THE INFLUENCE OF AVAILABLE FLUID ON THE PRODUCTION OF EXPERIMENTAL HEMOGLOBINURIC NEPHROSIS IN RABBITS

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It has been reported in a previous paper that hemoglobinuric nephrosis can be consistently produced when rabbits are deprived of water prior to repeated intravenous injections of hemoglobin (1). Since dehydration favors the precipitation of pigment casts in the kidney tubules of rabbits given hemoglobin, studies of fluid changes in which sodium thiocyanate dissolves before and after dehydration seemed desirable. Such studies might indicate whether there is a relationship between changes in available fluid and the occurrence of hemoglobinuric nephrosis.

Method

Animals.—Male and female rabbits from pedigreed New Zealand White stock weighing 2500 to 4200 gm. were used.

Preparation of Hemoglobin.—The original method of preparation of hemoglobin was modified by the addition of ether equivalent to 1 per cent of the total volume of water and centrifuged erythrocytes. This was done to delay alteration of the hemoglobin when solutions had to be kept as long as 1 week at 4–6°C. Following the last injection, the absorption properties of the hemoglobin were determined at different wave lengths in a Coleman spectrophotometer to determine whether alterations had occurred.

State of Hydration and Method of Injection.—Water and rabbit pellets[1] were withheld for 3 days. During the following 3 days each animal received 9 or 10 intravenous injections of hemoglobin which totaled 1.8 gm./kg. During the injection period the animals were not given any food. The morning following the last injection and for 6 days thereafter the rabbits were given 30 ml./kg. of drinking water and food as desired. After the 7th day water consumption was not restricted. From the 3rd to the 6th day the control group of rabbits received water to drink equivalent to the volume given intravenously to the test group.

Blood Studies.—The method of Crandall and Anderson (2) for the determination of available fluid was modified to make the $Fe(SCN)_3$ determinations in a Coleman spectrophotometer at 460 mμ. The fluid measured by sodium thiocyanate will hereafter be referred to as available fluid as recommended by Gregersen and Stewart (3).

The non-protein nitrogen (NPN) determinations were done before dehydration and on the 8th day after the initiation of the experiment.

Urine Studies.—In 7 test rabbits the daily output of urine, specific gravity, urinary pH, and the reaction of urine to heat and 5 per cent acetic acid were recorded.

Gross Observations and Histologic Studies.—The animals which died were autopsied and studied as previously described (1). The rabbits which survived were subjected to surgical

[1] Master mix rabbit pellets (McMillen Feed Mills, Division of Central Soya Company, Inc., Fort Wayne).

removal of the left kidney 11 to 16 days after the initial injection of hemoglobin. The kidney which was removed adequately demonstrated the degree of pigment cast accumulation since the lesions are uniformly bilateral. These animals, which had been operated upon, will be studied subsequently to determine the end results of hemoglobinuric nephrosis.

TABLE I

The Effect of Reduction in Available Fluid on the Development of Hemoglobinuric Nephrosis

Rabbit No.	Sex	Normal			After dehydration			Added observations in control and test groups			
		Weight	Available fluid volume	Per cent of body weight	Per cent of body weight	Weight loss	Total hemoglobin injected	Highest NPN recorded	Gross*	Combined kidney weight	Pigment casts observed‡
		gm.	*ml.*			*gm.*	*gm./kg.*	*mg. per cent*	*days*	*gm.*	
1	M	4225	1370	32.3	22.6	563	0	47	7	20.0	—
2	M	3442	1180	35.4	30.3	429	0	45	7	15.0	—
3	M	3361	1243	36.9	30.6	239	0	47	7	18.0	—
4	F	3616	1057	29.2	28.8	467	0	50	6	20.5	—
5	M	3958	1233	31.1	24.1	412	0	49	7	17.0	—
6	M	2584	875	32.9	21.2	254	1.8	300+	7§	30.5	3+
7	M	3441	1370	38.9	25.2	378	1.8	300	8§	38.6	3+
8	M	2934	882	30.1	22.0	262	1.8	51	17	17.0	2+
9	M	3457	1310	39.2	25.3	331	1.8	300+	7§	28.8	3+
10	M	3122	968	31.0	27.2	294	1.8	82	14	20.2	3+
11	M	4059	1290	31.7	26.4	353	1.8	72	19	22.4	2+
12	M	3225	1103	35.0	29.2	260	1.8	67	16	20.2	2+
13	F	3017	910	30.2	28.1	382	1.8	53	19		2+
14	F	3297	1005	31.8	26.8	431	1.8	49	14	16.4	1+
15	F	4025	1110	27.6	23.6	264	1.8	124	16	19.8	2+
16	F	3772	867	23.5	21.1	339	1.8	300+	8§	31.2	3+
17	F	3527	1212	34.4	31.4	327	1.8	56	16	17.6	2+
18	F	4097	982	24.0	23.9	357	1.8	265	6§	37.0	3+
19	F	3870	955	24.7	23.6	371	1.8	260	19	23.6	3+
20	F	3148	784	24.8	24.4	244	1.8	48	19	14.8	1+

* Includes 3 days of water deprivation.

‡ 1+ = less than one cast; 2+ = 1 to 5 casts; 3+ = 5 to 15 casts; average of 10 low power fields.

§ Rabbit died in uremia.

Results of Water Deprivation and Intravenous Injections of 1.8 Gm./Kilo of Hemoglobin

The significant data obtained on 5 control and 15 test rabbits are tabulated in Table I. Examination of column 4 reveals that there is a wide variation of available fluid in different animals. The 9 female rabbits exhibited still greater variability; 5 of these (Nos. 15, 16, and 18 to 20) had persistently low per cent fluid volumes in repeated tests. Such low available fluid volumes were not observed in male rabbits. Gregersen and Stewart (3) in similar studies found mongrel female dogs usually had more available fluid and exhibited wider individual fluctuations than the males. They pointed out that since humidity, water intake, age, diet, and breed had not been controlled, the cause for the variation in available fluid remained unknown. In our study, all of the rabbits were of the same stock and approximately the same age; they were kept under direct observation in separate cages during the experiment, at which time they had adequate quantities of water and ate the same food. In spite of these controlled conditions, female rabbits normally exhibited wider individual fluctuations in their available fluid, and some of these had less fluid than the males.

Study of columns 5 and 6, in which the available fluid volume is expressed as percentage of body weight before and following dehydration, reveals that the rabbits respond differently to water deprivation. Eleven rabbits (Nos. 2 to 5, 10 to 15, and 17) with optimal initial values suffered minimal to moderate depletion of available fluid. Eight of these received hemoglobin and did not develop a fatal hemoglobinuric nephrosis. Five rabbits (Nos. 1 and 6 to 9) with optimal quantities of fluid initially sustained an excessive depletion (in excess of 8 per cent). Three of these 4 which received hemoglobin died from hemoglobinuric nephrosis. Four female rabbits (Nos. 16 and 18 to 20) with low available fluid volumes initially had a minimal to no depletion during dehydration. In this group 2 died of hemoglobinuric nephrosis, one developed an NPN of 260 mg. per cent and recovered, whereas the last rabbit was apparently unaffected. Two (Nos. 8 and 20) of 8 rabbits, therefore, with low available fluid volumes following dehydration did not develop fatal hemoglobinuric nephrosis or a significant elevation of NPN.

There is a good relationship between the combined kidney weight and the animals which died in uremia. The combined weight of the kidneys of rabbits dying of hemoglobinuric nephrosis is in excess of 28 gm. The kidneys from neither the control nor the test rabbits with transient hemoglobinuric nephrosis ever equaled this weight (column 11). Column 12 indicates that pigment casts were demonstrable in all of the test animals 3 to 16 days after the injection of hemoglobin. In this group 5 of 15 rabbits died of fatal hemoglobinuric nephrosis (column 10). Of the 10 rabbits which survived, all were able to withstand a nephrectomy 11 to 16 days following injections of hemoglobin without any apparent deleterious effect.

The postmortem findings generally agreed with those previously reported (1). An additional finding of considerable interest was minimal to extensive central necrosis of the liver, observed in 3 of 5 rabbits dying in uremia. None of the control rabbits had necrosis of the liver. Only 1 rabbit (No. 9) had increased amounts of pigment in the liver. There was vacuolar degeneration of liver cells in 5 rabbits which died in uremia and in 2 control rabbits. Pulmonary edema was seen in only 3 rabbits, and these died in uremia. Focal necrosis of tubular epithelium was present in 5 rabbits which died in uremia, in 1 which survived, and in none of the control group. Swollen epithelial cells with a coarsely granular cytoplasm were present in 5 rabbits which died and in 3 which survived. Dilatation of proximal convoluted tubules was present in 3 and absent in 2 rabbits which died. Dilatation was also evident in 6 of the animals which survived, and to a minimal degree in 1 of the control rabbits.

Urinary Changes Observed.—Urine studies were made on 7 of 15 test rabbits and are tabulated in Table II. When the rabbits were allowed water and rabbit pellets at desire, the reaction of the urine was always alkaline. During the dehydration period the urine output decreased. The specific gravity and pH changes were variable. Only 2 of 7 rabbits developed an acid urine. No protein was found under normal conditions or during dehydration. Following the injections of hemoglobin, 2 rabbits developed anuria, 5 rabbits continued to

TABLE II

Urine Changes Observed Following Food and Water Deprivation and Intravenous Injections of Hemoglobin

Rabbit No.	Sex	Control for 3 days			Water deprivation for 3 days			Hemoglobin injected for 3 days			
		Total urine output	Lowest specific gravity	Lowest pH	Total urine output	Lowest specific gravity	Lowest pH	Total urine output	Lowest specific gravity	Lowest pH	Proteinuria
		ml.			*ml.*			*ml.*			
6	M	250	1.040	8.2	181	1.025	6.9	0	0	0	0
7	M	456	1.030	8.2	135	1.057	8.1	0	0	0	0
8	M	261	1.030	8.2	130	1.047	6.6	111	1.037	5.6	+
10	M	371	1.035	7.8	94	1.030	7.8	180	1.026	6.0	+
11	M				234	1.034	7.9	198	1.024	5.7	+
15	F	305	1.038	8.2	95	1.042	8.2	249	1.012	5.9	+
16	F				291	1.037	7.7	306	1.013	5.7	+

Rabbit numbers correspond to those given in Table I.

urinate. One of these 5 rabbits died in uremia (No. 16). Comparison of the urinary output columns during dehydration and after injections of hemoglobin shows that in 3 animals more urine was excreted following hemoglobin injection. The specific gravity after hemoglobin is usually lower than in the previous periods. In 2 rabbits (Nos. 15 and 16) the specific gravity dropped to 1.013 and 1.012. One of these rabbits survived whereas the other died in uremia. During the period of hemoglobin injection all of the rabbits which urinated had an acid urine and a proteinuria

DISCUSSION

There is close agreement of the postmortem findings in this experiment with those previously observed (1). Additional findings, not previously described, are central necrosis and vacuolar degeneration of the liver as well as pulmonary edema. The conditions of the first experiment are not comparable to this study, since both the dosage of hemoglobin given and the period of water deprivation varied in different rabbits. The arbitrary conditions in this experiment are more severe and were identical for each rabbit. The shorter survival periods in those animals which died, the finding of central necrosis of the liver, and pulmonary edema are probably due to the modifications which were introduced.

Tubular dilatation was observed in 3 rabbits in the previous report and in 3 rabbits in this experiment which died in uremia. The presence of tubular dilatation probably indicates a mechanical obstruction and also, that filtration of fluid into the renal tubules persists for a period thereafter. Uremia which develops in such animals might be wholly attributed to the blockage of the tubules. However, the observation in 2 of 5 rabbits, in the present study, in which death occurred without tubular dilatation, suggests that other mechanisms besides mechanical blockage of renal tubules are probably functioning in hemoglobinuric nephrosis. Apparently, therefore, the widespread degeneration of tubular epithelium and the foci of tubular necrosis play a greater rôle in the production of uremia than was originally suspected.

Bywaters and Stead (4) and Flink (5) have stressed the importance of the quantity of hemoglobin injected in the production of hemoglobinuric nephrosis. In the present study a constant quantity of hemoglobin (1.8 gm./kg.) was purposely used. The results which were obtained in 15 test rabbits adequately demonstrate that in addition to the quantity of hemoglobin given, which unquestionably exerts a direct influence on the production of this syndrome, other factors of equal importance are functioning simultaneously. One factor of importance during hemoglobinemia is the presence of adequate reserves of available fluid in the organism. Flink (5) has demonstrated that intravenous injections of hemoglobin into dogs not deprived of water always produced a hemodilution. Whether the hemodilution resulted from the hemoglobin or other compounds formed during the hemolysis of erythrocytes is not known. Nevertheless, this is probably one of the means by which the organism hastens the elimination of hemoglobin. In the urine studies intravenous hemoglobin solutions were observed to exert a diuretic effect in 3 of 7 rabbits. However, when the available fluid compartment is first depleted sufficiently by water deprivation, the elimination of hemoglobin is either delayed or completely inhibited. Under such circumstances, then, the hemoglobin enters the kidney tubules, pigment casts are formed, degeneration and focal necrosis of tubular epithelium appear, and uremia follows.

Keith (6) and Elkinton and Taffel (7) demonstrated that water deprivation exerts pronounced physiologic alterations. Yorke and Nauss (8) originally pointed out that feeding dry oats to rabbits first increased the severity of hemoglobinuric nephrosis after intravenous injections of hemoglobin. Bywaters and Stead (4) by combining dehydration with acid diets and compression of leg muscles in rabbits, prior to injections of human myohemoglobin, were able to produce myohemoglobinuric nephrosis in their animals. Rigdon and Cardwell (9) found water deprivation in rabbits enhanced their susceptibility to sucrose nephrosis. In the present study it was possible to demonstrate a close relationship between the depletion of available fluid and the occurrence of hemoglobinuric nephrosis in 13 of 15 rabbits. Two rabbits with available fluid volumes in the critical range after water deprivation survived. The findings in these animals suggest that other factors besides available fluid volume are important in the elimination of hemoglobin by the organism. The mechanism by which the depletion of the available fluid exerts its influence on the production of this syndrome remains to be determined.

SUMMARY

The importance of previous dehydration on the production of hemoglobinuric nephrosis is substantiated. Hemoglobinuric nephrosis regularly occurred in rabbits 3 to 16 days following the injections of hemoglobin. Five of 15 animals died of fatal hemoglobinuric nephrosis; the combined kidney weight in these exceeded the weight of the kidneys of control rabbits and of those which survived. Additional observations, not previously made, are focal necrosis of the liver and pulmonary edema in some of the rabbits which died. A relationship was evident between the quantity of available fluid and the severity of the hemoglobinuric nephrosis which developed after injections of hemoglobin.

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