EXPERIMENTAL ACUTE NEPHRITIS: THE ELIMINATION OF NITROGEN AND CHLORIDES AS COMPARED WITH THAT OF PHENOLSULPHONEPHTHALEIN.*

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This investigation was undertaken, at the suggestion of Dr. Pearce, as complementary to the studies of one of us,[1] on the elimination of phenolsulphonephthalein in a variety of experimental renal lesions. Its object has been to determine whether or not the elimination of this dye parallels that of the chlorides and the total nitrogen. The determination of this point is of importance, not only for the interpretation of the clinical value of the phenolsulphonephthalein test, but also as a means of adding to our knowledge of the normal physiology of the kidney.

Methods.—The dog has been used in all the experiments. Several forms of experimental nephritis were selected for study; namely, those due to uranium nitrate or to potassium chromate, representatives of the so-called tubular (or epithelial) nephritis type; and that caused by cantharidin, usually considered to be a nephritis of the vascular type. The various poisons have been given in small doses, since the use of large doses has invariably resulted in vomiting, diarrhea, or loss of appetite, thus rendering the nitrogen and chloride estimations relatively valueless. With small doses, the

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[1] Eisenbrey, A. B., A Study of the Elimination of Phenolsulphonephthalein in Various Experimental Lesions of the Kidney, *Jour. Exper. Med.*, 1911, xiv, No. 5 (November).

lesions caused by cantharidin have not been severe; those due to uranium nitrate and potassium chromate have, however, been marked. All animals were kept in the conventional metabolism cages and, after a preliminary period of observation for the detection of spontaneous lesions, were placed on a constant diet. This consisted of beef heart, casein, lard, and crackers, in such proportions that, when in nitrogen equilibrium, the daily elimination of nitrogen in the urine approximated one gram per kilo of body weight. The object was thus to bring about, without causing digestive disturbances or loss of appetite, as high a daily nitrogen elimination as possible. The importance of such a high nitrogen elimination will be discussed in connection with the experiments with uranium. To this diet was added, daily, one gram of sodium chloride and one gram of bone ash, and a measured quantity of water. The nitrogen and chloride estimations were made each day on the total twenty-four hour urine from catheterized animals. On the days when the phenolsulphonephthalein elimination was determined, two sets of estimations were made: one on a composite sample from the urine collected during the two hours required for the phenolsulphonephthalein test, the other from the urine collected during the remaining twenty-two hours. The Kjeldahl method was used for the determination of nitrogen, and the modified Volhard method for chlorides. In making the nitrogen determinations, coagulable protein was first removed by boiling and acidulation with acetic acid. Control estimations to determine the effect of the administration of phenolsulphonephthalein upon the nitrogen or chloride elimination showed no alteration in either. The elimination of phenolsulphonephthalein was determined by the original method of Rowntree and Geraghty.[2]

Experiments with Uranium Nitrate.—Uranium nitrate was used in 0.75 per cent. aqueous solution and injected hypodermically into the flank, as indicated in the tables.

It will be seen that during the two days following the administration of uranium nitrate a fall in the nitrogen occurred, comparable

[2] Rowntree, L. G., and Geraghty, J. T., An Experimental and Clinical Study of the Functional Activity of the Kidneys by Means of Phenolsulphonephthalein, *Jour. Pharmacol. and Exper. Therapeutics,* 1909, i, 579.

TABLE I.

Experiment I. Dog 19, weighing 10,420 gms., was placed, on May 28, on a constant diet of beef heart, casein, cracker, and lard, containing 10.58 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 500 c.c. of water. The urine contained no albumin or casts from this time until June 7.

Date.	Weight in gms.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
June 5	10,370			490	7.90	2.00	0	
" 6	10,430	69.0 %		500	8.07	2.12	0	
" 7	10,465		3 P.M. 0.015 gm.	520	6.87	1.88	Trace	Few fine granular casts.
" 8	10,430	72.8 %	Noon 0.01 gm.	478	5.19	1.37	0.6 gm.	Few fine granular casts.
" 9	10,225	56.6 %						Slight vomiting.
" 10	9,940	14.0 %						Vomiting. Chloroformed.

On microscopic examination of the kidneys, the glomeruli appeared normal. The epithelium of the convoluted tubules showed "granular" degeneration and fat vacuoles; the nuclei were pale. The medullary loops contained granular casts. The vessels were not congested.

to that noted by Pearce, Hill, and Eisenbrey[3] and by Siegel,[4] in the dog, and by Schlayer and Takayasu[5] in the rabbit. The vomiting during the third day rendered further analyses worthless. The chlorides of the urine showed a distinct drop; this also was noted by the above mentioned investigators. The fall in the phenolsulphonephthalein elimination was less prompt than the fall in the nitrogen and chlorides, but eventually more marked. Synchronously with this fall in the phenolsulphonephthalein elimination, the condition of the dog became poor, and vomiting and loss of appetite set in. During the experiment, the animal was placed on a diet containing somewhat more than one gram of nitrogen per kilogram of body weight and showed, in consequence, a high nitrogen elimination during the control period. A comparison with experiment

[3] Pearce, R. M., Hill, M. C., and Eisenbrey, A. B., Experimental Acute Nephritis: The Vascular Reactions and the Elimination of Nitrogen. *Jour. Exper. Med.*, 1910, xii, 196.

[4] Siegel, W., Ein Stoffwechselversuch bei Urannephritis am Hunde, *Ztschr. f. exper. Path. u. Therap.*, 1907, iv, 561.

[5] Schlayer and Takayasu, Untersuchungen über die Funktion kranker Nieren., *Deutsch. Arch. f. klin. Med.*, 1910, xcviii, 17.

II shows that such a high nitrogen diet is a necessary condition for the study of the power of the kidney to eliminate nitrogen.

TABLE II.

Experiment II. Dog 1, weighing 8,415 gms., was placed, on March 14, on a constant diet of beef heart, casein, cracker, and lard, containing 3.61 gms. nitrogen, with 1 gm. bone ash and 350 c.c. water. The urine from that date until March 21 was free from albumin and casts.

Date.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.		Notes.
			Amount in c.c.	Total nitrogen in gms.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
				2.94	No albumin or casts.
Mar. 19	77.8 %		228	3.28	No albumin or casts.
" 21		11 A.M. 0.015 gm.		3.04	Albumin 1/6 by volume; few fine granular casts.
" 22	25.3 %		415	3.39	Albumin 1/6 by volume; few fine granular casts.
" 23	4.1 %		485	3.37	Albumin 1/6 by volume; few fine granular casts. Very slight vomiting.
" 24	3.51 %				Vomiting marked.
" 25	1.8 %				Vomiting marked.
" 26	1.55 %				Vomiting marked. Chloroformed.

Microscopic examination of the kidney showed congestion of the glomeruli and intertubular spaces of both cortex and medulla and the presence of subcapsular and interstitial hemorrhages. The cells of the convoluted tubules were swollen and turbid and the lumina of these and of the medullary loops contained hyaline and fine granular casts.

In this experiment, the diet was much lower in nitrogen than in experiment I, although the amount of nitrogen was still adequate for the requirements of the animals, and no fall in the elimination of nitrogen occurred. The dog in experiment I, even at the time when its nitrogen elimination was impaired, excreted 5.19 grams; the dog in experiment II, on the other hand, because of the low nitrogen diet, had never more than 3.39 grams of nitrogen to excrete and continued to maintain the nitrogen elimination undiminished in spite of renal injury. The impairment which doubtless did exist in the excretory power of the kidney remained undemonstrated. In this instance it is evident that the nitrogen retention was more readily determined in an animal on a high nitrogen equilibrium than in one on a low equilibrium. In all the other experiments reported, we employed, therefore, a high nitrogen diet.

The phenolsulphonephthalein test in experiment II showed a somewhat more rapid fall than in experiment I. It is of interest that on the day when the elimination had fallen to the very low figure of 4.1 per cent., the kidney still eliminated readily 3.37 grams of nitrogen and an increased quantity of water. It is also of interest that vomiting began, as in experiment I, when the functional test had reached a low figure. This fact will be referred to later.

In experiment III, the attempt was made to produce a nephritis with less gastric disturbance, and for this purpose only half the previous dose of uranium was employed.

TABLE III.

Experiment III. Dog 17, weighing 7,450 gms., was placed, on May 16, on a constant diet of beef heart, casein, cracker, and lard, containing 7.32 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 300 c.c. of water. The urine contained no albumin or casts from this time until May 23.

Date.	Weight in gms.	Phenol-sulph. test, 10 A. M.	Uranium nitrate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A. M. on date specified.
May 20	7,450	79.5 %		210	5.75	1.53	0	
" 22	7,450	78.0 %		285	5.79	1.71	0	
" 23	7,360		2 P.M. 0.0075gm.	291	5.91	1.99	0	
				250	6.27	1.77	Faint trace	Few pale granular casts.
" 24	7,350	78.8 %		404	6.78	2.20	Light cloud	Many pale granular casts.
" 25	7,230	68.6 %		536	7.32	1.31	0.5gm.	Many pale granular casts.
" 26	7,050	44.4 %						Vomiting.
" 27	6,720	12.8 %		646	7.06	0.73	0.7gm.	Very little vomiting.
" 28	6,440							Chloroformed.

Microscopic examination showed the glomeruli and interstitial tissue of the cortex and medulla to be moderately congested. The cells of the convoluted tubules showed cloudy swelling. Their nuclei were well stained; the lumina contained granular debris. The medulary loops were filled with hyaline and granular casts.

The experiment of using the smaller dose of uranium nitrate was repeated three times and the same rise in the nitrogen elimination always occurred. The explanation of this increased nitrogen excretion after a small dose is uncertain, but, in view of the dis-

tinct loss of body weight, it would appear that an increased katabolism is caused by uranium, while at the same time the eliminative function of the kidney is not sufficiently impaired by the small dose to interfere with the excretion of the excess of waste nitrogen. An early increase in the elimination of chlorides associated with diuresis occurred, followed by a reduced chloride elimination. Whether this fall is to be considered as compensatory for the earlier excessive elimination, or as a retention from diminished excretory power is a question. The delay in the elimination of the phenolsulphonephthalein was quite similar to that observed with the larger doses of uranium, and a very low figure was reached at a time when the nitrogen elimination was still above normal.

Experiments with Potassium Chromate.—A 3 per cent. aqueous solution of potassium chromate was injected hypodermically as shown in the tables. The dose employed was the largest that could be given without encountering the risk of early vomiting.

Microscopic examination showed the glomeruli to be normal. The tubular epithelium was the seat of fatty degeneration and the nuclei were large and pale. The lumina contained granular debris. A moderate interstitial edema was present in the medulla.

In this experiment a transient fall in the nitrogen occurred after each injection, within either twenty-four or forty-eight hours. The chlorides showed a similar fall, followed by a compensatory rise. Pearce, Hill, and Eisenbrey noted a similar fluctuation in the nitrogen elimination of the dog after chromate; and Green,[6] following the use of a very large dose of the poison, obtained in the rabbit a similar though somewhat more prolonged fall in both nitrogen and chlorides during the first three days. The delay in the phenolsulphonephthalein elimination developed more slowly and was less marked than in the experiments with uranium. It will be noted that some of the lowest figures for the functional test were synchronous with a high nitrogen and chloride elimination. Vomiting began, as with uranium nephritis, when the functional test had reached a low figure.

[6] Green, J. L., Experimental and Clinical Observations on the Urine and Blood in Nephritis, *Jour. Path. and Bact.*, 1909, xiii, 296.

TABLE IV.

Experiment IV. Dog 4, weighing 6,370 gms., was placed, on April 10, on a constant diet of beef heart, casein, cracker, and lard, containing 7.18 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 300 c.c. of water. The urine was free from albumin and casts from this date until April 14.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Potassium chromate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
April 12	6,370	72.0 %		245	6.51		0	
" 13	6,275			233	7.01		0	
" 14	6,270		Noon 0.03 gm.	237	6.88		0	
				275	5.82		0.1 gm.	Few fine granular casts.
" 15	6,300	66.2 %		413	6.26		0.6 gm.	Few fine granular casts.
" 16		63.0 %		446	6.46		0.3 gm.	Few fine granular casts.
" 17	6,000	65.2 %					Cloud	
" 18	6,040	63.8 %	Noon 0.03 gm.				Cloud	
								Error in diet. Intermission.
May 3	5,570						Trace	Constant diet resumed.
" 5	5,670			320	6.26		Faint trace	Occasional granular casts.
" 6	5,535			320	5.98		Trace	Occasional granular casts.
" 7	5,650			330	6.03	1.52	Trace	Occasional granular casts.
" 8	5,650	66.4 %		330	6.56	1.75	Faint trace	Occasional granular casts.
" 9	5,660	72.2 %	2 P.M. 0.03 gm.	355	6.40	1.75	Light cloud	Many granular casts.
" 10	5,645	58.2 %		378	5.89	0.97	0.7 gm.	Many granular casts.
" 11	5,645	46.3 %		380	6.95	1.96	0.1 gm.	Many granular casts.
" 12	5,660	59.3 %	Noon 0.03 gm.	300	6.08	1.58	0.6 gm.	Many granular casts.
" 13	5,720	51.2 %		340	6.77	1.77	0.6 gm.	Many granular casts.
" 14		45.6 %	2 P.M. 0.03 gm.	426	6.92	2.15	0.5 gm.	Many granular asts.
" 15	5,630	45.6 %						Trace of vomitus.
" 16	5,570	42.5 %						Trace of vomitus.
" 17	5,690							Chloroformed.

In order to secure with this poison a more rapid fall in the phenolsulphonephthalein elimination, two doses of chromate were given on successive days. The result is shown in table V.

Microscopic examination showed the glomeruli to be normal. The tubular epithelium was the seat of marked cloudy swelling, with slight fatty degenera-

tion; the lumina were filled with finely granular debris. A slight interstitial edema was noted in the medulla.

TABLE V.

Experiment V. Dog 20, weighing 5,460 gms., was placed, on May 27, on a constant diet of beef heart, casein, cracker, and lard, containing 6.43 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 350 c.c. of water. The urine was free from albumin and casts from this date until June 11.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Potassium chromate.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
June 8	5,430			350		1.37	0	
" 9	5,430	78.9 %		370	6.17	1.32	0	
" 10	5,470	70.9 %		550	6.11		0	
" 11			Noon 0.03 gm.	380	5.52	1.27	Faint trace	Few granular casts.
" 12	5,450	40.9 %	2 P. M. 0.03 gm.					Vomiting.
" 13	5,465	3.54 %						Chloroformed.

In this experiment, a prompt fall in nitrogen was evident, but it was somewhat less marked than in experiment IV. A drop in chlorides was not observed during the first twenty-four hours. The fall in the phenolsulphonephthalein elimination was, however, more prompt and marked than in dog IV, as was also the vomiting.

Experiments with Cantharidin.—With this drug two kinds of experiments were made, according as a single large dose, or daily small doses were given. The cantharidin was administered hypodermically in the form of a saturated solution in acetic ether.

The first type is illustrated in table VI.

Microscopic examination of the kidneys showed the cortex, medulla, and especially the glomeruli to be intensely congested. The epithelium of the convoluted tubules showed a marked granular degeneration, with entire loss of cellular outline and very pale swollen nuclei. The epithelium of the other tubules was swollen and turbid. The lumina of the convoluted tubules and of the medullary loops were completely occluded by the swelling of the epithelium. The medulla showed moderate interstitial edema.

In this experiment, in the first twenty-four hours, with free diuresis, a fall in the nitrogen elimination occurred. That this fall was not due to the very slight vomiting was proved by the fact that the total

TABLE VI.

Experiment VI. Dog 6, weighing 5,760 gms., was placed, on April 2, on a diet of beef heart, casein, cracker, and lard, containing 6.88 gms. nitrogen, with 1 gm. bone ash and 300 c.c. water on April 2. The urine was free from albumin and casts from this time until April 16.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Cantharidin.	Urine.			Notes.
				Amount in c.c.	Total nitrogen in gms.	Coagulable protein in gms., Esbach.	The urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
April 12	5,715			295	5.36	0	
" 14	5,695			320	5.54		
" 15	5,765	66.0 %		275	5.73		
" 16	5,710		5 P.M. .02 gm.	560	3.24	0.1	Very slight vomiting. Hematuria.
" 17	5,285	9.1 %					Marked vomiting; animal refuses food; anuria. Chloroformed.

nitrogen of urine and vomitus during the twenty-four hours was only 3.74 grams. The fall in the phenolsulphonephthalein elimination was prompt and very marked and coincided with the onset of vomiting.

The second type of experiment is illustrated in table VII.

Microscopic examination of the kidneys showed congestion of the glomeruli and a deposit of precipitated serum between the tuft and the capsule of Bowman. The cortex and medulla were moderately congested. The epithelium of the convoluted tubules was the seat of marked granular degeneration with fatty vacuoles; the nuclei were pale and swollen and the lumina were distended with granular debris. The walls of the medullary tubules were in apposition, the lumina being occluded, and the medulla showed slight interstitial edema.

In this experiment the nitrogen excretion was increased. The loss of weight, conspicuous after small doses of uranium, was very slight, and therefore the interpretation of the increased nitrogen excretion is less clear. The excess of diuresis in a given day bore no relation to the amount of nitrogen eliminated, but was followed, in a general way, at least for a few days, by the chloride elimination. The phenolsulphonephthalein elimination was only very slightly affected and not until after the sixth injection. No vomiting occurred. In spite of marked anatomic alteration in the kidney, the elimination of nitrogen, chlorides, and phenolsulphonephthalein

TABLE VII.

Experiment VII. Dog 16, weighing 10,620 gms. was placed, on May 16, on a constant diet of beef heart, casein, cracker, and lard, containing 10.59 gms. nitrogen, with 1 gm. bone ash, 1 gm. sodium chloride, and 400 c.c. water. The urine was free from albumin and casts from this date until May 22.

Date.	Weight in gms.	Phenol-sulph. test, 10 A.M.	Cantharidin.	Urine.				Notes.
				Amount in c.c.	Total nitrogen in gms.	Sodium chloride in gms.	Coagulable protein, Esbach.	Urinary data refer to twenty-four hour specimens beginning 9 A.M. on date specified.
May 19				220	8.52	1.52	0	
" 20	10,620	72.1 %		330	8.83	1.72	0	
" 21	10,640	80.7 %		285	8.30	1.56	0	
" 22	10,680		2 P.M. 0.006 gm.	390	9.17	2.69	0	
" 23	10,450	81.0 %		296	10.62	1.91	0	
" 24	10,550	87.5 %	2 P.M. 0.006 gm.	602	9.47	3.06	Trace	Erythrocytes in urine.
" 25	10,420	75.0 %	2 P.M. 0.006 gm.	312	10.58	1.07	Trace	Erythrocytes and few granular casts.
" 26	10,440	81.9 %	2 P.M. 0.006 gm.	492	11.29	2.88	Faint trace	Erythrocytes and few granular casts.
" 27	10,420	79.1 %	2 P.M. 0.006 gm.	509	9.73	1.75	Faint trace	Erythrocytes and few granular casts.
" 28	10,170		2 P.M. 0.006 gm.	420	9.45	0.84	Light cloud	Erythrocytes and few granular casts.
" 29	10,080		2 P.M. 0.013 gm.				Light cloud	Animal refuses part of food; erythrocytes and few granular casts.
" 30	9,975	67.5 %	2 P.M. 0.013 gm.				Light cloud	Animal refuses part of food; erythrocytes and few granular casts.
" 31		65.7 %					Faint trace	Animal eats all of food again; erythrocytes, few granular casts.
June 3		57.8 %		460	7.20	1.32	0	Animal eats all of food again; erythrocytes and few pale granular casts.
" 4		78.8 %		445	8.82		Faint trace	Animal eats all of food again; erythrocytes and few pale granular casts.
" 5	9,880							Animal does not eat. Chloroformed.

was excellent, and the only symptom of intoxication observed was slight loss of appetite.

Experiments with arsenious acid were attempted, but were unsatisfactory, in that doses large enough to cause a frank nephritis were followed by immediate vomiting which prevented metabolic

study. Such lesions as were produced did not, however, alter the elimination of phenolsulphonephthalein.

CONCLUSIONS.

1. Uranium and cantharidin, in the smallest doses capable of producing a distinct nephritis, tend to increase the elimination of nitrogen, probably by stimulating tissue katabolism.

2. Uranium, cantharidin, and potassium chromate, in larger doses, impair the power of the kidney to eliminate nitrogen; but this may not be evident unless the animal is on a high nitrogen diet, and the impairment, when due to potassium chromate, may not persist more than a day.

3. Small doses of uranium and of cantharidin cause a transient increase of chloride elimination which corresponds in a general way to the excess of diuresis.

4. Large doses of uranium and of chromate cause a fall, usually transient, in the chloride elimination. The chloride elimination may, however, be diminished forty per cent. for twenty-four hours without evidences of intoxication (vomiting).

5. The anatomic appearance of the kidney varies somewhat with the poison used and greatly with the period of survival after administration of the poison, but bears no definite relation to the nitrogen, chloride, or phenolsulphonephthalein elimination; marked anatomic alteration is compatible with normal elimination of all these substances and with freedom from symptoms of intoxication (vomiting).

6. The decrease in the elimination of phenolsulphonephthalein, which occurs in uranium, chromate, and cantharidin nephritides, and which, in a general way, is proportional to the dose of the poison, bears no constant relation to the changes in the nitrogen or chloride elimination.

7. A marked decrease in the elimination of the phenolsulphonephthalein occurs synchronously, as a rule, with the onset of the symptoms of intoxication (vomiting), and therefore the phenolsulphonephthalein test would seem to be a better indicator of the ability of the kidney to eliminate the toxic substance responsible for the symptoms of renal insufficiency than are either the anatomic changes or the elimination of total nitrogen or of chlorides.