

Pharmacokinetics of Drugs in Patients with the Nephrotic Syndrome

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ABSTRACT Since the binding of drugs to plasma proteins can significantly alter the intensity of pharmacological and toxicological effects of drugs, we studied the pharmacokinetics of three drugs in patients with hypoalbuminemia secondary to the nephrotic syndrome, but with relatively normal renal function. No significant differences were seen in the pharmacokinetic parameters observed for antipyrine, a drug which is less than 10% bound to plasma proteins. The percentage of unbound diphenylhydantoin, a highly plasma protein-bound drug, was found in patients with the nephrotic syndrome to be twice that of healthy individuals (19.2 vs. 10.1%, $P < 0.001$). However, there was also a lower steady-state plasma concentration of diphenylhydantoin (2.9 ± 0.6 vs. 6.8 ± 0.6 $\mu\text{g/ml}$, $P < 0.001$) secondary to an increase in the plasma clearance (0.048 ± 0.019 vs. 0.022 ± 0.006 liter/kg·h, $P < 0.001$) in the nephrotic patients. The net effect is no difference in the absolute concentration of unbound diphenylhydantoin in healthy individuals (0.69 ± 0.05 $\mu\text{g/ml}$) and patients with the nephrotic syndrome (0.59 ± 0.06 $\mu\text{g/ml}$). Qualitatively, similar differences were observed with clofibrate. The dose of these drugs need not be routinely reduced in patients with the nephrotic syndrome as long as they have reasonably normal renal function (creatinine clearance greater than 50 ml/min). With all highly bound acidic drugs, knowledge of the concen-

tration of unbound drug is essential to the proper interpretation of total blood levels and subsequent treatment of the patient.

INTRODUCTION

Binding of drugs to plasma proteins can profoundly influence their pharmacodynamic or toxicological actions, as well as their disposition (1). Minor interindividual changes in the degree of binding of highly protein-bound drugs can produce significant changes in the amount of unbound drug; abnormal binding in disease states such as uremia (2, 3) and cirrhosis of the liver (4) may lead to even higher levels of unbound drug. The binding of diphenylhydantoin (DPH)¹ was observed to decrease as the concentration of albumin, which binds many drugs, was reduced in in vitro studies with diluted plasma (5). The effects of reduced binding secondary to low albumin concentrations in vivo on pharmacokinetics of drugs have not been studied extensively.

Elevated concentrations of unbound drug secondary to hypoalbuminemia may be associated with increased toxicity. For example, Bridgman, Rusen, and Thorp (6) reported that five out of six patients with the nephrotic syndrome developed adverse effects to clofibrate when treated for the associated hyperlipoproteinemia. The Boston Collaborative Drug Surveillance Program (7) reported an increased incidence of toxicity from DPH in patients with hypoalbuminemia and suggested that these patients have increased circulating levels of unbound DPH.

¹ *Abbreviations used in this paper:* app V_d , apparent volume of distribution; CPIB, chlorophenoxyisobutyric acid; DPH, diphenylhydantoin; HPPH, *p*-hydroxyphenylphenylhydantoin; K_{e1} , elimination rate constant; 4-OH A, 4-hydroxyantipyrine.

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The present work was designed to study the pharmacokinetics and metabolism of two highly protein-bound drugs, DPH and clofibrate, and of antipyrine, which is not significantly bound to plasma proteins, in patients with the nephrotic syndrome.

METHODS

Subjects

12 patients (9 men and 3 women) with the nephrotic syndrome and 18 healthy volunteers (14 men and 4 women) were studied (Table I). Their ages ranged between 20 and 58 yr. Before entering the study, each subject had a complete history, physical examination, and laboratory tests which included complete blood count, urinalysis, plasma protein and protein electrophoresis, protein content of 24-h urine, creatinine clearance, bilirubin, alkaline phosphatase, and serum glutamic oxaloacetic transaminase. Written, informed consent was obtained from each volunteer after an explanation of the risks, hazards, and inconveniences reasonably to be expected.

The patients were not selected according to the etiology of their nephrotic disease; only patients with an albumin concentration in plasma equal to or less than 3 g/dl and

excretion of protein in urine greater than 3 g/24 h were accepted. Individuals with creatinine clearance less than 50 ml/min or evidence of liver function impairment were arbitrarily excluded. To prevent interference with necessary treatment, patients were not taken off their current medication. Thus, patients on diuretics were accepted, but the above criteria excluded patients with marked peripheral edema. The control group was selected to match the ages of the patients with nephrosis.

Study design

10 of the 12 nephrotic patients initially received antipyrine as a single dose. Subsequently, six were given DPH and four clofibrate for 14 days. Two patients received chronic clofibrate treatment only. All patients of the DPH group in addition received a single intravenous dose of DPH at least 2 wk after completion of the chronic treatment period. Control subjects were tested in three groups of six with one drug only; those on DPH also received the intravenous dose. A small breakfast was allowed 2 h before the single-dose studies.

Antipyrine. 10 mg antipyrine/kg was given orally dissolved in 250 ml water after an overnight fast. 10-ml blood samples were obtained at 0, 3, 6, 9, 12, and 24 h after the dose. All urine excreted was collected in fractions

TABLE I
Weight, Concomitant Drug Therapy, Drug Studied, and Clinical Laboratory Data for the Volunteers in This Study

Patients	Body weight	Concomitant therapy	Plasma proteins	Plasma albumin	Creatinine clearance	Protein excretion in 24-h urine
	kg		g/dl	g/dl	ml/min	g
DPH						
1	72	Prednisone, 15 mg q.o.d.	6.4	2.05	85	3.7
2	64	Furosemide, 50 mg; cyclophosphamide, 50 mg	5.8	2.9	108	3.0
3	85	Furosemide, 40 mg; digitoxin, 0.05 mg	6.5	2.3	52	17.1
4	76	Spironolactone, 100 mg	6.4	2.5	63	6.3
5	78	—	5.7	2.6	101	5.5
6	63	Cyclophosphamide, 75 mg; prednisone, 15 mg	4.6	2.2	93	8.4
Clofibrate						
7	74	—	5.0	2.75	61	12.4
8	57	Digoxin, 0.25 mg	6.8	2.5	112	5.9
9	49	Spironolactone, 50 mg; cyclophosphamide, 100 mg	5.5	2.3	83	10.4
10	53	Furosemide, 60 mg	3.9	1.67	68	7.6
11	97	—	6.1	3.0	76	4.2
12	104	Spironolactone, 75 mg	2.9	1.2	88	6.4
All patients						
Mean	72.8	—	5.5	2.33	82.5	7.3
SE	4.7	—	0.3	0.14	5.3	1.2
Control subject (n = 18)						
Mean	70.7	—	7.1	3.5	101.0	—
SE	3.1	—	0.16	0.11	6.8	—

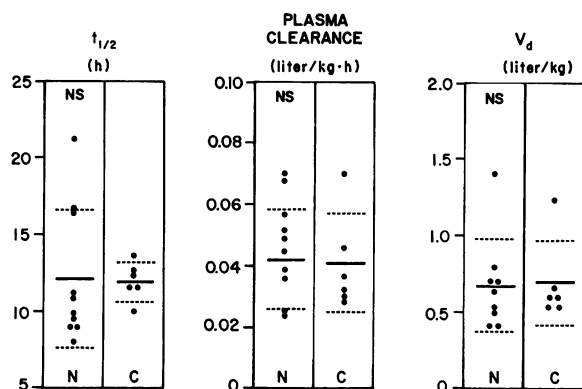


FIGURE 1 Pharmacokinetic parameters of antipyrine in healthy individuals (C) and patients with the nephrotic syndrome (N). V_d , apparent volume of distribution. NS, not significant.

over 0-12, 12-24, and 24-48 h. Plasma and urine were assayed for antipyrine and urine for 4-hydroxyantipyrine (4-OH A), the major metabolite.

DPH. Three 100-mg capsules (Dilantin®, Parke, Davis & Co., Detroit, Mich.) were given orally daily for 14 days. 3-ml blood samples were obtained the last 3 days just before the dose and then 4, 8, 12, 24, 30, 36, 48, and 72 h after the final dose. 250 mg DPH was administered on a separate occasion by infusion over 5 min. Blood samples were obtained 10, 20, 30, 45, 60, 90 min and 2, 3, 4, 8, 12, 24, 36, and 48 h after the infusion.

Urine was collected in 24-h aliquots the final 2 days during chronic dosing and in 0-6, 6-12, 12-24, and 24-48-h fractions after the intravenous dose. Urine and blood were assayed for DPH, urine also for *p*-hydroxyphenylphenylhydantoin (HPPH).

Clofibrate. 1 g clofibrate (Atromid-S®, Ayerst Laboratories, New York) was given twice daily for 14 days. 10-ml blood samples were obtained just before the morning dose on days 13 and 14 and 4, 8, 12, 24, 30, 36, and 48 h after the final dose. Two 12-h urine samples were collected on day 13. All samples were assayed for chlorophenoxyisobutyric acid (CPIB), and urine for CPIB glucuronide in addition.

Analytical procedures

Antipyrine and 4-OH A. Antipyrine was measured by a gas chromatographic method (8) with phenacetin (9) as an internal standard. 4-OH A was measured as the silylated derivative, also by gas chromatography (8).

DPH and HPPH. DPH was assayed by a recently developed, very sensitive and highly specific radioimmunoassay (10).³ HPPH was measured by a slight modification of the method of Atkinson, MacGee, Strong, Garteiz, and Gaffney after methylation with tetramethyl ammonium hydroxide (11).

CPIB and CPIB glucuronide. During absorption and in the plasma clofibrate (ethyl chlorophenoxyisobutyrate) is rapidly hydrolyzed to CPIB. The latter was estimated by a method developed in this laboratory. To 1 ml of plasma or

urine, 1 ml of 0.5 N HCl was added and the CPIB extracted into 8 ml of chloroform. After shaking and centrifuging the sample, the aqueous layer was aspirated and 7 ml of the organic layer was transferred to another tube and evaporated to dryness. The residue was dissolved in 1 ml of freshly prepared 0.5% potassium carbonate in methanol (wt/vol) and 0.1 ml dimethyl sulfate added. Methylation was achieved by heating the mixture for 10 min in a water bath at 70°C. After addition of 1 ml 0.2 M acetate buffer, pH 5.6, the CPIB was extracted into 8 ml of chloroform containing 1 µg clofibrate/ml as an internal standard. After shaking, centrifugation, and aspiration of the aqueous phase, 7 ml of the organic phase was transferred into conical tubes and evaporated under a gentle stream of air to a volume of 0.1-0.2 ml. Evaporation should not be done in a water bath even at room temperature since the cooling temperature produced during evaporation is essential to prevent hydrolysis of clofibrate. Gas chromatographic analysis was performed by injecting 1-3 µl onto a 6-ft column packed with 3% SE30 on G.A.S.-Chrom Q (80-100 mesh, Applied Science Labs, Inc., State College, Pa.) in a Barber-Colman 5000 gas chromatograph (Barber-Colman Company, Rockford, Ill.). The temperature of the oven was 185°C, the injection port 240°C, and the flame ionization detector 270°C. Retention times of CPIB and clofibrate were 2.0 and 2.5 min, respectively. Concentrations as low as 3 µg/ml were easily detectable. Recovery of CPIB was 94±2%.

CPIB glucuronide was measured as CPIB after enzymatic hydrolysis of urine samples diluted 1:10 with 0.1 M acetate buffer, pH 5.0. 0.1 ml (20,000 Fishman U) β-glucuronidase-sulfatase solution from *Helix pomatia* (Sigma Chemical Co., St. Louis, Mo.) was added to 4 ml diluted urine and incubated for 12 h at 37°C.

During validation of our analytical procedures we noted poor recovery due to inadequate hydrolysis of all the above metabolites added to the urine of nephrotic patients. If the urine were boiled for 5 min and the resulting precipitate removed by centrifugation, recovery of the metabolites in the supernate was essentially complete.

Protein binding. Protein binding was determined by equilibrium dialysis (3). 2 ml of plasma from a blood sample obtained 4 h after the final dose of either DPH or clofibrate was dialyzed over 16 h at 37°C against 10 ml of a 0.05 M phosphate buffer, pH 7.4. Equilibration of DPH and clofibrate was attained by 12-16 h.

Pharmacokinetics. The plasma elimination half-life ($t_{1/2}$) was calculated from the linear portion of the log concentration-time curve obtained by the least-squares method starting at 6 h (antipyrine), 24 h (DPH), and 12 h (CPIB) after the last dose. Plasma clearance was obtained by dividing the dose by the area under the plasma concentration-time curve calculated by the trapezoidal rule. Apparent volume of distribution (app V_d) was calculated for antipyrine, assuming complete absorption, by the relationship: app V_d = plasma clearance/elimination rate constant (K_{e1}). App V_d for DPH was calculated by assuming a two-compartment (12) instead of a one-compartment open model as for antipyrine.

RESULTS

The relevant laboratory data and concomitant drug therapy in the nephrotic patients and healthy controls are recorded in Table I. Although the albumin concentration of every patient was less than 3 g/dl only two showed concentrations below 2 g/dl. Thus, the patient

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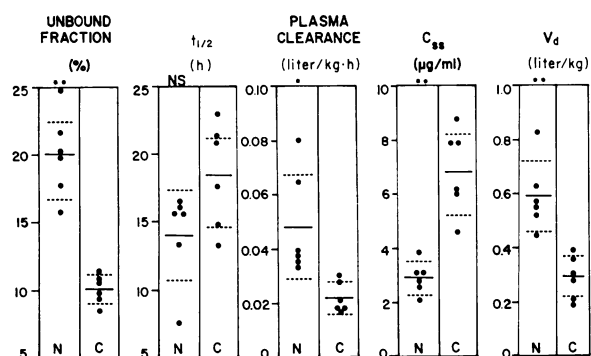


FIGURE 2 Pharmacokinetic parameters of DPH in healthy individuals (C) and patients with the nephrotic syndrome (N). V_d , apparent volume of distribution. C_{ss} , steady-state plasma concentration. NS, not significant. * $P < 0.005$. ** $P < 0.001$.

group mainly represents moderate rather than severe hypoalbuminemia.

Antipyrine. There was no significant difference in the $t_{1/2}$ of antipyrine between the control group and patients with nephrosis, although the variation was considerably greater among the nephrotic patients (Fig. 1). The plasma clearances and app V_d also did not vary. Complete absorption of antipyrine was assumed in calculating these parameters. The 48-h excretion of antipyrine (3.8 ± 1.3 vs. $3.9 \pm 1.5\%$ of dose) and 4-OH A (41.2 ± 8.0 vs. 36.4 ± 7.0) in urine was similar in the nephrotic patients and control.

Diphenylhydantoin. The pharmacokinetic parameters obtained for steady-state and single intravenous dose administration of DPH are summarized in Fig. 2. Protein binding was significantly reduced ($P < 0.001$) from a mean of 89.9 to 80.8%, resulting in a doubling of the fraction of unbound drug. However, the concentration of unbound DPH was not different in the nephrotic patients (0.59 ± 0.06 $\mu\text{g/ml}$) and controls (0.69 ± 0.05 $\mu\text{g/ml}$). An inverse correlation between the albumin concentration in plasma and the percentage of unbound drug was found for the nephrotic patients and the controls (Fig. 3), the correlation coefficient being 0.96 ($P < 0.01$).

The $t_{1/2}$ of DPH was decreased in nephrotic patients, but this difference failed to reach statistical significance ($t = 2.19$; $\nu = 10$), due in part to a large variation between individuals. The plasma clearance, obtained from the single intravenous studies, of 0.048 ± 0.019 in the nephrotic patients compared to 0.022 ± 0.006 liters/kg·h in controls was significantly different ($P < 0.005$).

The steady-state plasma concentration following treatment with 300 mg of DPH for 14 days was 2.9 ± 0.6 $\mu\text{g/ml}$ in the patients, whereas control subjects had 6.8 ± 1.6 $\mu\text{g/ml}$. The app V_d of DPH was significantly

increased in nephrotic patients. If we assume the percentage of unbound drug in the sample obtained 4 h after the dose remains constant at other plasma levels, the app V_d for unbound drug can be estimated. The app V_d of unbound DPH (calculated as a fraction of the bound drug) was similar in nephrotic patients (3.4 ± 0.62 liters/kg) and controls (2.96 ± 0.67 liters/kg).

The excretion of DPH and HPPH in urine during steady state and of HPPH during the 48 h following a single intravenous dose is shown in Table II. No significant differences were found between nephrotic patients and controls in the excretion of DPH or HPPH in urine.

Clofibrate. The pharmacokinetic parameters determined after chronic administration of clofibrate are recorded in Fig. 4. Plasma protein binding was reduced from 96.4 to 88.8% in nephrotic patients, a change qualitatively similar to that observed with DPH. The $t_{1/2}$ of CPIB was considerably shortened in nephrotic patients to as low as 5.2 h in one patient in whom protein binding was reduced to 83%. No data were obtained for plasma clearance and app V_d of CPIB since single-intravenous dose studies could not be performed due to a lack of an intravenous dosage form of clofibrate.

The steady-state plasma concentration of CPIB was lower in nephrotic patients; however, the concentration of unbound drug was similar in both groups (5.1 ± 0.6 vs. 4.7 ± 0.5 $\mu\text{g/ml}$). In normal volunteers receiving

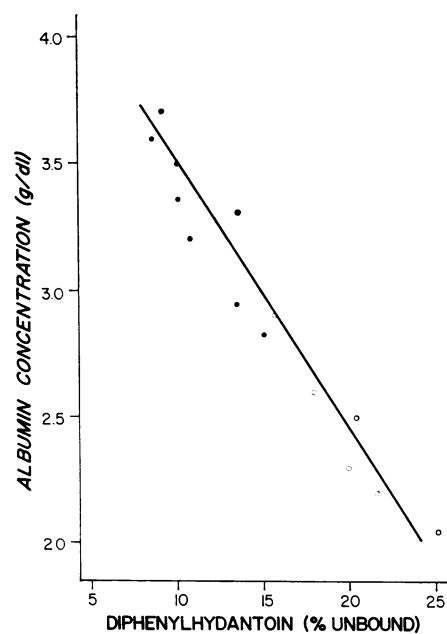


FIGURE 3 Relationship between plasma albumin concentration and unbound fraction of DPH. Each point represents values obtained in healthy individuals (●) and patients with the nephrotic syndrome (○) receiving 300 mg DPH for 14 days.

TABLE II
Excretion of DPH and HPPH in the Urine of Healthy Individuals (Control) and Patients with the Nephrotic Syndrome after Administration of 300 mg DPH Daily

	DPH		HPPH			
	Nephrotic	Control	Nephrotic	Control	Nephrotic	Control
	mg/24 h*		mg/24 h*		mg/48 h†	
	1.4	2.9	171	168	123	107
	2.4	1.2	144	168	98	100
	1.7	1.4	156	282	116	139
	0.9	1.6	201	248	151	154
	3.2	2.3	226	222	151	135
	5.5	2.2	151	228	144	120
Mean	2.5	1.9	175	219	129	126
SD	1.7	0.6	32	45	21	21
	NS		NS		NS	

NS, not statistically significant.

* Chronic dosing studies.

† Single-dose study.

1 g clofibrate twice daily, an inverse correlation was found between body weight and the steady-state plasma concentration (Fig. 5). The steady-state concentration of CPIB in several hyperlipoproteinemic patients from our clinic on the same dose is also included in the figure. Similar parameters of the nephrotic patients were clearly below the regression line (Fig. 5). When viewed as individual patients in this manner, the pronounced difference is more obvious than from the cumulated data.

The excretion of CPIB and CPIB glucuronide in urine is recorded in Table III. No significant difference was found between nephrotic patients and the controls in the excretion in urine of either CPIB, CPIB

glucuronide, or CPIB/CPIB glucuronide ratio in the steady state.

DISCUSSION

It is well recognized that in uremic patients DPH steady-state concentrations are lower than in normal volunteers (2, 13, 14). The decreased binding in this disease appears to be related to inherent alteration in the plasma proteins (3) whereas a change in the protein concentration per se seems to be of minor importance (2). Therefore, we deemed it necessary to study nephrotic patients without uremia to exclude possible

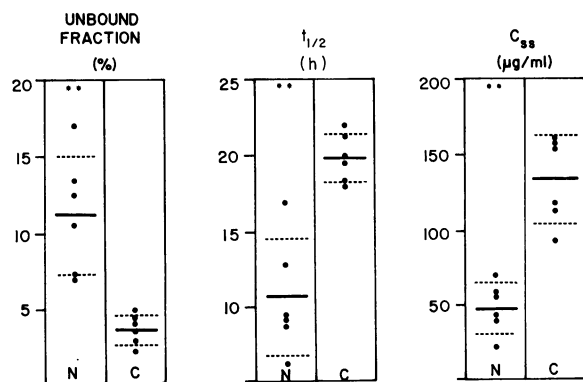


FIGURE 4 Pharmacokinetic parameters of clofibrate in healthy individuals (C) and patients with the nephrotic syndrome (N). C_{ss} , steady-state plasma concentration. ** $P < 0.001$.

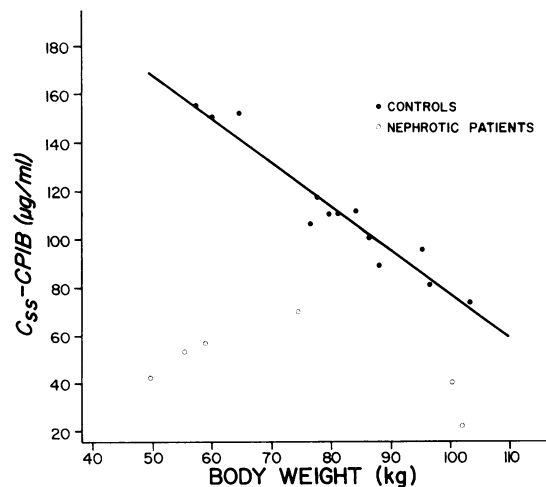


FIGURE 5 Relationship between body weight and steady-state plasma concentration of clofibrate in healthy individuals (●) and patients (○).

changes in protein binding and metabolism caused by severe renal failure. We arbitrarily excluded individuals with a creatinine clearance less than 50 ml/min.

The majority of the patients studied were also receiving additional drug therapy, mostly diuretics (Table I). Of the drugs given, however, only spironolactone is known to be an agent that induces hepatic microsomal drug oxidation (15). Furosemide is considerably bound to plasma proteins and some displacement of DPH and CPIB from their binding sites by this drug cannot be excluded, although a significant effect is rather unlikely considering the doses used and the short half-life of furosemide (16). In addition, we have found no displacement of DPH (6 µg/ml) from protein binding sites in the presence of furosemide (6 µg/ml).

Antipyrine, a drug less than 10% bound to plasma proteins, is handled by nephrotic patients in a manner similar to that observed in healthy individuals. From the data obtained it may be generalized that the overall ability of these patients to metabolize drugs is not altered by their disease. The changes observed with DPH and CPIB are secondary to alterations in protein binding and will be discussed in detail below. A lack of increase in the app V_a of antipyrine indicates the total body water to be unchanged (17), confirming our clinical evaluation that no appreciable edema was present in the patients at the time of the investigation.

Protein binding of DPH and CPIB was reduced consistently in nephrotic patients. This is not an unexpected finding, since Lunde, Rane, Yaffe, Lund, and Sjöqvist (5) showed by *in vitro* studies that dilution of plasma (albumin) reduces the binding of DPH. In the present study a good inverse correlation was found

between the patient's plasma albumin concentration and the percentage of unbound DPH. For clinical purposes, by using the data in Fig. 3, complicated techniques to measure protein binding can be avoided and the percentage of unbound DPH determined for every albumin concentration. As a rule of thumb, for every 0.1 g/dl decrease in plasma albumin, the unbound DPH concentration increases 1%.

The percentage of unbound DPH in the nephrotic patients was about twice that found in normal subjects and the plasma total DPH concentration at steady state was an average of 50% lower. Thus, identical concentrations of unbound DPH were found in nephrotic patients and controls. Similar results were obtained with CPIB, the difference being only quantitative. A three-fold increase in the unbound fraction was observed in the patients receiving clofibrate which was compensated for by a reduction in the steady-state level to almost one-third of the normal concentration. No difference in binding of DPH was observed when equal concentrations of albumin from plasma of normal and nephrotic patients were studied.

The decreased steady-state concentration in nephrotic patients receiving DPH appears to be due to a consistent increase in app V_a , the K_{e1} being higher although not statistically significant due to the large interpatient variation. In patients receiving clofibrate, the increased rate of elimination appears to be a major mechanism responsible for the decreased steady-state levels. Since an intravenous dosage form is not available, the contribution of changes in app V_a could not be ascertained.

TABLE III
Excretion of CPIB and CPIB Glucuronide in the Urine of Healthy Individuals (Control) and Patients with the Nephrotic Syndrome after Administration of 1.0 g Clofibrate Every 12 h for 2 wk

	CPIB		CPIB glucuronide		CPIB CPIB glucuronide	
	Nephrotic	Control	Nephrotic	Control	Nephrotic	Control
	<i>mg/12 h</i>		<i>mg/12 h</i>			
	221	83	879	273	0.25	0.30
	65	99	525	961	0.12	0.10
	87	92	612	614	0.14	0.15
	102	289	252	421	0.40	0.68
	73	162	443	621	0.16	0.26
	127	189	630	518	0.20	0.36
Mean	113	152	557	568	0.20	0.31
SD	58	79	209	233	0.11	0.23
	NS		NS		NS	

NS, not statistically significant.

The excretion of antipyrine, DPH, and CPIB in urine did not vary in nephrotic patients from the controls. These drugs, however, are extensively metabolized in man. It would be of interest to study a drug which is highly protein bound and excreted primarily unchanged to determine whether loss of large amounts of protein in urine increases the excretion of the drug in the urine.

Since the concentration of unbound drug in nephrotic patients is not different from that found in healthy subjects, modification of the dose of highly bound acidic drugs such as DPH and clofibrate is not necessary. This statement seems to be in contradiction to the reports on increased toxicity from DPH in patients with low albumin concentration (7) and from clofibrate in patients with the nephrotic syndrome (6). The Boston Collaborative Drug Surveillance Program study (7) includes all hypoalbuminemic patients without regard for the underlying disease. Although the authors state there was no evidence the results were due to a preponderance of patients with chronic liver disease, the numbers in each group were rather small. If the liver disease is significant, the majority of these individuals would have impairment of drug metabolism with resulting high rather than low steady-state drug levels. Similarly, all but one of the nephrotic patients described by Bridgman et al. (6) had a severe reduction in renal function with serum creatinine concentrations higher than 4 mg/dl. We find that uremic patients (creatinine clearance < 20 ml/min) have a decreased K_{e1} of CPIB caused by a reduction in the rate of metabolism of the drug.³ The toxicity seen in nephrotic patients with severely compromised renal function is, therefore, most likely due to an increased concentration of unbound CPIB secondary to one or more of three abnormalities: (a) decreased levels of albumin; (b) increased concentrations of unbound acidic drugs associated with the abnormal albumin from uremic patients; and (c) decreased K_{e1} producing elevated total CPIB levels in plasma.

In those nephrotic individuals with impaired renal function (creatinine clearance < 20 ml/min) the dose of clofibrate should be adjusted downward whereas no change is necessary in those with relatively normal renal function. The nephrotic syndrome is associated with hyperlipidemia which appears to be significantly related to the increased incidence of the coronary heart disease observed in patients with this disorder (18). Therefore, our observations take on even greater importance in view of the tendency of physicians to treat the hyperlipidemia of the nephrotic syndrome with clofibrate.

³ Gugler, R., C. V. Manion, and D. L. Azarnoff. Unpublished observations.

It is axiomatic among pharmacologists that both effect and toxicity are better related to the level of unbound drug in plasma than to total level, dose, or body burden. Proof of this concept has been obtained for sulfonamides (19). Although in individuals with normal protein binding the concentration of total DPH in plasma correlates with antiepileptic activity, adequate effect has been reported in newborns and uremic individuals at very low levels and is thought due to an increased concentration of unbound DPH (20). The adverse effects of DPH similarly correlate best with the level of unbound DPH (21). With all highly bound acidic drugs, knowledge of the concentration of unbound drug is essential to the proper interpretation of total blood levels and subsequent treatment of the patient.

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