TABLE IV. Insoluble Dermal Hexosamine (Hex) and Hydroxyproline (Hypro) in μMoles/mMole Total Nitrogen and Nitrogen as % of Total in the Skin of Rats Receiving Various Doses of Vit. A.

Dosage	Hex	Nitrogen	Hypro
0	.80	53	27
1	.79	57	24
2	.74	63	24
3	.76	62	24
4	.76	59	26

massive doses of Vit. A. Since the "ground substance" of rat skin contains only glucosamine and *no* galactosamine(11) the mucopolysaccharide disappearing from the "ground substance" must be hyaluronic acid and perhaps heparin. Similarly, decreases in concentration of hexosamine in the 0.5 M NaCl extract occurred initially without parallel losses of nitrogen and therefore presumably represent a loss of the mucopolysaccharide content of this fraction with Vit. A administration. Since this fraction has been shown to contain only galactosamine and no glucosamine(11), the mucopolysaccharides lost from this fraction presumably was chondroitin sulfate.

Associated with hypervitaminosis A was a marked increase in dermal concentration of citrate soluble hydroxyproline, or collagen. Despite this change in the concentration of soluble collagen, hypervitaminosis A was without significant effect upon the chemistry of the insoluble portion of the connective tissue.

Summary. In general, the initial primary effect of hypervitaminosis A upon the chemistry of the connective tissue appeared to be a marked loss of hyaluronic acid and chondroitin sulfate from the saline soluble components of the tissue. With further doses of Vit. A there appeared to be a significant decrease in the glycoprotein content of these fractions. To this degree these chemical studies essentially confirm the effects of Vit. A described by others for cartilage *in vitro,* upon the soft connective tissues *in vivo.*

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Breakdown Products of Urea and Uremic Syndrome.* (28990)

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During the past 150 years the accumulation of urea, ammonium cyanate(1), amino acids, guanidine, uric acid, phenols and other substances, both organic and inorganic, in the blood of uremic animals and patients has lead to their being implicated in the uremic syndrome(2). No definitive proof has been presented that any of these substances are directly responsible for the symptoms of uremia.

Urea, since it is the major metabolic end product found in uremic blood, has been the substance most often considered to be responsible for the symptoms of uremia. A proof to the contrary was offered by Merrill(3) who observed no difference in the extent of clinical improvement in the uremic syndrome following extracorporal dialysis even if the

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patient's blood urea level remained unchanged after dialysis. Over 1300 patients in the University Hospitals have received hypertonic solutions of urea for reduction of intracranial or intraocular pressure. We have demonstrated that these patients (none of whom had marked loss of renal or hepatic function) tolerate a daily dose of 1.0-2.0 g/kg body weight of urea for a short period without any apparent ill effects(4). Others (5) have administered up to 8.0 g of urea per kg body weight to dogs intravenously in a 30% solution every 24 hours over a period of 7 days without any manifestation of toxicity.

Paradoxically, Grollman and Grollman(6) reported that bilaterally nephrectomized dogs dialyzed with a commercial peritoneal lavage solution to which 5-30 g of urea were added per liter of solution developed progressively severe symptoms of uremia and expired within a few days while similar animals previously reported by one of the authors(7) did not receive urea and lived an average of 39 days. The blood urea nitrogen (BUN) averaged 200 mg per 100 ml at onset of symptoms and rose as high as 790 mg per 100 ml in some animals dialyzed with the urea solution.

The observations of Grollman and Grollman indicate that continuous exposure to higher than normal levels of urea in the absence of kidneys leads to an accelerated onset of symptoms that resemble those of uremia by a mechanism which is unknown. From the data provided one could hypothesize that the symptoms may be due to any or all of the following: (a) tissue hyperosmolarity, (b) a low level of inactivation of all enzymes due to the presence of relatively high amounts of urea (a protein denaturing agent known to inhibit some enzymes at low levels *in vitro*)(8), (c) carbamylation of proteins and free amino acids(9) (isocyanate ion is formed from urea in aqueous solution)(10), or (d) inability to detoxicate ammonia produced by bacterial breakdown of the large amounts of urea. The latter two mechanisms are the subject of this study.

Methods. Three groups of mongrel dogs were bilaterally nephrectomized under sodium pentobarbital anesthesia using a retroperitoneal approach with a 2-3 day interval between the two operations. The animals had no special care after recovery from anesthesia. Each animal was given 200,000 units of penicillin and 1 g of streptomycin intramuscularly the day of surgery and 100,000 units of penicillin on each of the first 2 postoperative days. Twenty-five mg of tetracycline were added to each liter of the sterile commercial peritoneal lavage solution (Dianeal)† utilized for dialysis. The animals received a total of 4.5-6.0 liters of solution, depending on the size of the dog, in 3 one hour exchanges daily. A Duke Trocar placed through a midline incision approximately 1 inch below the umbilicus was utilized to position the catheter prior to dialysis each day.

Group I was dialyzed with the solution mentioned above; 10 g of urea were added to each liter of lavage solution given to Group II, and Group III received 150 mg (1.85 meq) of potassium isocyanate in each liter of dialysis solution. Our observations with urea indicated that the dialysis fluid must be five times higher in concentration of a given material than the final concentration desired in the serum. The velocity of isocyanate formation may double *in vivo* due to the presence of amino and sulfhydryl groups which react with isocyanate, removing it from solution and causing the equilibrium to shift. To achieve a tissue fluid level of isocyanate approximating that formed by a 1% aqueous urea solution at 37°C in 24 hours, the lavage solution must have a concentration of isocyanate 10 times greater than that found in a 1% aqueous urea solution at 37°C *in vitro* after 24 hours(10).

Blood samples were drawn 5 times a week prior to dialysis and analyzed for Cl, CO_2, Na, K, and urea nitrogen in all dogs, and NH_3 in 4 dogs from Group I, 8 dogs from Group II, and 3 dogs from Group III.

Sodium and potassium determinations were done on a spectrophotometer with a flame photometer attachment, chloride by the titra-

† Supplied by Travenol Laboratories, Morton Grove, Ill.

tion technique of Schales and Schales(11). Carbon dioxide combining power was assayed by the method of Van Slyke and Cullen(12), and blood urea nitrogen was estimated with an automatic analyzer. Blood ammonia was determined according to the method of Reinhold and Chung(13).

All animals, commencing about the third day after bilateral nephrectomy, exhibit signs of anorexia; therefore, tube feeding (30 Cal./kg/day) was instituted after the second surgical procedure.

Results. The control group consisting of 6 dogs lived an average of 14 (SE ± 0.17) days. In general the serum electrolyte pattern (Table I) in these animals was normal. No potassium was added to the lavage solution, and serum potassium levels remained relatively stable initially with a tendency to decrease as the study progressed. Some potassium may have been lost the last few days of the study because of vomiting. The animals lost 100-200 g a day; approximately 50 g of this loss was due to excess withdrawal of fluid after lavage. Since serum sodium levels were within the normal range, most of the weight loss was attributed to catabolism rather than dehydration. The BUN, prior to dialysis, averaged 86 mg per 100 ml the first 8 days of the study. The mean serum potassium level was 4.53 meq/l during the same period. Blood ammonia ranged from 0.26-1.34 μg/ml. A decrease in number of samples due to death of experimental animals caused the standard error reported in Tables I-III to increase as the study progressed.

The 12 dogs in Group II lived an average of 7 (SE ± 0.60) days. Serum electrolytes (Table II) were normal except for the potassium which became elevated and remained elevated through the 7th day. The mean serum potassium level was 5.56 meq/l the first 8 days. An average of 100 g more fluid was recovered each day than was instilled into the abdominal cavity. This loss plus an earlier onset of vomiting accounted for the greater weight loss (200-250 g/day) observed in Group II. The animals exhibited no signs of dehydration. Blood urea nitrogen steadily increased reaching an average of 589 mg per

TABLE I. Serum Electrolytes, Urea Nitrogen and Ammonia in Group I.

	Days after second nephrectomy														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
NH_3 μg/ml	.26	.62	.35	.85	1.34	—	—	1.01	.65	.71	.74	.78	—	—	.66	.57
S.E.	±.05	±.13	±.09	±.13	±.32	—	—	±.13	±.05	±.08	±.02	±.04	—	—	±.18	—
CO_2 meq/l	28.16	28.00	24.75	30.50	27.80	33.00	—	31.68	31.30	31.50	31.50	32.37	36.00	33.50	26.50	28.25
S.E.	±.92	±1.77	±1.22	±2.00	±1.70	±.49	—	±2.23	±2.07	±2.87	±2.72	±1.33	—	—	±2.86	±4.23
Cl meq/l	108.00	102.33	97.00	95.25	98.60	105.00	—	95.00	96.60	99.00	100.00	98.00	98.00	102.00	97.00	101.00
S.E.	±1.52	±2.90	±1.11	±1.33	±1.25	±1.00	—	±1.25	±1.61	±1.50	±2.72	±2.45	—	—	±3.02	—
Na meq/l	143.50	139.83	142.25	148.00	141.00	136.50	—	145.20	146.80	145.00	148.00	143.50	140.00	150.00	146.66	138.50
S.E.	±.91	±1.67	±1.56	±.89	±1.61	±4.49	—	±2.70	±2.70	±1.10	±4.42	±1.33	—	—	±1.50	±5.49
K meq/l	4.60	4.25	5.18	5.03	4.96	3.97	—	4.40	3.92	4.35	4.61	3.26	2.85	3.00	3.11	3.90
S.E.	±.24	±.21	±.26	±.54	±.29	±.67	—	±.16	±.21	±.10	±.32	±.23	—	—	±.25	±1.10
BUN mg /100 ml	17.66	67.83	91.50	103.50	88.00	97.50	—	73.60	81.40	80.00	83.66	57.00	54.00	54.00	92.33	98.50
S.E.	±1.22	±2.90	±10.26	±14.50	±12.95	±21.00	—	±14.93	±8.00	±14.52	±25.72	±6.69	—	—	±20.57	±31.46

TABLE II. Serum Electrolytes, Urea Nitrogen, and Ammonia in Group II.

	Days after second nephrectomy									
	0	1	2	3	4	5	6	7	8	9
NH_3 μg/ml	.76	.80	1.10	.99	.78	—	1.09	.98	—	—
S.E.	±.05	±.12	±.10	±.07	±.08	—	—	±.06	—	—
CO_2 meq/l	27.54	27.81	30.41	29.85	28.00	29.75	30.20	28.25	27.50	23.50
S.E.	±.93	±1.06	±2.06	±1.60	±1.06	±1.00	±1.61	±1.45	—	—
Cl meq/l	102.75	99.66	98.71	94.00	100.25	102.50	103.50	104.75	98.50	104.00
S.E.	±2.84	±2.06	±1.86	±2.13	±1.90	±.67	±4.12	±5.13	±2.49	—
Na meq/l	145.41	145.25	147.42	143.00	146.12	141.00	143.50	147.00	149.50	138.00
S.E.	±4.60	±2.58	±2.80	±1.20	±.71	±1.12	±.45	±6.02	±3.49	—
K meq/l	4.67	5.25	5.55	7.09	5.67	5.19	5.48	5.66	4.97	4.80
S.E.	±.39	±.24	±.03	±.36	±.40	±.55	±.49	±.81	±.12	—
BUN mg/100 ml	22.66	160.00	322.14	412.42	472.50	520.00	560.83	588.50	557.50	545.00
S.E.	±2.32	±5.60	±12.00	±20.00	±14.31	±32.50	±38.97	±75.00	±17.47	—

100 ml on the 7th day. Blood ammonias ranged from 0.76-1.10 μg/ml in the animals studied.

Group III, composed of 8 dogs, lived an average of 6 (SE ± 0.53) days. Approximately 100 g of the 200-250 g each animal in this group lost per day was due to recovery of excess fluid from the peritoneal cavity. The remainder was lost due to vomiting and catabolism. There were no signs of dehydration. The BUN (Table III) averaged 134 mg per 100 ml the first 8 days, and mean serum potassium level was 5.70 meq/l during the same period. Blood ammonia in these animals varied from 0.51-1.23 μg/ml.

Onset of symptoms was more gradual in Group I, but all animals in this study eventually became drowsy or comatose. The first symptoms were weakness and anorexia with the animals refusing raw hamburger and canned dog food. They became progressively more ill and tended to be apathetic even during periods of dialysis. Many animals had diarrhea with subsequent hemorrhage from the bowel. The body temperature steadily declined in animals receiving isocyanate or urea, reaching an average temperature of 33°C (rectally) in Group II animals living 7 days. Nearly all animals developed seizures prior to death.

Discussion. In bilaterally nephrectomized dogs similar symptoms can be elicited with urea and potassium isocyanate, providing tissue isocyanate concentration is roughly comparable to the expected formation in a 1% aqueous solution of urea at 37°C *in vitro*.

It would appear doubtful that ammonia is intimately involved in the toxicity observed in bilaterally nephrectomized dogs dialyzed with a solution containing urea. The animals

TABLE III. Serum Electrolytes, Urea Nitrogen, and Ammonia in Group III.

	Days after second nephrectomy								
	0	1	2	3	4	5	6	7	8
NH_3 μg/ml	.74	.51	.45	—	1.23	—	—	.66	.52
S.E.	±.06	±.19	±.08	—	±.42	—	—	±.46	±.18
CO_2 meq/l	25.33	24.70	—	—	27.60	27.00	26.13	26.66	34.50
S.E.	±.90	±1.56	—	—	±1.60	—	±1.99	±2.11	—
Cl meq/l	109.25	104.28	—	—	95.80	96.00	97.33	99.00	95.00
S.E.	±1.56	±1.60	—	—	±2.33	±4.83	±3.63	±1.81	—
Na meq/l	140.87	142.57	—	—	140.00	147.00	142.00	151.00	150.00
S.E.	±.71	±.66	—	—	±1.43	±5.44	±1.81	±6.35	—
K meq/l	3.94	4.85	—	—	6.27	6.48	5.60	6.20	4.85
S.E.	±.17	±.14	—	—	±.69	±.55	±.49	±1.17	—
BUN mg/100 ml	22.25	81.57	—	—	175.20	—	136.00	149.00	127.00
S.E.	±3.69	±6.13	—	—	±23.39	—	±45.39	±47.20	—

receiving urea did not have ammonia levels significantly higher than those of the control animals, even though bacterial breakdown of urea must have given them considerably larger amounts of ammonia to detoxicate. The presence of tetracycline in the lavage solution may have contributed to the unexpectedly low blood ammonia levels in the animals receiving urea, but it did not affect the severity of the symptoms.

One often finds increased protein catabolism and elevated serum potassium levels in disease states, such as uremia, which are associated with regressive changes in cells. Groups II and III, in general, have significantly higher levels of serum potassium than the controls. The higher serum potassium level associated with a significantly higher endogenous production of urea in Group III and presumably in Group II, is believed to be indicative of an accelerated protein breakdown brought about by the presence of urea and potassium isocyanate.

While urea is a known protein denaturant, the level attained in these studies (0.15 M) is not generally believed to cause protein denaturation. The formation of ammonium isocyanate in aqueous urea solutions gives both urea and potassium isocyanate solutions the capacity to carbamylate epsilon aminos, sulfhydryls and N terminal aminos without enzymatic intervention. The resulting denaturation may cause an increase in the rate of protein breakdown in Groups II and III. It is possible that the toxicity of urea in nephrectomized dogs may be due in part to formation of ammonium isocyanate and subsequent carbamylation reactions.

The LD_{50} of potassium isocyanate is approximately 300 mg per kilogram body weight in rats and dogs with normal renal function, while 60 mg per kilogram over a 5-7 day period is fatal to bilaterally nephrectomized dogs. The seemingly greater toxicity of isocyanate in nephrectomized animals is undoubtedly due, in part, to their inability to excrete the material, a factor permitting nearly complete reaction of the isocyanate present.

Summary. The course of uremic syndrome can be accelerated in bilaterally nephrectomized dogs treated with peritoneal lavage solution containing either 1% urea or 0.015% potassium isocyanate. It is suggested that ammonium isocyanate formed from urea in aqueous solutions may contribute to the uremic syndrome via carbamylation reactions with free amino acids and proteins. Blood ammonia levels were not significantly elevated in any of the animals tested.

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