Table V—Distribution of Polychlorinated Biphenyl Mixture Components in Swine Tissues

Component	Mean Concentration, ppm Relative to Standard						Percent Total Polychlorinated Biphenyl						
	Fat	Liver	Kidney	Muscle	Spleen	Blood	Fat	Liver	Kidney	Muscle	Spleen	Blood	Standard
70	0.96	2.57	0.26	0.99	0.34	0.04	7.9	24.8	11.5	20.3	8.2	5.05	16.1
83	3.81	8.08	2.26	4.93	4.27	0.73	15.9	39.6	51.0	51.6	52.2	47.0	8.2
85	0.00	2.63	0.00	0.00	0.00	0.31	0.0	12.9	0.0	0.0	0.0	20.0	8.2
99	3.11	1.27	0.82	0.98	1.49	0.20	16.0	7.7	22.8	12.6	22.4	15.9	10.1
105	0.00	0.00	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0	0.0	0.0	10.1
127	2.12	0.76	0.07	0.16	0.12	0.02	18.0	7.6	3.2	3.4	3.0	2.6	16.7
149	2.06	ND	0.08	0.17	0.19	0.02	14.4	ND[a]	3.0	3.0	3.9	2.2	13.7
176	3.61	0.35	0.11	0.19	0.15	0.04	15.1	1.7	2.5	2.0	1.8	2.6	8.2
208	3.56	0.23	0.13	0.27	0.30	0.02	3.8	0.3	0.8	0.7	0.9	0.3	2.1
253	3.47	0.16	0.09	0.23	0.32	0.02	3.4	0.2	0.5	0.6	0.9	0.3	1.9
286	4.79	1.44	0.43	2.43	1.63	0.04	1.5	0.5	0.7	1.9	1.5	0.2	0.6
332	5.36	12.93	ND	ND	8.04	0.03	0.3	0.8	ND	ND	1.2	Trace	0.1
Total	1.66	1.59	0.34	0.78	0.58	0.12							

[a] Not determined due to interference.

tissues above the relative levels present in the mixture administered. Otherwise, the peaks of longer relative retention time (more highly chlorinated) accumulated in fat while blood carried the peaks of shorter relative retention time. Other tissues were intermediate, except that the liver maintained high levels of component 70, which is rapidly distributed or eliminated from swine and sheep fat and blood (1, 2, 4, 5); the level of peak 253, which is rapidly eliminated by fish (13), was lower in the liver than in any other tissue.

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Renal Function Testing: Differentiation between a Nephrotoxic Agent and Diuretic Drugs

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Abstract □ Preliminary studies indicate that it may be possible to differentiate the effects of a nephrotoxic substance from those of diuretic agents by the measurement of both urine and plasma osmolality. The nephrotoxic substance, mercuric chloride, decreases urinary osmolality and increases plasma or serum osmolality. The diuretic agents, at exceedingly high dosages, may show a dose-related decrease in urine osmolality. However, serum osmolality either remains unchanged or is only slightly lowered. This difference in the serum response of animals treated with a nephrotoxin or diuretic agents may allow for the differentiation in toxicological studies.

Keyphrases □ Osmolality, urine and plasma—effects of nephrotoxic and diuretic agents compared, dogs □ Renal function testing—differentiation between nephrotoxic and diuretic agents by urine and plasma osmolality measurements, dogs □ Nephrotoxic agents—mercuric chloride, effect on urine and plasma osmolality compared to various diuretic agents, dogs □ Diuretics, various—effects on urine and plasma osmolality compared to nephrotoxic agent, dogs □ Mercuric chloride—effect on urine and plasma osmolality compared to various diuretic agents, dogs

The evaluation of nephrotoxicity in safety evaluation studies has always posed a problem to the toxicologist. The need to determine histopathological changes in the urinary bladder prohibits catheterization because of possible irritant effects. The clinical evaluation of nephrotoxicity evolves about the determination of blood (serum) urea

Table I—Drug Dosage for 3 Days

Compound	Milligrams per Kilogram per Day
Hydrochlorothiazide	12.5, 25, 50
Chlorothiazide	125, 250, 500
Acetazolamide	10, 20, 40
Ethacrynic acid	2.5, 5.0, 10.0[a]

[a] Administered as two 5-mg/kg successive doses.

nitrogen and urine analytical studies. Osmolality measurements have been used extensively in clinical situations to assess renal function. The concept, physiology, and clinical application of this procedure were described previously (1).

One of the most easily studied functions of the kidney is its capacity to conserve water under dehydration conditions. In these circumstances, water is reabsorbed from the tubular fluid against an osmotic gradient through the influence of endogenous antidiuretic hormones, and the maximum concentration of the solutes is used as an indication of the kidney's capacity to perform osmotic work (2).

Low protein diets and habitual consumption of large quantities of fluid may result in the excretion of urine of low specific gravity or inhibit the concentrating ability of the kidneys. Since diuretics also can reduce the renal concentrating capacity, preliminary studies were undertaken to determine if osmolality measurements of urine and serum can differentiate between pharmacodynamic and nephrotoxic effects of chemical agents. This report presents the initial findings.

EXPERIMENTAL

Eighteen purebred male and female beagle dogs were individually housed in metabolism cages in air-conditioned and humidity-controlled quarters and were maintained on a standard laboratory chow. Osmolality measurements were made according to the following procedures. At approximately 1 pm on the test day, all food and water were withdrawn from the cages. At 4:30 pm, a clean urine-collecting vessel was placed beneath the cage. A filter was positioned above the vessel to prevent contamination of the urine with feces, hair, *etc.*

Urine was collected from each animal until 8:30 am the following morning, at which time aliquots were taken for the determination of osmolality. Blood, with or without anticoagulant, was obtained from representative dogs by venipuncture. Following centrifugation, 2 ml of plasma or serum was used for the determination of osmolality. Osmolality measurements[1] were performed by the freezing-point method.

Physiological measurements included the volume, sodium, potassium, chloride, and specific gravity of urine and the sodium, potassium, chloride, urea nitrogen, alkaline phosphatase, and glutamic oxaloacetic transaminase of plasma or serum.

Six dogs were divided into three groups (one male and one female in each) and dosed with the diuretic agents according to the schedule shown in Table I; a table of random numbers was used for assignments to drug and dosage level. Seven days were allowed to elapse between each drug or dosage change to obviate measurable drug interactions. Twelve dogs were given two intravenous doses of mercuric chloride for 2 consecutive days. Dosage levels were 0.5, 1.0, 2.0, and 3.0 mg/kg. Following collection of urine and plasma for the various physiological measurement, these animals were sacrificed and examined in detail at necropsy, and histomorphological studies of renal tissues were made.

RESULTS

Urine osmolality measurements made on 88 colony dogs revealed values ranging from 975 to 2904 mOsm/kg. The results also indicated a linear correlation with specific gravity. Plasma osmolality measurements were made on 46 dogs and resulted in a mean value of 325 ± 3.7 (*SD*) mOsm/kg. Serum values were somewhat less, about 307 mOsm/kg. The ratio of urine osmolality to plasma osmolality (for 24 dogs on which both measurements were performed) was 7.51 ± 1.49 (*SD*).

[1] Advance Instrument osmometer with direct readouts.

Table II—Effect of Intravenous Mercuric Chloride on Blood Chemistry

Dog Number (Sex)	Dose, mg/kg	Urea[a] Nitrogen, mg %	Alkaline[a] Phosphatase Units	Transaminase[a] Units
313 (F)	0.5	16/21	15/11	23/17
267 (F)	0.5	12/18	20/20	16/19
315 (F)	1.0	10/16	24/14	20/21
265 (F)	1.0	12/15	12/12	15/17
266 (M)	2.0	15/17	18/16	16/13
393 (M)	2.0	9/247	10/72	16/1070
392 (M)	3.0	13/131	16/12	14/183
280 (M)	3.0	16/139	14/14	18/147
630 (F)	3.0	17/129	18/13	15/108
493 (F)	3.0	11/133	13/24	14/111
494 (M)	3.0	10/156	13/39	11/129
495 (F)	3.0	14/174	11/12	17/75

[a] The numerator is the predrug value; the denominator is the postdrug value.

The effects of mercuric chloride administration on blood chemistry and osmolality measurements are shown in Tables II and III. At doses of 0.5 and 1.0 mg/kg for 2 consecutive days, no overt renal toxicity was evident. Values for urea nitrogen, transaminase, and urine and serum osmolality were within the normal ranges. The urine–plasma (U/P) ratio was reduced but also remained within normal limits.

One of two dogs at 2.0 mg/kg and all dogs at 3.0 mg/kg showed clinical evidence of nephrotoxicity as manifested by elevated urea nitrogen and serum osmolality values, marked inhibition in renal concentrating ability with marked reduction in urine osmolality, and reduction of U/P ratios to unity. Alkaline phosphate values were not altered; transaminase was markedly elevated. Histopathological examinations showed evidence of renal tubular pathology consistent with the clinical findings.

The results of administration of the diuretic agents resulted in slight to marked reductions in urine osmolality. These data are shown in Tables IV, V, VI, and VII for hydrochlorothiazide, chlorothiazide, acetazolamide, and ethacrynic acid, respectively. Acetazolamide and ethacrynic acid were most potent in decreasing urine osmolality. Dogs given hydrochlorothiazide or chlorothiazide generally had urine osmolality levels within the normal range. If the measurements had been obtained under circumstances whereby treatment was not known, the values would have been considered as normal.

Serum osmolality was slightly decreased following administration of hydrochlorothiazide or chlorothiazide, whereas variable responses were seen in dogs administered ethacrynic acid or acetazolamide. The U/P ratio of the dogs treated with hydrochlorothiazide, although reduced, was still greater than 3, which clinically has served as an indication of normality of the renal concentrating ability. Only one dog, Dog 4, in the group treated with chlorothiazide showed a U/P ratio of less than 3. Ethacrynic acid and acetazolamide resulted in marked decreases in U/P ratios in most instances. In general, the posttreatment values were less than 3.

Table III—Effect of Intravenous Mercuric Chloride on Osmolality of Urine and Plasma

Dog Number (Sex)	Dose, mg/kg	Specific[a] Gravity (1.0 +)	Urine[a], mOsm/kg	Serum[a], mOsm/kg	U/P[a]
313 (F)	0.5	60/50	2470/2199	298/304	8.3/7.2
267 (F)	0.5	35/40	1624/1591	297/303	5.5/5.3
315 (F)	1.0	55/48	2413/1914	300/296	8.0/6.5
265 (F)	1.0	52/36	2304/1449	284/299	7.8/4.8
266 (M)	2.0	37/30	1605/1316	292/295	5.5/4.5
393 (M)	2.0	35/06	1563/294	294/397	5.3/0.7
392 (M)	3.0	50/00	2223/252	297/342	7.5/0.7
280 (M)	3.0	40/10	1605/356	300/335	5.4/1.1
630 (F)	3.0	35/10	1596/285	297/332	5.4/0.9
493 (F)	3.0	60/15	2399/261	310/321	7.7/0.8
494 (M)	3.0	45/03	2275/417	316/387	7.2/1.1
494 (F)	3.0	35/05	2098/401	320/387	6.6/1.0

[a] The numerator is the predrug value; the denominator is the postdrug value.

Table IV—Effect of Hydrochlorothiazide on Serum Urea Nitrogen, Electrolytes, and Urine and Serum Osmolality

Dog Number	Dose, mg/kg	Sodium, mEq/liter	Potassium, mEq/liter	Chloride, mEq/liter	Blood Urea Nitrogen, mg %	Urine[a], mOsm/kg	Serum[a], mOsm/kg	U/P[a]
1	12.5	147/144	4.8/4.5	105/99	15/17	2118/1760	305/299	6.95/5.90
2	12.5	147/146	5.1/4.8	105/98	15/17	2940/2475	307/298	9.55/8.28
3	25	150/145	5.0/4.1	105/96	13/16	1225/975	308/293	4.07/3.34
4	25	148/146	5.0/4.4	105/95	15/16	1675/1045	308/296	5.45/3.57
5	50	148/146	4.6/3.7	109/99	15/15	2025/1460	298/298	6.80/4.89
6	50	147/146	4.7/4.8	104/99	19/19	1000/1055	302/294	3.33/3.60

[a] The numerator is the predrug value; the denominator is the postdrug value.

In an attempt to analyze the data with regard to pharmacological effects, urinary excretion of sodium, potassium, and chloride ions was determined after treatment with the respective diuretic agents. While some pre- and postdrug values differed markedly, considerable overlap was seen and no definite increase in the excretion of electrolytes was observed. These findings are shown in Table IV for hydrochlorothiazide, which served as the prototype agent. Similar patterns were seen in chronic toxicity studies with diuretics when urine was collected on the 3rd day of dosing. This response is not unusual and appears to reflect the homeostatic mechanisms of the animal to maintain normal electrolyte balance.

No significant changes were found in sodium levels subsequent to the administration of the diuretic agent. Administration of chlorothiazide and acetazolamide tended to reduce blood potassium-ion levels, while animals given hydrochlorothiazide and chlorothiazide generally showed a slight reduction in the blood chloride-ion levels. However, the values recorded are within the normal limits for the species. In general, only dogs given ethacrynic acid demonstrated slight elevations in blood urea nitrogen values.

Microscopic examination of renal tissue from these animals revealed no pathological changes attributable to the administration of diuretic agents.

DISCUSSION

These studies indicate that the use of plasma or serum and urine osmolality tests may be useful in evaluating renal function during subacute or chronic toxicity studies with diuretic agents.

Mercuric chloride administration resulted in an apparent dose–response in renal pathology. At doses less than 2.0 mg/kg, no clinical evidence of nephrotoxicity was evident; however, U/P osmolality ratios were reduced in a manner consistent with diuretic activity. This finding appears to reflect the mercuric-ion effect on sulfhydryl enzymes at these subtoxic levels (3). At higher dosages (2 and 3 mg/kg), the dogs showed renal toxicity that could be correlated with marked elevations of urea nitrogen, serum osmolality, and histopathology affecting the proximal renal tubule.

Table V—Effect of Chlorothiazide on Osmolality

Dog Number	Dose, mg/kg	Urine[a], mOsm/kg	Serum[a], mOsm/kg	U/P[a]
1	125	1675/1475	288/276	5.85/5.32
2	125	2075/2475	280/287	7.40/8.61
3	250	1300/875	290/279	4.50/3.19
4	250	1300/650	296/275	4.46/2.36
5	500	1475/1225	281/268	5.25/3.58
6	500	1650/1125	279/276	5.92/4.09

[a] The numerator is the predrug value; the denominator is the postdrug value.

Table VI—Effect of Acetazolamide on Osmolality

Dog Number	Dose, mg/kg	Urine[a], mOsm/kg	Serum[a], mOsm/kg	U/P[a]
1	40	2427/575	289/293	8.35/1.97
2	40	NS/475	298/307	— /1.52
3	20	2025/275	315/298	6.45/0.92
4	20	1400/600	298/291	4.72/2.06
5	10	1475/1575	293/314	5.05/5.01
6	10	1725/550	298/282	5.80/1.95

[a] The numerator is the predrug value; the denominator is the postdrug value.

Table VII—Effect of Ethacrynic Acid on Osmolality

Dog Number	Dose, mg/kg	Urine[a], mOsm/kg	Serum[a], mOsm/kg	U/P[a]
1	2.5	2125/800	309/316	6.89/2.54
2	2.5	2825/2750	307/308	9.17/8.95
3	5.0	1775/925	328/314	5.40/2.95
4	5.0	1625/1175	318/308	5.13/3.83
5	10.0	1825/750	293/304	6.22/2.47
6	10.0	1350/600	304/312	4.45/1.92

[a] The numerator is the predrug value; the denominator is the postdrug value.

Mercuric chloride toxicity causes severe tubular necrosis with acute oliguric renal failure (4) but sparing of the glomeruli (5). Renal blood flow and glomerular filtration continue at rates which, in chronic renal failure, should provide adequate volumes of urine. However, it is postulated that oliguria results from filtrate disappearance by nonselective back-diffusion through damaged tubules (5).

Since serum osmolality is primarily dependent upon electrolyte concentration and concentrations of urea and glucose, the high levels seen in these nephrotoxic dogs may be attributed to the oliguric state and consequent uremia resulting from mercuric chloride toxicity.

Although diuretics can reduce the renal concentrating ability of the kidney and likewise alter the U/P ratio in a manner similar to that caused by the nephrotoxin mercuric chloride, the determination of plasma or serum osmolality may serve as the basis for the differentiation of effects. In cases of renal disease, serum or plasma osmolality is generally elevated; however, in the present program, serum osmolality remained unchanged or was slightly decreased subsequent to the administration of diuretic agents. Serum sodium concentrations and urea nitrogen levels were not elevated.

The data indicate that this method appears to be of value in assessing and differentiating renal function subsequent to diuretic agents and a nephrotoxic agent. While these results are preliminary, they can serve as a basis for extending the scope of testing by evaluating a series of less potent nephrotoxins during subacute oral toxicity studies and comparing these data to those obtained subsequent to more prolonged administration of a diuretic agent.

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