THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 196, No. 3

Printed in U.S.A.

PHARMACOKINETICS OF ACETAMINOPHEN ELIMINATION BY ANEPHRIC PATIENTS[1, 2]

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Accepted for publication October 8, 1975

ABSTRACT

LOWENTHAL, DAVID T., SVEIN ØIE, JOHN C. VAN STONE, WILLIAM A. BRIGGS AND GERHARD LEVY: Pharmacokinetics of acetaminophen elimination by anephric patients. J. Pharmacol. Exp. Ther. **196:** 570–578, 1976.

The pharmacokinetics of acetaminophen elimination were determined in five surgically anephric and five physiologically anephric patients on an interdialysis day, and in three normal adult volunteers. There was no significant difference in the biologic half-life and no apparent difference in the volume of distribution of acetaminophen between the three groups of subjects but the anephrics, unlike the normal subjects, showed pronounced accumulation of acetaminophen glucuronide and sulfate in plasma. The apparent volume of distribution for conjugated acetaminophen is considerably smaller than that for the unmetabolized drug even though neither acetaminophen nor its glucuronide or sulfate is significantly bound to plasma proteins. The results of this study indicate that the kidneys do not contribute significantly to the elimination of acetaminophen in man. Since acetaminophen is eliminated largely by conjugation with glucuronic acid and sulfate, it can be concluded that the kidneys do not contribute significantly to the formation of these metabolites.

It is generally appreciated that the biologic half-life of drugs which are eliminated largely by renal excretion in normal subjects is substantially increased in patients with renal failure (Bennet *et al.*, 1973; Dettli, 1974). Much less is known about the contribution of the kidneys to the biotransformation of drugs. One group of investigators inferred from indirect studies in normal animals and man that the kidneys may account for a large fraction of the body's conjugation of benzoic acid with glycine, the acetylation and glycine conjugation of *p*-aminobenzoic acid and the glycine conjugation of salicylic acid (Von Lehmann *et al.*, 1973; Wan and Riegelman, 1972; Wan *et al.*, 1972). They cautioned against the possible toxic effects in anephric patients of drugs which are ordinarily metabolized primarily by the kidneys unless the dosage of these drugs is reduced appropriately. On the other hand, our own subsequent direct studies in anephric patients indicated that the kidneys do not contribute significantly to the conjugation of salicylic acid with glycine (Lowenthal *et al.*, 1974). Another

Received for publication August 21, 1975.
[1] This work was supported in part by Grant GM 20852 from the Institute of General Medical Sciences, National Institutes of Health.
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group of investigators concluded from the results of their study of the pharmacokinetics of pindolol in patients with impaired renal function that the extrarenal (biotransformation) rate constant for this drug increases in proportion to the degree of renal impairment (Ohnhaus *et al.*, 1974). A re-examination and pharmacokinetic analysis of their data by two of us revealed no evidence for such a conclusion and suggested that the nonrenal clearance of pindolol is not influenced by renal function (Øie and Levy, 1975). Many investigators have shown that the plasma or serum protein binding of some drugs is decreased in renal failure (Andreasen, 1973; Kramer *et al.*, 1974; Lowenthal *et al.*, 1974); this phenomenon may be responsible for the increased metabolic clearance of certain highly plasma protein bound drugs by patients in renal failure (Cutler *et al.*, 1974).

The investigation to be described here was initiated to study the pharmacokinetics of acetaminophen in anephric patients. Acetaminophen is widely used as an analgesic and antipyretic in these patients and excessive accumulation of the drug due to impaired elimination would be undesirable. Since acetaminophen is eliminated largely by formation of glucuronide and sulfate conjugates (Cummings *et al.*, 1967; Levy *et al.*, 1975; Levy and Regårdh, 1971; Levy and Yamada, 1971; Mitchell *et al.*, 1974; Mrochek *et al.*, 1974) a comparison of its biologic half-life in normal subjects and anephric patients can provide an indication of the magnitude of the renal contribution to these two biotransformation processes. Such an assessment is feasible because the metabolic clearance of acetaminophen is not liver blood flow limited (see "Discussion") and is not complicated by renal disease-related changes in plasma protein binding since, as will be shown, acetaminophen is not significantly bound to plasma proteins in the usual concentration range. *In vitro* studies have shown that the mammalian kidney can form phenolic glucuronide and sulfate conjugates (Boström and Wengle, 1967; Stevenson and Dutton, 1962), but *in vivo* investigations are needed to determine the quantitative importance of these renal processes

Methods

Five surgically anephric patients, five physiologically anephric patients (creatinine clearance < 5 ml/min) and three normal volunteers (members of the medical staff) gave their informed consent and participated in this investigation. The patients were maintained on regular hemodialysis, three times a week, and are described in table 1. They were studied on an interdialysis day.

All subjects received a single oral dose of acetaminophen, 1 g per 1.73 m^2 of body surface area (Tylenol elixir) in the morning on an empty stomach. Food was permitted after 1 hour. Blood samples were obtained in heparin-treated containers usually at 0, 0.5, 1, 3, 5, 7 and 9 hours after drug administration, and plasma was separated by centrifugation. Mixed saliva was

TABLE 1

Description of anephric patients

Patient No.	Age	Sex	Type of Anephric[a]	Body Surface Area	Blood Urea Nitrogen	Plasma Creatinine Conc.	Total Plasma Protein Conc.	Plasma Albumin Conc.
	yr			*m^2*	*mg/100 ml*	*mg/100 ml*	*g/100 ml*	*g/100 ml*
1	53	M	P	1.75	54	7.3	6.4	3.5
2	47	M	P	1.49	68	12.9	6.5	4.2
3	48	F	P	1.70	57	7.5	6.7	3.3
4	57	M	P	1.90	30	8.2	6.4	3.8
5	64	M	P	1.77	73	7.3	6.0	3.5
6	55	M	S	1.78	100	17.0	6.2	3.4
7	28	F	S	1.45	65	14.2	7.0	3.1
8	43	F	S	1.90	69	24.0	7.2	4.1
9	42	F	S	1.39	45	10.7	5.5	3.2
10	24	F	S	1.45	65	12.5	6.4	4.6

[a] P, physiologic; S, surgical. Surgical anephrics: both kidneys surgically removed due to refractory hypertension, chronic pyelonephritis, or antiglomerular basement membrane antibody-associated glomerular nephritis. Physiologic anephrics: kidneys *in situ* but nonfunctional (endogenous creatinine clearance < 5 ml/min).

obtained at the time of blood collection by having the subjects chew on a piece of Parafilm and salivate into a glass vial for 3 minutes. The plasma and saliva samples were stored at −18°C until they were assayed.

The concentration of acetaminophen in plasma and saliva was determined by the gas chromatographic method of Prescott (1971) with the following modifications. The drug was extracted into ether after addition of 0.5 ml 1 M sodium and potassium phosphate buffer, pH 7.4 and an excess of sodium chloride to 1 ml of plasma or saliva; derivatization was carried out with 5 μl of pyridine and 10 μl of butyric anhydride. The column packing was 3% OV-17 on Chromosorb W-HP, 80/100 mesh, and the column length was 6 feet. Recovery of acetaminophen from "spiked" plasma and saliva was 90 ± 5% and 94 ± 5% (mean ± S.D.), respectively. Blank values were less than 0.1 μg/ml.

The total metabolite concentration (*i.e.*, acetaminophen glucuronide and sulfate) in plasma was determined by incubating 1 ml of plasma with 0.5 ml of 0.4 M sodium acetate buffer, pH 5.0 (to give a final pH of 5.2) and 50 μl of Glusulase (mixture of β-glucuronidase and sulfatase, Endo Laboratories, Inc., Garden City, N.Y.) for 24 hours[5] at 37°C. After incubation, the pH was adjusted to ~ 7.4 by adding 0.1 ml 2 M sodium hydroxide and 0.5 ml 1 M phosphate buffer, pH 7.4, and this solution was extracted and assayed for acetaminophen as described in the previous paragraph. The total metabolite concentration was calculated by subtracting from the plasma concentration determined by this procedure the separately determined concentration of unmetabolized acetaminophen.

The concentration of acetaminophen glucuronide in plasma was determined by incubating 1 ml of plasma with 0.25 ml of Ketodase (β-glucuronidase solution, Warner-Chilcott Laboratories, Morris Plains, N.J.) and 0.25 ml of 0.5 M acetate buffer, pH 4.0 (to give a final pH of ~ 4.5) for 4 hours[5] at 37°C, then adjusting the pH to 7.4 as described in the previous paragraph, and extracting and assaying for acetaminophen. The results thus obtained were corrected for unmetabolized acetaminophen to yield the concentration of acetaminophen glucuronide.

The concentration of acetaminophen sulfate in plasma was calculated by subtracting the concentration of acetaminophen glucuronide from the total metabolite concentration. All concentrations of acetaminophen metabolites are reported here in terms of unmetabolized acetaminophen.

Plasma protein binding of acetaminophen and its metabolites was determined by equilibrium dialysis of 2 ml of plasma pooled from samples of an individual subject against an equal volume of 0.16 M trometha-mine buffer, pH 7.4, at 37°C for 14 hours, using Plexiglas dialysis cells and a cellophane membrane (Union Carbide Corporation, Oak Ridge, Tenn.). Preliminary studies had shown that 14 hours were sufficient for equilibration. The concentrations of acetaminophen and its conjugates in the plasma and buffer phases were determined as described in the preceding paragraphs, except that sample and reagent volumes were halved.

The biologic half-life ($T_{1/2}$) of acetaminophen was determined from the slope of the least-squares nonweighted regression line for the log-transformed plasma concentrations in the terminal exponential phase of plasma concentrations as a function of time, using the relationships slope = $-k_{el}/2.30$ and $T_{1/2} = 0.693/k_{el}$. The area under the plasma concentration-time curve (AUC) was calculated by means of the equation

$$\text{AUC} = \int_0^{t_e} C_p\, dt + C_p^{t_e}/k_{el} \qquad (1)$$

where the first term on the right of the equal sign is the AUC up to the time of the last blood sample (t_e) and C_p is the plasma concentration (Gibaldi and Perrier, 1975). The apparent volume of distribution (V_d) can be calculated from the equation

$$V_d = fD/(k_{el}\text{AUC}) \qquad (2)$$

where D is the dose and f is the fraction of the dose entering the systemic circulation as unchanged drug (Gibaldi and Perrier, 1975). Since f is unknown, only V_d/f values are reported and serve mainly for comparative purposes. An estimate of V_M, the apparent volume of distribution of acetaminophen metabolites (*i.e.*, a hybrid constant which is the weighted average of the volumes of distribution for acetaminophen glucuronide and sulfate) was obtained in the anephric patients from the relationship $V_M \simeq 0.8\ D/C_M^{t_e}$ (where $C_M^{t_e}$ is the concentration of total acetaminophen metabolites in the plasma at time = t_e). The basis for and the assumptions underlying this relationship will be detailed under "Discussion."

Results

Figures 1 to 3 show the time course of acetaminophen concentrations in plasma and saliva, the time course of acetaminophen total metabolite (glucuronide and sulfate) concentrations in plasma and the relationship between acetaminophen concentration in saliva and plasma for three representative subjects: a normal volunteer, a physiologically anephric patient and a surgically anephric patient. Acetaminophen concentrations in plasma decreased exponentially with time in all subjects. Pharmacokinetic constants for the distribution and elimination of acetaminophen in the physiologically and surgically anephric patients and in the normal volunteers are listed in tables 2 to 4.

[5] Longer periods of incubation with the enzyme yielded essentially identical results.

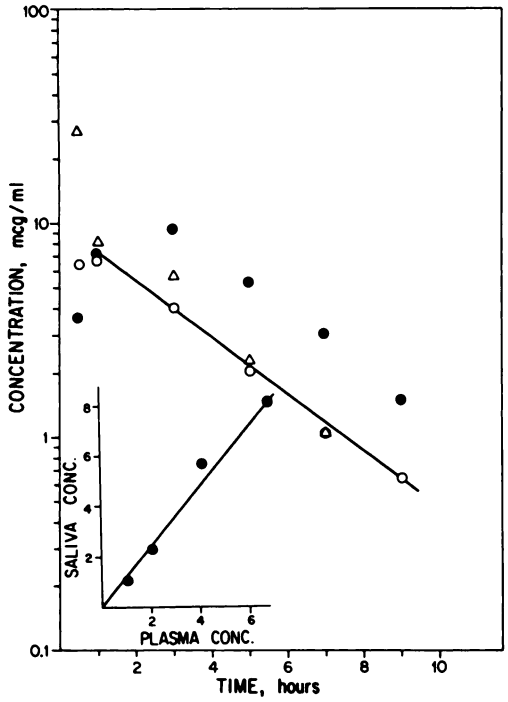

FIG. 1. Time course of acetaminophen concentrations in plasma (○) and saliva (△) and of total metabolite concentrations in plasma (●) of a normal subject (No. 12) after an oral dose of 1 g of acetaminophen per 1.73 m^2 of body surface area. The inset shows the relationship between the saliva and plasma acetaminophen concentrations for samples obtained at 1 hour and later.

There was no significant difference between the three groups in biologic half-life and V_d/f. The time course of plasma acetaminophen concentrations was therefore similar in these groups (figs. 4 and 5). The AUC values for acetaminophen were much more variable in the surgical anephrics than in the other two groups and this is reflected also in the large coefficient of variation (51%) for V_d/f in these patients (table 3).

Acetaminophen total metabolite concentrations in plasma declined exponentially in parallel with plasma-acetaminophen concentrations in normal subjects (fig. 1). In the anephric patients, total metabolite concentrations increased for about 6 hours and then remained essentially constant until the time of the last blood withdrawal which was usually at 9 hours (figs. 2 and 3). In one patient (No. 3), blood samples were obtained at 7¼ and 24 hours and the plasma total metabolite concentrations in these two samples were found to be identical (53.7 and 53.3 μg/ml). The average (± S.D.) maximum concentrations of acetaminophen total metabolites in the plasma of normal volunteers, physiologic anephrics and surgical anephrics were 11.9 ± 2.5, 58.4 ± 9.3 and 42.1 ± 3.8 μg/ml, respectively, each being statistically significantly different from the other. The estimated apparent volume of distribution of acetaminophen total metabolites (V_M) is 5.5 times smaller, on the average, than V_d/f. The values of V_M in the surgically anephric patients are somewhat higher than in the physiologic anephrics, for unknown reasons. The former group was younger and consisted largely of women whereas the other consisted largely of men. However, the one male (No. 6) in the surgically anephric group and the one female (No. 3) in the physiologically anephric group had V_M values near the average of their respective groups, and there is no significant correlation between age and V_M in the 10 anephric patients ($P > .1$).

There was an excellent linear correlation between acetaminophen concentrations in sa-

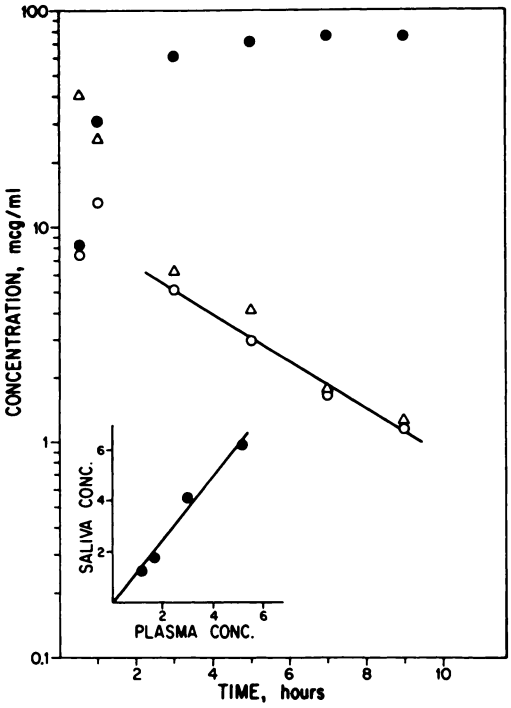

FIG. 2. Time course of acetaminophen concentrations in plasma (○) and saliva (△) and of total metabolite concentrations in plasma (●) of a physiologically anephric patient (No. 2) after an oral dose of 1 g of acetaminophen per 1.73 m^2 of body surface area. The inset shows the relationship between the saliva and plasma acetaminophen concentrations for samples obtained at 3 hours and later.

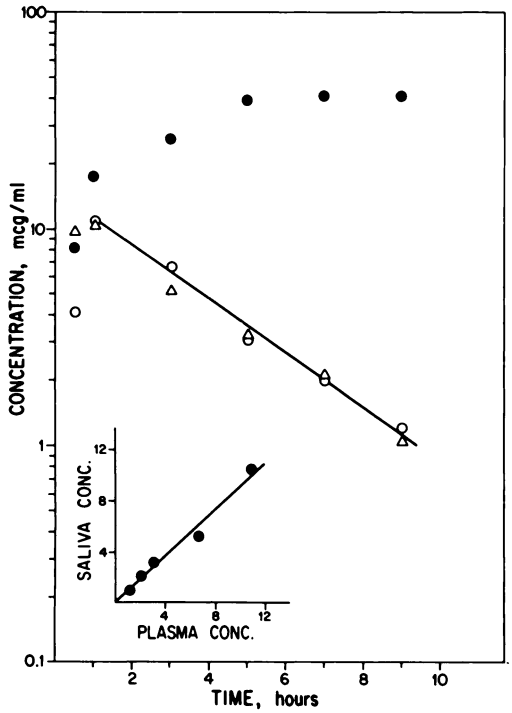

FIG. 3. Time course of acetaminophen concentrations in plasma (O) and saliva (Δ) and of total metabolite concentrations in plasma (●) of a surgically anephric patient (No. 10) after an oral dose of 1 g of acetaminophen per 1.73 m^2 of body surface area. The inset shows the relationship between the saliva and plasma acetaminophen concentrations for samples obtained at 1 hour and later.

liva and plasma (figs. 1-3) except for the first 1 to 3 hours when the concentrations in saliva were relatively much higher due to retention of some of the orally ingested drug in the oral cavity, presumably by adsorption on the mucosa. The mean concentration ratio, saliva/plasma, ranged from 0.91 to 1.30 in eight individuals, with an average ratio (± S.D.) of 1.08 ± 0.16 for the group. No saliva collections were made from three subjects and the saliva from two others produced interfering peaks on gas chromatograms which prevented quantitative analysis.

Equilibrium dialysis of plasma pooled from plasma samples from each of several subjects showed no evidence of plasma protein binding of acetaminophen, acetaminophen glucuronide and acetaminophen sulfate. Subsequent equilibrium dialysis experiments with acetaminophen-spiked plasma revealed 5 to 10% binding when the plasma was equilibrated with phosphate or barbital buffer and no binding when tromethamine buffer was used. Insufficient plasma was available to repeat the dialysis studies with plasma obtained during the pharmacokinetic study. Portions of a composite plasma made up of the remainder of five patients' samples were dialyzed against all three buffer solutions. Dialysis against tromethamine buffer again yielded no evidence of protein binding; dialysis against phosphate and barbi-

TABLE 2

Pharmacokinetic constants for acetaminophen distribution and elimination in physiologically anephric patients

Subject No.	Biologic Half-life	AUC	Maximum Metabolite Concentration in Plasma		V_d/f[a]	Estimated V_M[b]
			Glucuronide	Sulfate		
	min	*(μg/ml) hr*	*μg/ml*		*l/1.73 m²*	*l/1.73 m²*
1	113	32.6	38.1	16.8	83.4	14.6
2	164	43.1	49.1	25.6	91.5	10.7
3	127	31.7	34.5	19.2	96.4	14.9
4	147	51.3	27.0	24.9	68.4	15.4
5[c]	121	34.7	16.7	39.9	83.9	15.0
Mean	134	38.7	33.1	25.3	84.7	14.1
S.D.	21	8.4	12.1	9.0	10.6	1.9

[a] V_d in this and the next two tables is the apparent volume of distribution of acetaminophen.

[b] V_M in this and the next table is the apparent volume of distribution of acetaminophen glucuronide and acetaminophen sulfate (combined).

[c] Mean of two studies.

TABLE 3

Pharmacokinetic constants for acetaminophen distribution and elimination in surgically anephric patients

Subject No.	Biologic Half-life	AUC	Maximum Metabolite Concentration in Plasma		V_d/f	Estimated V_M
			Glucuronide	Sulfate		
	min	*(μg/ml) hr*	*μg/ml*		*l/1.73 m²*	*l/1.73 m²*
6	101	21.1	29.4	9.1	115	20.8
7	137	18.5	26.2	12.6	178	20.6
8	98	27.9	27.2	17.6	84.5	17.8
9	128	70.7	38.6	8.7	43.5	16.9
10	150	47.5	23.2	17.9	75.9	19.4
Mean	123	37.1	28.9	13.2	99.4	19.1
S.D.	23	21.9	5.9	4.4	50.8	1.7

TABLE 4

Pharmacokinetic constants for acetaminophen distribution and elimination in healthy male subjects

Subject No.	Age	Body Surface Area	Biologic Half-life	AUC	Maximum Total Metabolite Concentration in Plasma	V_d/f
	yr	*m²*	*min*	*(μg/ml) hr*	*μg/ml*	*l/1.73 m²*
11	33	1.98	146	47.0	11.8	74.7
12	35	2.18	139	30.3	9.5	110
13	32	1.88	142	47.5	14.4	73.0
Mean			142[a]	41.6	11.9	86.0
S.D.			4	9.8	2.5	21.1

[a] The average biologic half-life of acetaminophen in five published studies on a total of 39 healthy adults ranged from 114 to 132 minutes. The shortest and longest individual half-life values were 90 and 180 minutes, respectively (Levy *et al.*, 1975).

tal buffer solutions indicated between 5 and 10% protein binding of acetaminophen and less than 5% binding of acetaminophen glucuronide and sulfate.

Discussion

In normal subjects, less than 5% of a dose of acetaminophen is usually excreted in the urine while the rest is eliminated largely as the glucuronide and sulfate conjugates (Cummings *et al.*, 1967; Levy and Regårdh, 1971; Levy and Yamada, 1971; Mitchell *et al.*, 1974; Mrochek *et al.*, 1974). A substantial contribution of the kidneys to the formation of either or both of these metabolites may be expected therefore to be reflected by a measurable increase in the biologic half-life of acetaminophen in anephric patients. The results of this investigation reveal no such increase and suggest that the renal contribution to the biotransformation of acetaminophen in man is quantitatively negligible.

The apparent volume of distribution (V_d) of acetaminophen could not be determined directly since the drug was administered orally and its systemic bioavailability (f) is not known. Oral doses of acetaminophen administered as tablets have been quantitatively accounted for in the urine (Mrochek, 1974) so that complete absorption of the drug from solution, the dosage form used in this investigation, may be assumed. Indirect evidence (Levy, 1971) suggests that the first-pass effect of acetaminophen is very small. Theoretical considerations (Gibaldi *et al.*, 1971) indicate that it is about 20%. Thus, V_d/f is probably a reasonable approximation of V_d. The apparent lack of effect of renal failure or

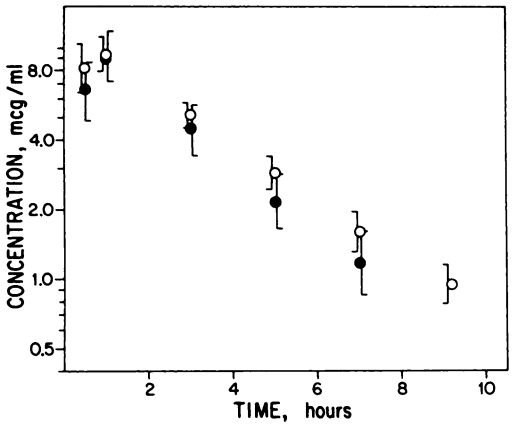

FIG. 4. Time course of the log-averaged plasma concentrations of acetaminophen in three normal subjects (O) and five surgical anephrics (●). The bars indicate one standard deviation in each direction.

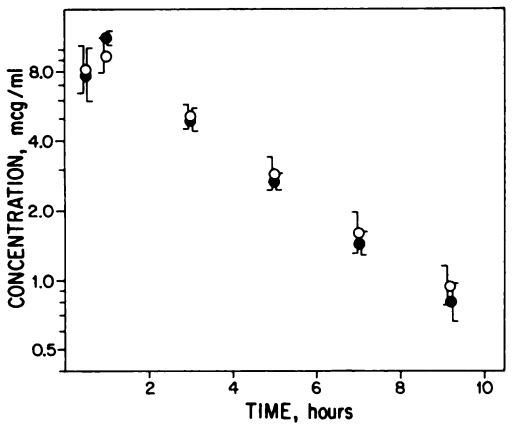

FIG. 5. Time course of the log-averaged plasma concentrations of acetaminophen in three normal subjects (O) and five physiologic anephrics (●). The bars indicate one standard deviation in each direction. The 9 hour value for one of the patients was obtained by extrapolation.

absence of kidneys on V_d/f found in this study is consistent with the negligible protein binding of the drug. The only other available information on the V_d of acetaminophen comes from an unpublished study by Prescott and Wright (1973) who found values of 0.85 ± 0.04 l/kg in healthy subjects who received the drug intravenously. This is somewhat smaller than the V_d/f value obtained in this study (1.49 ± 0.73 l/kg; mean ± S.D., n = 13)[6] but both values are of an order of magnitude consistent with the observation in dogs that acetaminophen concentrations are similar in plasma, liver, kidneys, heart, spleen, lungs, brain and muscle, although much lower in fat (Gwilt *et al.*, 1963).

An apparent volume of distribution of about 70 to 80 liters and a biologic half-life of about 2 hours is equivalent to a total body clearance of less than 0.5 l/min, *i.e.*, considerably less than the normal plasma flow rate through the liver of an adult (about 1 l/min). The metabolic clearance of acetaminophen is therefore not hepatic blood flow limited and the increased hepatic plasma flow rate in anephric patients due to their higher cardiac output (Corvol, 1974) should not affect the elimination kinetics of acetaminophen. For the same reason, a loss of drug-metabolizing tissues (such as the kidneys) should be evident as a decrease in biologic half-life of acetaminophen if the contribution of these tissues is substantial.

The one significant difference between normal subjects and anephric patients noted in this investigation is the pronounced accumulation of acetaminophen metabolites in the plasma of the anephric patients. Similar observations were made by Dubach (1968) in patients with decreased renal function after administration of phenacetin. There was no evidence of elimination of these metabolites during the 9-hour experiment and even during 24 hours in the one patient studied. Since acetaminophen is more than 90% metabolized in 9 hours, and since there appears to be little or no elimination of the metabolites during this period of time, an *estimate* of the apparent volume of distribution of acetaminophen metabolites (V_M) can be obtained by dividing 80% of the dose of acetaminophen by the maximum metabolites concentration in the plasma. The available evidence from normal subjects indicates that approximately 80% of the dose of acetaminophen is converted to the glucuronide and sulfate. Unlike the V_d for acetaminophen calculation, the estimation of V_M does not require consideration of a possible first-pass effect since an oral dose of acetaminophen can be totally recovered in the urine in the form of unchanged drug and various metabolites (Mrochek *et al.*, 1974).

V_M, as determined in this study, is a weighted average of the apparent volumes of distribution of acetaminophen glucuronide and acetaminophen sulfate. The reported V_M values are based on the assumption that 80% of the dose of acetaminophen is converted to the glucuronide

[6] These results are affected by an unusually high value (3.73 l/kg) for subject 7; deletion of this value results in an average ± S.D. of 1.30 ± 0.29 l/kg. If the value of f is about 0.8, then V_d is about 1 l/kg, i.e., similar to that reported by Prescott and Wright (1973).

and sulfate and that the elimination of these metabolites is negligible during the first 9 hours. These assumptions introduce an uncertainty of about ± 10% in the estimations. An even greater uncertainty would be introduced if apparent volumes of distribution had been estimated separately for each of the two conjugates since the reported values for the glucuronide and sulfate fractions are quite variable. The following data are offered therefore strictly as a rough approximation: based on the average maximum concentrations of acetaminophen glucuronide (31.0 ± 0.2 μg/ml, mean ± S.D.) and acetaminophen sulfate (19.2 ± 9.2 μg/ml) in the plasma of the anephric patients, and assuming 55% of the dose to be converted to the glucuronide and 25% to the sulfate, the respective average apparent volumes of distribution are 18 and 13 l/1.73 m^2.

The statistically significant ($P < .005$) difference between the V_M values obtained in surgically and physiologically anephric patients (tables 2 and 3) may be an artifact. Blood sampling was stopped at about 7 hours in four of the five surgically anephrics while the last blood sample in all but one of the physiologically anephric patients was obtained at 9 hours. Estimation of V_M based on the 7-hour plasma concentrations in both groups yield values of 18.8 l/1.73 m^2 and 15.1 l/1.73 m^2 for the surgically and physiologically anephric patients, respectively, with the difference being not statistically significant ($P > .05$). In any event, the lower mean V_M values in the physiologically anephric patients are due almost entirely to their higher maximum acetaminophen sulfate concentrations (tables 2 and 3), which differ significantly ($P < .05$) from those in the surgical anephrics.

The available data do not permit calculation of V_M for the normal subjects but they do show that, just as in the anephric patients, V_M is considerably smaller than the V_d for acetaminophen since the maximum concentration of the acetaminophen conjugates in plasma equalled or exceeded the maximum concentration of acetaminophen itself (for example, fig. 1). Considering the fact that only about 80% of the dose of acetaminophen is converted to the glucuronide and sulfate, and that this conversion proceeds slowly, maximum metabolite concentrations in plasma should be lower than maximum acetaminophen concentrations if the respective volumes of distributions are similar.

As already observed by others in normal subjects (Glynn and Bastain, 1973), there is an excellent linear correlation between the concentration of acetaminophen in plasma and saliva (figs. 1 to 3), except for the first 1 to 3 hours after oral ingestion of the drug when some of the ingested acetaminophen remaining in the oral cavity causes unusually high concentrations in the saliva. The saliva/plasma concentration ratio is about unity, and there is no apparent difference in this ratio between normal subjects (Glynn and Bastain, 1973) and anephric patients (table 5).

The results of the equilibrium dialysis study (table 6) indicate no (< 5%) plasma protein binding of acetaminophen, its glucuronide or its sulfate. Gazzard *et al.* (1973) observed some, but very little (< 10%) binding of acetaminophen in spiked plasma at concentrations below 100 μg/ml, and less than 5% binding at concentrations below 90 μg/ml in plasma from patients who had taken the drug. Since it was suspected that the tromethamine buffer used in our study may have had a displacing effect (Crooks and Brown, 1973), additional studies were carried out with phosphate and barbital buffers. The results of these studies differed very little from those obtained in the initial dialysis study.

The lack of appreciable plasma protein binding of acetaminophen and its glucuronide and sulfate conjugates is of interest in that it rules out differences in plasma protein binding as a possible reason for the pronounced differences in their respective apparent volumes of distribution. It may be that the smaller volume of distribution of acetaminophen glucuronide and sulfate is due to poor penetration of these very polar compounds through lipoid barriers and a consequent restriction of their distribution space in the body.

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