Liver	.0376	.5358	.0112	.1301
Lungs	.0564	.0902	.0116	.0179
Testis	.0065	.0193	.0012	.0031
Brain	.0027	.0039	.0005	.0009
Spleen	.0659	.0955	.0039	.0018
Heart	.0199	.0215	.0065	.0067
Kidneys	.0769	.1752	.0106	.0206
Blood	.0664	-	.0171	-
Thyroid	.0012	-	-	.0005
Muscle	.0124	-	.0572	-
Thymus	-	.0009	-	-
BODY WT. IN g.	256.5		321.0	

THE FATE OF PHENOL, o-PHENYL PHENOL AND DISOPHENOL IN RATS

by

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AN ABSTRACT OF A MASTER'S THESIS

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Three widely-used phenolic compounds which animals or human may be exposed to were investigated: Phenol (P), molecular weight 94, is used in disinfectant preparations and as a starting compound for chemical formulations; disophenol (DNP), molecular weight 391, is used as an anthelminthic for the treatment of canine hookworms; and o-phenyl phenol (OPP), molecular weight 170, is used in household germicides and disinfectants. The effect of molecular weight on metabolism and excretion, the influence of pentobarbital on metabolism, the biliary excretion and enterohepatic circulation, and the tissue distribution of these compounds were studied in male rats.

Radioassay was used to determine plasma disappearance, enterohepatic circulation, urinary and biliary excretion, and tissue residues of P, DNP and OPP. Descending irrigation chromatography was used to separate the metabolites, and a radiochromatogram scanner and integrator was used to quantitate urinary and biliary metabolites.

P and OPP rapidly disappeared from the blood, but DNP disappearance was very slow and remained almost constant throughout the five-hour experimental period. The rate of disappearance of P, DNP and OPP from blood was faster in pentobarbital anaesthetized rats than in unanaesthetized rats; an enzyme induction effect was proposed. DNP appeared to be the most protein bound followed, in decreasing order, by OPP and P.

OPP exhibited the highest biliary excretion followed by P and DNP. Five biliary metabolites were found in P, DNP and OPP-treated rats; the major biliary metabolites in each case were glucuronide conjugates. Enterohepatic circulation was detected for P, OPP and their metabolites but none was found over the two-hour experimental period for DNP and its metabolites.

Renal excretion of P and OPP was more than 90% completed 48 hours after iv adminstration, but only 47% of the DNP dose was recovered in urine in four days. Glucuronide and sulfate conjugates were the two almost equal urinary metabolites of P. The major urinary metabolite of OPP and DNP was the glucuronide conjugate. Significant amounts of unchanged OPP and DNP occurred in the urine.

The highest tissue residues two hours after administration were found in kidney and liver of P and OPP-treated rats and in muscle and blood in DNP-treated rats.

It was concluded that: (1) when compared to P and OPP, the excretion of DNP was slowest; (2) of the three phenolics, repeated administration of DNP is most likely to result in increased body burden; and (3) physico-chemical properties other than molecular weight play significant roles in a chemical's biliary excretion and metabolism.