

THE ROLE OF ACIDURIA IN THE DEVELOPMENT
OF HEMOGLOBINURIC NEPHROSIS
IN DEHYDRATED RABBITS

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Experimental (1-4) and clinical (5-7) studies on the production of hemoglobinuric or myoglobinuric nephrosis indicate that multiple variables are operating. It is not known whether the formation of pigment casts in the renal tubules precedes or follows the anuria which may be observed in this syndrome (1). There is general agreement that the oliguria or anuria which is encountered is usually not due to the accumulation of pigment casts within the renal tubules (6, 8-10). It is believed that hemoglobin is relatively nontoxic, because injections of hemoglobin into normal animals have not produced hemoglobinuric nephrosis or manifest symptoms (11-14).

Since neither hemoglobin nor cast accumulation in the tubules appears to be responsible for anuria, those factors which are known to be capable of reducing the urine volume should be critically appraised. There is experimental evidence to show that oliguria or anuria can be induced by: (a) severe peripheral vascular failure ("shock") (15, 16); (b) factors causing tubular degeneration and necrosis (17, 18); and (c) dehydration (19).

Dehydration and fasting prior to and during intravenous injections of hemoglobin favor the development of hemoglobinuric nephrosis (4, 12, 20). Animals with the lowest available fluid volumes also have a greater tendency to die following injections of hemoglobin. Studies on individual rabbits with severe reductions of extracellular fluid which survived following injections of hemoglobin suggest that fluid depletion acts in conjunction with some other factor or factors. In experiments which involve fasting and dehydration in association with intravenous injections of hemoglobin it has not been determined whether fluid depletion or the associated physiologic and biochemical alterations exert the greater effect on the production of this condition.

It is known that deprivation of food or water for 5 days will produce in rabbits variable degrees of acidosis and aciduria (19) in addition to depletion of available fluid (12, 20). Since aciduria has been considered contributory in the

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production of hemoglobinuric nephrosis by some investigators (1-3), it seemed logical to the present authors to combine fluid depletion and aciduria. In a previous experiment, in which animals were fed rabbit pellets,¹ marked aciduria (pH 5.4 or less) during the period of fasting and dehydration was observed in only a few of the urine samples (20). To increase the incidence and intensify the aciduria, rabbits have now been fed an acid-producing diet to obtain a better understanding of the relative importance of the different variables.

Method

The rabbits were fed either a mixture of 3 parts by weight of ground oats and 1 part alfalfa (diet A) or ground oats (diet B), with 1 per cent NaCl in each. A group of 11 control animals was studied to determine the combined effect of acid-producing diets, coupled with 5 days of fasting and dehydration, on the acidosis, aciduria, fluid depletion, and the kidneys. Water and food were withheld for 3 days prior to intravenous injections of 5 per cent glucose in the control, or hemoglobin solution (7 to 9 gm. per cent) in the test group. These latter received a total of 1.8 gm./kils of hemoglobin intravenously in divided daily doses during the next 3 days. The hemoglobin was prepared as previously described (12). Control rabbits received equivalent amounts of fluid intravenously as 5 per cent glucose. The available fluid was determined by the sodium thiocyanate method and the values expressed as percentage of body weight (21). The non-protein nitrogen (NPN) was determined in plasma or serum specimens obtained during the control period, at autopsy, or on the 5th day following the initial injection (22). The carbon dioxide-combining power was determined by the Van Slyke method during the control period and after 3 days of dehydration and fasting (23). The 24 hour urine volume, specific gravity, and pH were determined for 3 days of the control period and also during fasting and dehydration. The specific gravity was ascertained with a hydrometer and the pH with a glass electrode. Changes in weight were followed from the time the animals were placed on the diets until they were weighed at autopsy. Usually the rabbits consumed minimal quantities of oats or oats and alfalfa for the first 10 days, and only after 2 weeks was uniformity attained in food consumption and urine volume excretion. The effect of the urine on hemoglobin solubility was established *in vitro* by mixing 5 ml. of filtered urine with 0.5 ml. of the hemoglobin solution used for injection (24). After agitation the mixture was observed at room temperature for 30 minutes for turbidity (+) or the appearance of coarse aggregates (++) . Autopsies were performed on rabbits which died during the course of the experiment or which were killed within 13 days after the initial injection. Pigment cast accumulation was expressed as an average of the total numbers observed in ten low power fields in sections taken from each kidney.

RESULTS

It is evident from Table I that in the control animals the diets mentioned above, when combined with fasting and dehydration, produced significant losses in weight. The carbon dioxide-combining power was lowest in the animals which had been on diet B for 54 days. Two out of 11 rabbits died during the period of fasting and dehydration and one on the 5th day after. The highest NPN values occurred in the rabbits which died. Acid diets, fasting, and de-

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

hydration may cause death with or without necrotic changes in the kidneys and liver. Focal necrosis of the liver was encountered in 2 of the 3 rabbits which died, and focal hemorrhages in 2 of the rabbits which survived until sacrificed. Hyaline casts in the collecting tubules and interstitial medullary edema were observed in all the rabbits. Increased granularity, swelling, and vacuolization of

TABLE I
The Effect of Acid-Producing Diets and 5 Days of Fasting and Dehydration on the Organs of Rabbits in the Control Study

Rabbit No.	Diet	Weight loss	Time on diet	Autopsy*	CO ₂	Highest NPN recorded	Kidneys			Liver
							Combined weight	Tubular necrosis	Hyaline casts	
		<i>per cent body weight</i>	<i>days</i>	<i>days</i>	<i>vols. per cent</i>	<i>mg. per cent</i>	<i>gm.</i>			
1	A	14.0	17	6	30	41	13.6	—	+	—
2	A	16.7	17	6	28	43	13.7	—	+	Focal hemorrhage
3	A	17.9	17	6	22	25	12.2	—	+	Focal hemorrhage
4	A	28.1	17	4†		148	11.5	—	+	—
5	B	22.6	26	9	35	32	11.6	—	+	—
6	B	25.2	26	9	44	80	11.2	—	+	—
7	B	20.0	26	4†	32	90	13.8	+	+	Focal necrosis
8	B	24.0	26	9	44	56	15.6	—	+	—
9	B	29.4	54	6†	15	144	11.5	—	+	Focal necrosis
10	B	28.2	54	7	20	60	9.4	—	+	—
11	B	23.3	54	7	19	58	13.6	—	+	—

* Days counted from time of food and water deprivation.

† Indicates animals died.

epithelial cells of moderate to marked degree were also observed in every section of the proximal convoluted tubules and Henle's loops.

The influence of acid diets, fasting, and dehydration on urine pH, specific gravity, and *in vitro* hemoglobin solubility in the control rabbits is shown in Table II. The first 2 columns show values encountered during the control period, while the following 3 days represent data obtained during the period without food and water.

Examination of the data obtained during the control period shows that the acid diets produced an aciduria of 5.8 or less in 7 of the 11 rabbits. The animals with the greater aciduria were given only oats and were fed for relatively long periods of time (see Table I). When the rabbits had water *ad libitum*, the speci-

fic gravity of the urine was variable, but in 18 of 21 samples it was less than 1.030. With an unlimited water and food intake, hemoglobin, when mixed with the urine *in vitro*, gave rise to turbidity or aggregates in 3 samples. No effect on hemoglobin solubility was evident in 7 urine samples with a pH of 5.4 or less when the specific gravity was less than 1.030. During the fasting and dehydration period, the aciduria tends to become more pronounced and the specific gravity of the urine increases. Owing to these combined changes the urine acquires a greater ability to render hemoglobin insoluble. This is evident

TABLE II
Relationship of Urine pH, Specific Gravity, and in Vitro Hemoglobin Solubility in the Control Rabbits

Rabbit No.*	pH					Specific gravity					<i>In vitro</i> hemoglobin solubility					
	Control		No food or water			Control		No food or water			Control		No food or water			
	1	2	1	2	3	1	2	1	2	3	1	2	1	2	3	
	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>	<i>days</i>
1	7.8	7.9	6.3	0	5.7	1.012	1.027	1.019	0	1.032	-	-	-	-	-	+
2	7.5	7.7	6.7	5.0	5.3	1.020	1.016	1.028	1.028	1.036	-	-	-	++	+	
3	8.7	8.1	6.6	5.2	5.1	1.007	1.012	1.020	1.034	1.034	-	-	-	+	++	
4	7.5	8.6	6.6	5.5	6.5	1.010	1.017	1.024	1.033	1.026	-	-	-	-	-	
5	5.4	4.9	5.6	5.0	4.8	1.028	1.039	1.025	1.022	1.028	-	++	-	-	++	
6	5.8	5.2	5.0	4.9	5.0	1.031	1.030	1.033	1.032	1.032	-	+	++	+	++	
7	5.4	5.1	5.3	4.9	5.2	1.006	1.008	1.014	1.031	1.033	-	-	-	++	++	
8	5.0	4.9	5.1	4.9	4.9	1.012	1.022	1.017	1.013	1.031	-	-	-	-	+	
9	0	5.6	5.7	5.3	4.9	0	1.023	1.015	1.021	1.029	0	-	-	-	-	
10	5.0	5.2	5.3	0	4.9	1.012	1.011	1.005	0	1.030	-	-	-	0	++	
11	5.2	5.6	5.5	5.0	5.1	1.026	1.022	1.025	1.029	1.029	+	-	-	-	+	

0, no urine available; -, no reaction; +, turbidity; ++, coarse aggregates.

* Numbers of animals correspond to those observed in Table I.

in Table II, the hemoglobin coming out of solution in 9 of 10 samples on the 3rd day of fasting and dehydration. In a previous study concerning the influence of human urine on hemoglobin solubility *in vitro*, it was observed that turbidity or coarse aggregates usually appeared when the pH was 5.4 or less and the total anion concentration exceeded 125 m.eq./liter. In 29 rabbits from both groups of the present study the urine precipitated the hemoglobin in 51 of 172 samples. In these 51 urine tests the pH was 5.4 or less in 47, and less than 5.7 in 4. The studies show that urine may influence hemoglobin solubility directly and suggest that dehydration enhances the effect of aciduria by increasing the urine specific gravity.

The effect of diet alone in the control group shows these animals to have developed acidosis and aciduria; hyaline casts appeared in the collecting tubules;

and 3 of 11 rabbits died during the course of the experiment. In the rabbits which died the NPN did not exceed 148 mg. per cent and the combined kidney weight was 15.6 gm. or less. The criteria used in the diagnosis of hemoglobinuric nephrosis in the test animals were the presence of two or more of the following: an NPN of 190 mg. per cent or above, a combined kidney weight of 16.0 gm.

TABLE III
The Influence of Acid-Producing Diets, Fasting, and Dehydration on Intravenous Injections of Hemoglobin in Rabbits

Rabbit No.	Sex	Diet	Weight loss	Time on diet	CO ₂ after 3 days without food and water	NPN	Autopsy	Kidneys		
								Com- bined weight	Tubu- lar ne- crosis	No. of pigment casts*
			<i>per cent</i> <i>body weight</i>	<i>days</i>	<i>vols. per cent</i>	<i>mg. per cent</i>	<i>days</i>	<i>gm.</i>		
12	M	A	20.0	21		440	7‡	20.1	+	318
13	M	A	8.5	21		78	4‡	16.8	+	314
14	M	A	25.5	21		368	8‡	21.5	—	299
15	M	A	18.9	21		278	8‡	27.8	+	414
16	F	A	40.0	32	11	282	10‡	17.6	—	163
17	F	A	41.0	32	16	94	13‡	13.9	—	3
18	F	A	6.4	32	24	26	13	15.8	—	13
19	F	A	32.6	32	20	316	8‡	22.7	+	281
20	F	B	18.8	42	22	300+	8‡	19.2	+	238
21	F	B	27.5	42	14	91	4‡	10.5	—	10
22	M	B	21.6	42	38	98	4‡	14.1	—	17
23	M	B	31.2	42	33	275	11‡	22.6	—	145
24	M	B	23.8	17	48	48	10	15.8	—	54
25	F	B	23.5	17	33	198	5‡	19.4	—	166
26	F	B	34.8	17	24	225	7‡	19.2	—	281
27	F	B	22.1	17	40	32	10	14.2	—	0
28	F	B	26.0	17	23	135	5‡	23.6	—	329
29	M	B	23.2	17	48	38	10	15.1	—	2

* Number of casts represents total observed in 10 low power fields.

‡ Animal died. Others were killed on the day indicated. Days were counted from the initial hemoglobin injection.

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or more, and pigment cast counts of 145 or above; in their lack, the animal's death was attributed to acidosis and dehydration.

The results in 18 test rabbits are shown in Tables III and IV. These animals were subjected to the same conditions as the controls and in addition received a total of 1.8 gm./kilo of hemoglobin intravenously.

In the group which was maintained on acid diets for periods of 17 to 42 days preceding the final 5 days of fasting and dehydration, the weight loss varied from 6.4 to 41.0 per cent. The diets and fasting exerted a variable effect on the carbon dioxide-combining power. The NPN levels were in excess of

190 mg. per cent in 9 of 18 rabbits. Fourteen rabbits died, in 3 of which (Nos. 17, 21, and 22) the elevation of NPN, increase in kidney weight, and pigment cast accumulation were insufficient for their death to be ascribed to hemoglobinuric nephrosis. One of these animals had intestinal intussusception, the others presumably died of acidosis and dehydration. Rabbits 13 and 28 were

TABLE IV
The Relationship of Fatal Hemoglobinuric Nephrosis to Changes in the Available Fluid and in Vitro Hemoglobin Precipitation in Urine

Rabbit No.*	Sex	Fatal hemoglobinuric nephrosis	Available fluid per cent body weight		Hemoglobin precipitation in the urine					
			Control	After 3 days without food or water	Control			During no food or water		
					1	2	3	1	2	3
					days	days	days	days	days	days
12	M	+	21.9	22.1	-	-	-	-	-	-
13	M	+	22.6	22.3	-	-	-	-	+	++
14	M	+	27.8	28.2	-	-	-	-	++	+
15	M	+	34.4	31.3	-	-	-	-	-	+
16	F	+	33.8	28.0	-	-	-	-	-	+
17	F	-	34.2	24.7	-	-	-	-	-	-
18	F	-	31.0	26.2	-	-	-	-	+	+
19	F	+	28.4	25.5	-	-	+	-	-	+
20	F	+	21.2	20.5	-	-	+	-	-	+
21	F	-	29.0	26.2	-	-	-	-	-	-
22	M	-	27.4	24.9	-	-	-	-	-	-
23	M	+	20.2	24.2	+	+	++	++	++	++
24	M	-	21.2	23.2	-	-	-	-	-	-
25	F	+	22.7	24.9	-	-	-	-	-	-
26	F	+	21.3	-	-	-	-	-	-	+
27	F	-	20.9	23.3	-	-	-	-	-	+
28	F	+	20.3	22.3	-	-	-	-	-	-
29	M	-	23.6	23.6	-	-	-	-	-	-

* Numbers correspond to those in Table III.

included in the hemoglobinuric nephrosis group despite their low NPN values, because of the increased kidney weight and pigment cast counts. The combined kidney weights exceeded 16 gm. in 11 of 18 rabbits. Microscopic examination of the kidneys indicated that focal tubular necrosis occurred in 5 of 11 rabbits which died of hemoglobinuric nephrosis. The kidney sections of the other 6 rabbits showed the presence of moderate to severe swelling of the epithelial cells in the proximal convoluted tubules, with vacuolization and increased granularity of the cytoplasm. Interstitial medullary edema was observed in all sections. In 11 rabbits the average pigment cast counts were 145 or more,

whereas in the 4 rabbits which survived (Nos. 18, 24, 27, and 29) and the 3 which died of other causes (Nos. 17, 21, and 22) the cast counts varied from 0 to 54. In contrast to previous experiments, there were greater numbers of hyaline casts in the collecting tubules; these have not been included in the table. Dilated Henle's loops and proximal convoluted tubules were observed in only 2 of 11 rabbits.

Studies of available fluid and the *in vitro* effect of urine on hemoglobin solubility in the different animals are shown in Table IV. In contrast to previous studies (12, 20) in which the rabbits were fed rabbit pellets, a larger number (10 of 18 rabbits) in the present study had unusually low available fluid volumes prior to fasting and dehydration. This condition we attribute to the acidotic influence of the diet. These observations are in agreement with Kerpel-Fronius' conclusions that animals with hyponatremia can develop dehydration without water restriction (25). A second observation which is in contrast to previous findings is that the depletions in available fluid were not so great during fasting and dehydration. This may be attributable to the relatively lower available fluid volumes in 10 of 18 rabbits observed during the control period. In this study fluid depletion was complicated by greater degrees of aciduria than were previously encountered, and the relationship between low available fluid volumes and the development of fatal hemoglobinuric nephrosis was not so pronounced (12). This suggests that fluid reduction to the extent encountered in these experiments exerts a secondary influence on the production of hemoglobinuric nephrosis.

Urine tests were substituted for pH readings in Table IV because we wished to learn whether any relationship could be established between alterations in hemoglobin solubility *in vitro* and death after hemoglobin injections. Ten of 18 rabbits excreted a urine sufficiently acid (pH 5.4 or less) and of concentration sufficient (specific gravity 1.030 or more) either to render solutions of hemoglobin turbid or to form aggregates during the fasting and dehydration period. In 11 rabbits which died of fatal hemoglobinuric nephrosis the urine affected hemoglobin solubility in eight instances. In 7 rabbits which survived or died of other causes turbidity *in vitro* was observed in two instances. No definite conclusions can be reached regarding the effect of aciduria on the production of hemoglobinuric nephrosis from *in vitro* studies. However, it may be of interest to reaffirm that urine can render hemoglobin insoluble at times, and that such changes can conceivably expedite the formation of pigment casts in the renal tubules.

DISCUSSION

The microscopic changes observed in the kidney were similar to those seen in previous experiments (12, 20). A variation not previously encountered was the presence of greater numbers of hyaline casts. Tubular dilatation was neither

so prominent nor frequent as in a previous study (4). Since the histological description has been given in previous publications, it will not be considered in greater detail at present. We believe that a review of some of the theories which have been proposed to explain the occurrence of hemoglobinuric nephrosis would be more appropriate. With this purpose in mind, we wish to point out how the present results confirm some of the hypotheses which have been advanced.

In such an analysis the most suitable experiments seem to be those in which acceptable renal lesions were produced and the animals developed uremia prior to death. A survey of the literature, with the above restrictions in mind, demonstrates that fatal hemoglobinuric nephrosis has been produced with difficulty and inconsistently. We wish to emphasize this fact now, because it is believed that this point has an important bearing on the pathogenesis of hemoglobinuric nephrosis.

A summary of some of the results reported is tabulated in Table V. There are included in this table only those factors which are considered pertinent to the present discussion. In one instance there was insufficient evidence for the conclusion that the animals died of hemoglobinuric nephrosis (26). Associated factors which have been considered important in the production of hemoglobinuric nephrosis are: (a) peripheral vascular failure secondary to hemorrhagic "shock" (27-29, 14); (b) plasma hemoglobin concentration (26); (c) antecedent tubular injury (2, 30); (d) dehydration (4, 12, 20) or a reduced urine volume (14, 31); (e) aciduria alone (32) or associated with trauma (1). Experiments illustrating these hypotheses and the incidence of fatal hemoglobinuric nephrosis are shown in Table V.

Yorke and Nauss succeeded in producing fatal hemoglobinuric nephrosis in all the 9 rabbits that were fed an oat and bread diet and bled prior to massive injections (23 gm./kilo) of hemoglobin (27). They concluded that antecedent bleeding predisposed to hemoglobinuric nephrosis. Flink produced death in 2 of 6 dogs with aciduric urine that were given large doses (up to 6.15 gm./kilo) of intravenous homologous hemoglobin (26). It seems more likely that Yorke and Nauss' success was due to the massive doses of intravenous hemoglobin injected, rather than to the antecedent bleeding. The importance of the observations is questionable since, as pointed out by Yuile *et al.*, equivalent amounts of hemolysis in human beings are unusual (33). In support of the "shock" hypothesis, Corcoran and Page believe that hemorrhagic or tourniquet "shock" capable of producing tubular injury could predispose to hemoglobinuric nephrosis (28). Corcoran and Page, however, advanced this hypothesis after functional studies in which fatal hemoglobinuric nephrosis was not produced. Bywaters and Popjak were unable to produce myoglobinuric nephrosis by tourniquet "shock" alone (34). Phillips *et al.* did not succeed in producing uremia with traumatic or hemorrhagic "shock" (15). Bywaters and Stead succeeded only after combining tourniquet "shock" or aciduria with injections of myoglobin (1). Hemoglobinuric nephrosis did not develop when Hamilton, Hiller, and Van Slyke first bled their dogs

TABLE V
The Influence of Various Conditions on Fatal Hemoglobinuric or Myoglobinuric Nephrosis as Reported by Different Authors

Author and reference	Species	Diet	Water	Additional laboratory procedures	Material injected and dosage	Fatal hemoglobinuric nephrosis	
						Acid urine	Alkaline urine
Yorke and Nauss (27)	Rabbit	Oats or vegetables	Restricted for 12 hrs.	Bled unknown volume	<i>gm./kg.</i> Hemoglobin 23	9/9*	
Corcoran and Page (28)	Dog	Casein and meat	<i>Ad libitum</i>	NaH ₂ PO ₄ added	Methemoglobin 0.06-0.25.	0/10	
					Hemoglobin 0.19-0.65	0/3	
Hamilton <i>et al.</i> (35)	Dog	Fasted 18 hrs.	<i>Ad libitum</i>	Bled 50 ml./kg.	Hemoglobin 3.5	0/7	
Baker and Dodds (32)	Rabbit	Dry grain or vegetables	<i>Ad libitum</i>		Hemoglobin 10 ml./rabbit	0/1	0/1
DeGowin <i>et al.</i> (3)	Dog	Meat and vegetables	<i>Ad libitum</i>	NH ₄ Cl added	Hemoglobin 3-5	7/23	0/4
Bywaters and Stead (1)	Rabbit	Dry oats	<i>Ad libitum</i>	NH ₄ Cl added	Myoglobin 0.08-0.24	2/13	
Bywaters and Stead (1)	Rabbit	Dry oats	Limited to 100 ml./day	NH ₄ Cl added	Myoglobin 0.08-0.24	2/12	
Yuile <i>et al.</i> (2)	Rabbit	Purina chow or oats	<i>Ad libitum</i>	Unilateral nephrectomy and renal artery occlusion	Hemoglobin 0.45-0.74	2/4	0/3
Yuile <i>et al.</i> (2)	Rabbit	Same	<i>Ad libitum</i>	Unilateral nephrectomy and sodium tartrate	Hemoglobin 0.45-0.74	2/4	0/4
DeNavasquez (13)	Rabbit	Oats or vegetables	<i>Ad libitum</i>		Hemoglobin 0.7-1.05	0/13	0/5
Bing (11)	Dog	Not specified	<i>Ad libitum</i>	NH ₄ Cl added	Methemoglobin 3-13 gm./animal	13/15‡	0/7
Flink (26)	Dog	Not specified	<i>Ad libitum</i>	NH ₄ Cl or NaHCO ₃ added	Hemoglobin 2.3-6.1	2/6	0/7
Lalich (20)	Rabbit	Rabbit pellets	No water for 5 days	No food for 5 days	Hemoglobin 1.8	4/8	

* Fraction 9/9 signifies that 9 of 9 animals died of hemoglobinuric nephrosis.

‡ Animals became moribund before they were killed.

and subsequently injected 3.5 gm./kilo of hemoglobin solution (35). In contrast to clinical evidence which strongly supports antecedent hemorrhagic "shock" as a predisposing factor, experimental studies in support of this theory have been inconclusive or negative.

Yuile, Gold, and Hinds (2) and Badenoch and Darmady (30) observed collections of pigment casts only when unilateral nephrectomy and temporary occlusion of the renal artery of the remaining kidney preceded intravenous injections of hemoglobin. It is apparent from the above experiments and others that fatal hemoglobinuric or myoglobinuric nephrosis is hard to produce following intravenous injections since it occurs only after antecedent direct manipulation of the kidneys (2, 30), tourniquet "shock" or aciduria (1), or a protracted restriction of food and water (12, 20). These observations, we believe, suggest that the physiologic, chemical, and anatomic alterations which precede the hemoglobinemia are the deciding factors in the development of this syndrome. The hemoglobin which is injected probably aggravates the antecedent tubular injury. However, the manner in which this occurs is not evident.

Baker and Dodds were the first to demonstrate *in vitro* that acid urine renders hemoglobin insoluble. They proposed the view that the aciduria was directly responsible for the hemoglobinuric nephrosis (32). This assumption has been substantiated in part (1-3), with the reservation that aciduria is not the only contributing factor. In this respect it may be of interest to point out (see column 7 of Table V) that in experiments in which urine studies were made, fatal hemoglobinuric nephrosis occurred only in animals with acid urine. Other workers have studied the effect of aciduria under different conditions and have concluded otherwise (11, 13, 26). When one appreciates that the acidosis and aciduria in the different experiments were of variable duration and severity, that the quantities of injected hemoglobin differed, and that the fluid balances were not comparable, the cause of such contradictory conclusions is apparent.

With but a single exception the conditions in the present study were the same as in our previous experiments: an acid-producing diet was substituted for rabbit pellets prior to the fasting and dehydration. Acid-producing diets, when combined with 5 days' deprivation of food and water, resulted in lower available fluid volumes in more rabbits, and greater degrees of aciduria, as well as a higher mortality rate from hemoglobinuric nephrosis. Even though a few of the animals with aciduria survived, in the majority the severe and protracted aciduria predisposed to death from hemoglobinuric nephrosis. Since it is evident that aciduria, when acting in conjunction with fasting and dehydration, does predispose to hemoglobinuric nephrosis, a consideration of possible mechanisms whereby aciduria may exert its influence is indicated.

It has been shown that a concentrated (specific gravity 1.030 or more) and an acid (pH 5.4 or less) urine usually renders hemoglobin insoluble *in vitro*. There is evidence that acid-producing salts, for example oxalates (18), NH_4Cl (36), and CaCl_2 (37), when administered in sufficient concentration, cause tubular degeneration and necrosis. We have succeeded in producing tubular changes of less severity in our control animals by substituting acid diets and 5 days of

fasting and dehydration for the acid salts. The increased mortality observed under such circumstances over others (12, 20) is probably due to the antecedent tubular injury caused by fasting, dehydration, and protracted aciduria. The effects of protein or vitamin deficiency have not been excluded in this study. However, the experimental evidence here presented implicates aciduria as an important contributing factor in the production of this syndrome.

Many observations indicate that tubular damage is associated with the development of hemoglobinuric nephrosis. Tubular degeneration or focal necrosis has been an invariable finding in clinical (5, 7) and experimental studies (4, 12, 20). The functional significance of such microscopic changes is not clear, nor has it been determined whether the tubular alterations precede or are caused by pigment casts. It was possible to demonstrate in a previous experiment that a close relationship exists between oliguria during the injection period and fatal hemoglobinuric nephrosis (20). Of the 4 rabbits which died, 3 had focal necrosis of tubular epithelium and one had severe degeneration of the epithelial cells. In the present study focal necrosis of tubules was observed in 5 of 11 rabbits and moderate to severe degeneration in the others. Our studies suggest that hemoglobinemia is not the primary factor in hemoglobinuric nephrosis, since pigment casts apparently are formed only when the hemoglobinemia is associated with antecedent tubular injury.

Richards has shown that anuria caused by poisoning frog tubules with HgCl_2 is due to the uninhibited back diffusion of water and electrolytes from the renal tubules (17). Bywaters and Dible have explained anuria on the basis of functionally damaged tubular epithelium which permits the unselective reabsorption of glomerular filtrate (38). Our studies support this hypothesis and, along with the work of others (1, 2, 30), suggest that tubular injury is necessary for hemoglobinemia to produce hemoglobinuric nephrosis. In the experiments here reported the diet and the deprivation of food and water produced acidosis, aciduria, and fluid depletion, which changes are believed to cause tubular degeneration or necrosis and thereby predispose to hemoglobinuric nephrosis. The inability to produce this syndrome consistently in animals is due, we believe, to the erroneous assumption that tubular changes are caused by pigment casts rather than that the antecedent tubular injury predisposes to the precipitation of casts. It is concluded on the basis of literature (1, 2, 30) and our own studies that the antecedent tubular damage is of primary importance in the pathogenesis of hemoglobinuric nephrosis.

SUMMARY

The effect of acid diets, fasting, and dehydration on the urine pH and the kidneys was determined in 11 control animals. Hyaline casts in the collecting tubules and interstitial medullary edema were observed in the 8 that survived. The test rabbits were subjected to the same treatment as the controls but, in

addition, received 1.8 gm./kilo of intravenous homologous hemoglobin. Eleven of 18 animals died of hemoglobinuric nephrosis. The rabbits which died of hemoglobinuric nephrosis exhibited significant alterations in two or more of the following: kidney weight, pigment cast accumulation, and elevations of NPN.

The theories which have been advanced to explain the pathogenesis of hemoglobinuric nephrosis are evaluated in the light of the present observations. It is proposed on the basis of them that antecedent tubular damage is of primary importance in the pathogenesis of hemoglobinuric nephrosis.

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