HEMOGLOBIN PRECIPITATION IN RENAL TUBULES

A Study of Its Causes and Effects

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Renal failure develops frequently in association with transfusion reactions, blackwater fever (12, 20), the "crush syndrome" (5), and a variety of other conditions characterized by marked intravascular hemolysis (21). Three features are more or less common to all of these, namely, the presence of hemoglobin or related pigments circulating freely in the plasma, the coexistence of some factor or factors of a vascular, toxic, or chemical nature affecting the organism as a whole, and the occurrence of a relatively specific kidney lesion found at autopsy in the fatal cases. These renal lesions are remarkably similar and consist essentially of varying degrees of epithelial degeneration and necrosis in the tubules of the cortex, associated with the presence of pigmented casts in the ascending limb of Henle's loop, the distal convoluted segment, and the collecting tubules (2, 5, 12, 21). That the mechanism involved in the production of this type of renal failure has remained a mystery despite the efforts of many investigators would appear to be due, at least in part, to the difficulties encountered in attempting to produce a similar lesion in the kidneys of experimental animals.

Experimental attempts to elucidate the factors responsible for the production of these lesions have been concerned largely with the formation of casts of hemoglobin or related pigments in laboratory animals with normal kidneys. Successful results have been reported only in animals with acid urine, by Baker and Dodds (3) following the intravenous injection of hemoglobin into rabbits, by De Gowin and coworkers with repeated injections of large amounts of laked red blood cells into dogs (7, 8), by Bywaters injecting myoglobin into rabbits (6), by Anderson *et al.* with ferrihemate in dogs (1), and by Bing with methemoglobin in dogs (4). In sharp contrast to the above, de Navasquez (9) and the authors of the present study have both failed to reproduce the results of Baker and Dodds in rabbits, and Bing (4) was unable to detect any functional or pathological changes following the injection of hemolyzed red blood cells, crystalline hemoglobin, or metmyoglobin into dogs with acid urine. Although some of these discrepancies can probably be explained on the basis of differences in technique

or animal species, it seems safe to conclude that pure hemoglobin is precipitated with difficulty, if at all, in the normal kidney. It follows logically that hemoglobinemia *per se* is in all probability not the basic factor in the production of the renal lesions seen in the clinical states under consideration.

The associated tubular damage encountered in these lesions has been variously attributed to the effects of the precipitated pigments, to anaphylactoid disturbances (16), spasm of the renal vessels (17, 19), and to the nephrotoxic action of exogenous or endogenous substances. This aspect of the problem has given rise to much speculation, but has seldom been approached experimentally. Dunn *et al.* (10) discovered alloxan diabetes while attempting to use this chemical as a selective renal tubular poison before injecting hemoglobin. They reported briefly that pigment casts were formed. Scarff and Keele (22) demonstrated that severe tubular necrosis and profound functional disturbances occur following temporary occlusion of the renal circulation for periods of 60 to 120 minutes. The changes were stated to be very similar to those seen in human cases of "crush kidney syndrome," and these authors also suggested that similar changes might occur as the result of severe or prolonged partial renal ischemia. The fact that a marked reduction in blood flow through the kidney appears to exert its maximum effect on the highly specialized proximal convoluted segment is not surprising and would seem to be highly significant in view of the possibility of such an occurrence during the development of at least some of the lesions discussed above.

It is noteworthy that the histological appearance of the kidney following temporary occlusion of the renal circulation bears a striking resemblance to the acute degenerative tubular lesions produced by a variety of chemicals, particularly since a chemical nephrotoxic action is a definite probability in certain human cases with fatal hemoglobin reactions.

In the present study, hemoglobin has been injected into rabbits with alkaline and acid urine, whose renal tubules had previously been damaged to a moderate degree, (*a*) by the application of a clamp to the renal pedicle for a period of time considerably shorter than 1 hour, and (*b*) by the administration of a specific chemical poison, sodium tartrate. Under these conditions hemoglobin casts occurred regularly in the animals having acid urine, with the production of a pathological picture closely resembling the "transfusion kidney," thus permitting an evaluation of the functional disturbances resulting from hemoglobin precipitation.

Methods

For this study white domestic rabbits were employed. The average age of the animals ranged from 4 to 6 months, and their weight from 2.0 to 3.2 kilos (average 2.75). The animals were kept in metabolism cages, and were divided into two main groups according to diet. One group was maintained on rabbit chow (Purina) and vegetable scraps, resulting in the production of normal (alkaline) urine. The second group received only a mixture of oats and stale bread, and were thus rendered acidotic. Water *ad libitum* was allowed both groups. Urine volumes were measured for 24 hour periods and, in the case of the male animals in each group,

catheter specimens were obtained to determine the degree of acidity. This was measured by the use of nitrazine papers (Squibb) and was found to lie between levels of pH 5 and 5.5 for the acidotic animals and between levels of pH 8 and 8.5 for the normal rabbits.

Freshly citrated rabbit's blood, obtained by cardiac puncture from donor animals, was used to prepare hemoglobin free from cell stroma and other by-products of hemolysis, according to the method already described (18). The concentration of these solutions was determined photoelectrically, according to the method of Evelyn and Malloy (11). Doses of hemoglobin, ranging from 1.32 to 2.35 gm., contained in volumes with a corresponding range from 8 to 16 cc., were injected into marginal ear veins. These quantities were calculated to correspond to the amount released by complete intravascular hemolysis of from 200 to 400 cc. of blood in a human being weighing 150 pounds. The solutions were brought to isotonicity with rabbit's blood by the addition of the required amounts of a standardized sodium chloride solution. Alternately in some instances, and particularly if the volumes were small, the solutions were injected without added sodium chloride, on the assumption that the quantities injected were small in comparison with the total blood volume and would cause no additional hemolysis.

Selective tubular damage was obtained by two methods. In the first, a simple mechanical occlusion of the renal artery was performed by the application of a clamp to the renal pedicle for periods of 15 or 25 minutes. Hemoglobin was injected 5 minutes prior to the removal of the clamp. A preliminary series of experiments, with only the left kidney pedicle clamped, furnished material for histological studies, the right kidney providing the control. The definitive experiments, however, were carried out upon animals which had undergone unilateral nephrectomy (right) 1 to 2 weeks before the clamping procedures and had subsequently recovered with a stabilized level of blood non-protein nitrogen. All these occlusion experiments were performed on rabbits anesthetized with intravenous sodium nembutal and ether by inhalation.

The second method of causing selective tubular lesions was by the subcutaneous injection of a 20 per cent solution of sodium tartrate, in a dosage of 0.8 to 0.95 gm. per kilo of body weight, into non-anesthetized animals.

Non-protein nitrogen values upon daily samples of 0.5 cc. of blood obtained from the artery of the ear, were determined by a modification of the method described by Hoffman (14). All photoelectric determinations were made with a luxtrol colorimeter.

Tissues for histological study were fixed in 10 per cent formol-saline for staining with hematoxylin and eosin, and in a solution of 3 per cent potassium ferricyanide in 10 per cent formalin for the special hemoglobin stain described by Lison (15).

EXPERIMENTAL OBSERVATIONS

The possibility of precipitation in the *normal* kidney of the relatively pure hemoglobin solutions used in this study was eliminated by failing to find any evidence of renal cast formation within 24 hours following preliminary injections into two animals with acid and two with alkaline urine.

In the first experiments, designed to reproduce and to study only the morbid anatomy of the "transfusion kidney," the left renal pedicle was clamped for 15 minutes in animals with acid and alkaline urine respectively. Controls killed 24 hours later showed minimal changes in the clamped kidney. These lesions consisted of swelling, pallor, and granularity of the epithelium of the proximal convoluted tubules. The intensity of these changes varied slightly from one animal to another, but was independent of the pH of the urine. The kidneys which were not clamped appeared normal. In all animals with acid

urine, given hemoglobin intravenously 5 minutes before removal of the clamp and killed 24 hours later, and in two out of three in which it was alkaline, casts were found in the clamped kidneys, chiefly in the distal convoluted and collecting tubules. These casts were reddish-brown in color and granular in the ordinary histological preparations. They gave a negative Prussian blue reaction, but with special staining were strongly positive for hemoglobin. Hemoglobin casts, when present in the animals with alkaline urine, were never as numerous as in the acid group. The unclamped kidneys of the former also contained a small number of hemoglobin casts.

In a second series of experiments, the rabbits were again divided into acid and alkaline groups and given injections of sodium tartrate. Control animals were killed 48 hours after receiving the sodium tartrate, while the others were given hemoglobin intravenously at that time, and killed 15 to 18 hours later.

In an effort to obtain a moderate degree of tubular damage, an average optimum dosage of 0.85 gm. of sodium tartrate per kilo of body weight was determined by trial and error, based upon the work of Friedman and Kaplan (13). Considerable variation in the response of the individual rabbits with both acid and alkaline urine was noted. In general, however, the degree of damage was found to be consistently greater in the acid group, so that it was frequently necessary to administer up to 0.95 gm. per kilo to animals with alkaline urine in order to obtain comparable results. A satisfactory lesion was obtained when edema and minimal necrosis of convoluted tubular epithelium, together with some hyaline cast formation in the lower part of the nephron, were seen.

In the animals given hemoglobin, typical hemoglobin casts were found in the ascending limb of Henle's loop, in the distal convoluted tubules, and in the collecting tubules, in addition to the changes already described as due to the sodium tartrate. While these occurred in both groups of animals, they were much more numerous in those with acid urine than when it was alkaline. Another striking observation in this experimental series was the great scarcity of hemoglobin casts when the initial tartrate injury was severe, in contrast to the large numbers found when the damage was mild or moderate.

The remaining experimental observations were concerned with the disturbances in renal function which accompany the anatomical changes described in the preceding paragraphs. The experiments fall into three main divisions, in all of which blood non-protein nitrogen and, in most instances, urinary output were studied. In the first and second groups of these "functional" experiments, the left renal pedicle was clamped, in animals previously subjected to right unilateral nephrectomy, for periods of 15 and 25 minutes respectively. The third group comprised animals subjected to renal injury by the subcutaneous administration of sodium tartrate. In all three groups, the effect of hemoglobin injection was observed. In each test animals with alkaline as well as acid urines were employed.

Table I summarizes the results following application of the renal pedicle clamp for a period of 15 minutes. No significant rise was noted in the non-protein nitrogen values of the two rabbits with alkaline urine despite the fact that No. 3-1 received 1.6 gm. of hemoglobin. In the acid group, however, there was a striking difference between the control rabbit 3-3 whose non-protein nitrogen level remained unchanged, and rabbits 3-2 and 4-3 in which the values rose abruptly to maximum levels of 170 and 163 mg. per 100 cc. Text-fig. 1 graphically illustrates the course of the non-protein nitrogen concentration plotted against the day of the experiment in this series. The kidney of rabbit 4-3, accidentally killed on the 6th day, during recovery, showed hemoglobin

TABLE I

Summary of 15 Minute Clamping Experiments

Rabbit No.	Weight	Reaction of urine	Renal pedicle clamped	Hemoglobin injected	Effect on blood N.P.N.	Effect on urine volume	Remarks
	kg.		*min.*	*gm.*			
3-0	2.02	Alk.	15	0	Unchanged	Not determined	Recovered
3-1	3.18	Alk.	15	1.60	Unchanged	Not determined	Recovered
3-3	3.25	Acid	15	0	Unchanged	Not determined	Recovered
3-2	2.75	Acid	15	1.32	Initial level 58 mg. per cent. Maximum—day 2—170 mg. per cent. Return to normal 8th day	Not determined	Recovered
4-3	2.60	Acid	15	1.50	Initial level 47.5 mg. per cent. Maximum—day 3—163 mg. per cent. Day 6—falling—120 mg. per cent	Average normal vol. 50+ cc. Oliguria 2nd day—5 cc. Gradual return to normal	Accidentally killed 6th day. Hb casts +++

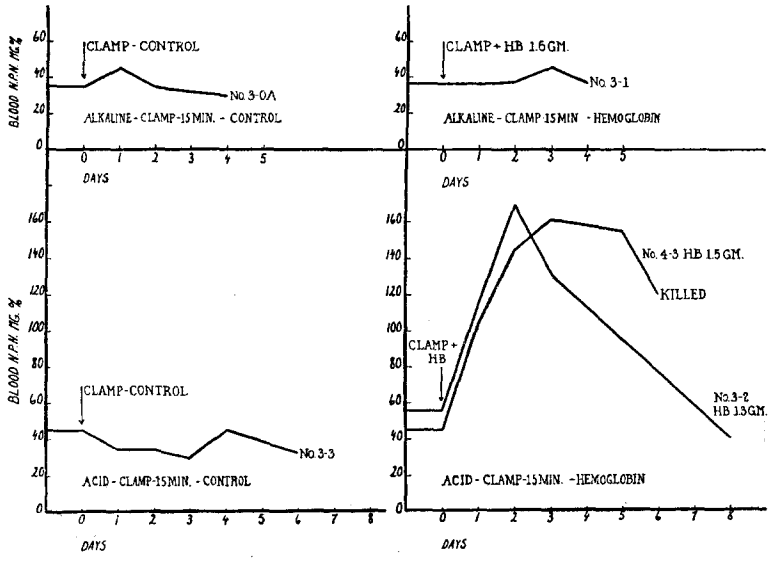
casts, tubular dilatation, and epithelial compression in many, but not all, nephrons (Fig. 1).

Since a 15 minute period of complete renal ischemia caused no elevation of non-protein nitrogen, unless combined with an injection of hemoglobin in a rabbit with acid urine, the clamp was applied in the second group for 25 minutes in order to obtain a greater degree of tubular injury as indicated by some functional disturbance in the control animals.

In Table II and Text-fig. 2 the results obtained following this procedure are recorded. Control animals in both acid and alkaline groups showed a transient rise in non-protein nitrogen concentration which reached variable levels, but in each instance returned to normal within 7 days. Short periods of oliguria or anuria occurred in most of these animals.

In the rabbits with alkaline urine which also received injections of hemoglobin, the rise in non-protein nitrogen was within the range observed in

the control animals, but the elevation was somewhat more persistent, lasting from 9 to 11 days. Periods of slight, transient oliguria were also noted. Two rabbits with acid urine, Nos. 4-6 and 5-7, showed a sharp rise in non-protein nitrogen concentration following application of the clamp and injection of hemoglobin. Death occurred in both instances on the 5th day, at which time non-protein nitrogen levels exceeded 200 mg. per 100 cc. of blood. Each of these animals was completely anuric throughout the course of the experiment. Fig.



TEXT-FIG. 1. Non-protein nitrogen curves in experiments in which the left renal pedicle of previously nephrectomized rabbits was clamped for 15 minutes in order to bring about tubular damage. Details of the various experiments are to be seen on the chart.

2 illustrates the extensive hemoglobin cast formation found at autopsy in both animals.

The results in rabbit 4-9*b* appear to be somewhat out of line with those in rabbits 4-6 and 5-7. This rabbit, however, was the subject of two separate experiments. On the first occasion (No. 4-9*a*), the clamp was applied for a control period of 25 minutes. After an interval of 1 month, a second clamping was performed (No. 4-9*b*), in conjunction with which hemoglobin was injected. At the time of the second clamping considerable scar tissue was encountered about the renal pedicle. This did not appear to encroach upon the hilar blood vessels but prevented their complete occlusion by the clamp. The outcome in this instance, with partial occlusion of the renal circulation for 25 minutes, was almost identical with that seen in acid animals infused with hemoglobin following a 15 minute period of complete ischemia.

Table III summarizes all the experiments in which functional studies were carried out following the administration of sodium tartrate, while the course of

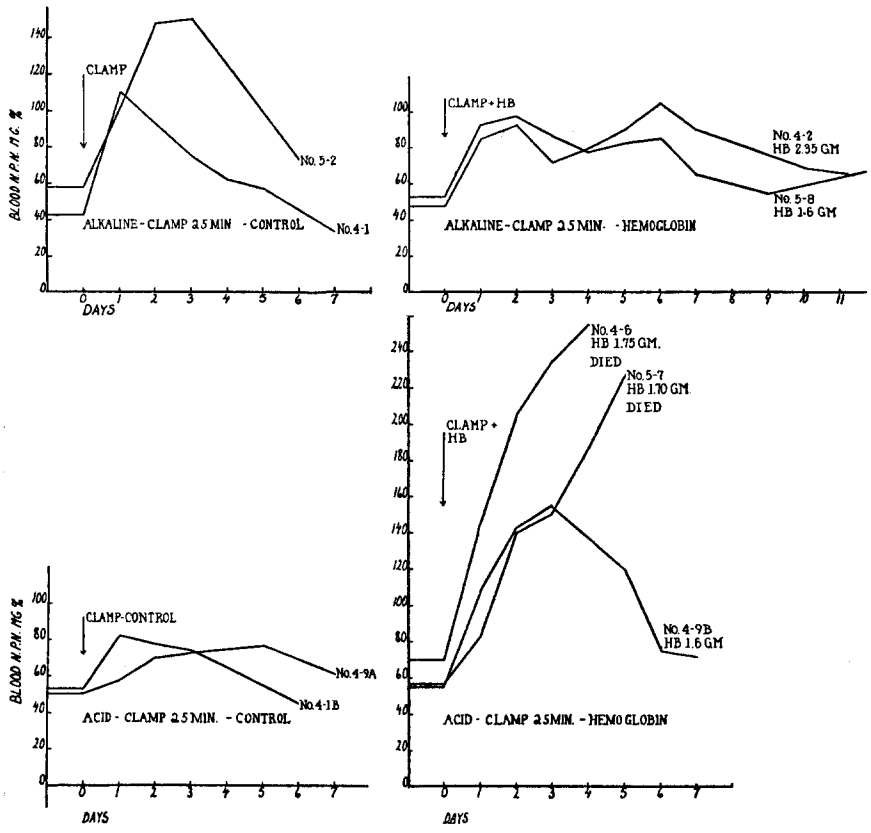
TABLE II

Summary of 25 Minute Clamping Experiments

Rabbit No.	Weight	Reaction of urine	Renal pedicle clamped	Hemoglobin injected	Effect on blood N.P.N.	Effect on urine volume	Remarks
	kg.		*min.*	*gm.*			
4-1*a*	2.70	Alk.	25	0	Initial level 43.6 mg. per cent. Maximum—day 1—110 mg. per cent. Returned to normal 6th day	Anuria—1st day. Immediate return to normal level 100–130 cc. per day	Recovered
5-2	3.10	Alk.	25	0	Initial level 57.8 mg. per cent. Maximum—day 3—151.6 mg. per cent. Normal—7th day	1st day—10 cc. 2nd day—0 cc. 3rd and subsequent days average 85 cc.	Recovered
4-2	3.20	Alk.	25	2.35	Initial level 48 mg. per cent. Irregular elevation to 105 mg. per cent 7 days. Approximately normal by 11th day	Slight oliguria on 1st and 2nd days only	Recovered
5-8	2.15	Alk.	25	1.60	Initial level 53 mg. per cent. Irregular elevation to 98 mg. per cent 6 days. Normal by 9th day	Oliguria 3 cc. 2nd day only	Recovered. Killed 12th day. Very occasional Hb casts.
4-1*b*	2.70	Acid	25	0	Initial level 53 mg. per cent. Maximum—day 1—81.6 mg. per cent. Normal on 6th day	Moderate oliguria 1st —6th day	Recovered
4-9*a*	2.70	Acid	25	0	Initial level 52 mg. per cent. Elevated to 80 mg. per cent ± 2nd to 5th days. Normal 6th day	Unchanged. Average 200 cc. per day	Recovered
4-6	3.00	Acid	25	1.75	Initial level 71.6 mg. per cent. Continuous rise to 256 mg. per cent on 4th day	Initial average 100 cc. per day. Complete anuria 1st to 5th day	Died 5th day. Hb casts ++++
5-7	2.77	Acid	25	1.70	Initial level 57 mg. per cent. Continuous rise to 227.5 mg. per cent on 5th day	Initial average 130 cc. per day. Complete anuria 1st to 5th day	Died 5th day. Hb casts ++++ (massive)
4-9*b*	2.70	Acid	25 (partial)	1.60	Initial level 56 mg. per cent. Maximum—day 3—156 mg. per cent. Decline by day 8 to 74 mg. per cent	Average over 100 cc. No appreciable change during experiment	Killed 8th day. Hb casts ++

the blood non-protein nitrogen in most of these animals is illustrated graphically in Text-fig. 3. In control animals with alkaline or acid urine the blood non-

protein nitrogen rose moderately following the treatment with sodium tartrate, but there was a prompt return to normal depending in some measure on the maximum level reached. Urinary output was altered slightly, if at all. Hemoglobin injections were given in most instances when the degree of nitrogen reten-



TEXT-FIG. 2. Non-protein nitrogen curves in experiments using a left renal pedicle clamp for 25 minutes in order to bring about tubular damage in rabbits which had previously been subjected to right nephrectomy. Details of the various experiments are to be seen on the chart.

tion caused by sodium tartrate was diminishing. It was felt that in this way alterations attributable to the hemoglobin *per se* would be more readily detectable.

Four of the rabbits with alkaline urine (Nos. 2-7, 3-8, 4-5 and 5-6) received doses of hemoglobin ranging from 1.25 to 2.32 gm. at intervals of 2 to 6 days after the administration of sodium tartrate. In rabbits 2-7, 3-8 and 4-5 non-

protein nitrogen levels continued to fall after the injection of hemoglobin and all three animals recovered promptly. The kidney of rabbit 3-8, killed

TABLE III

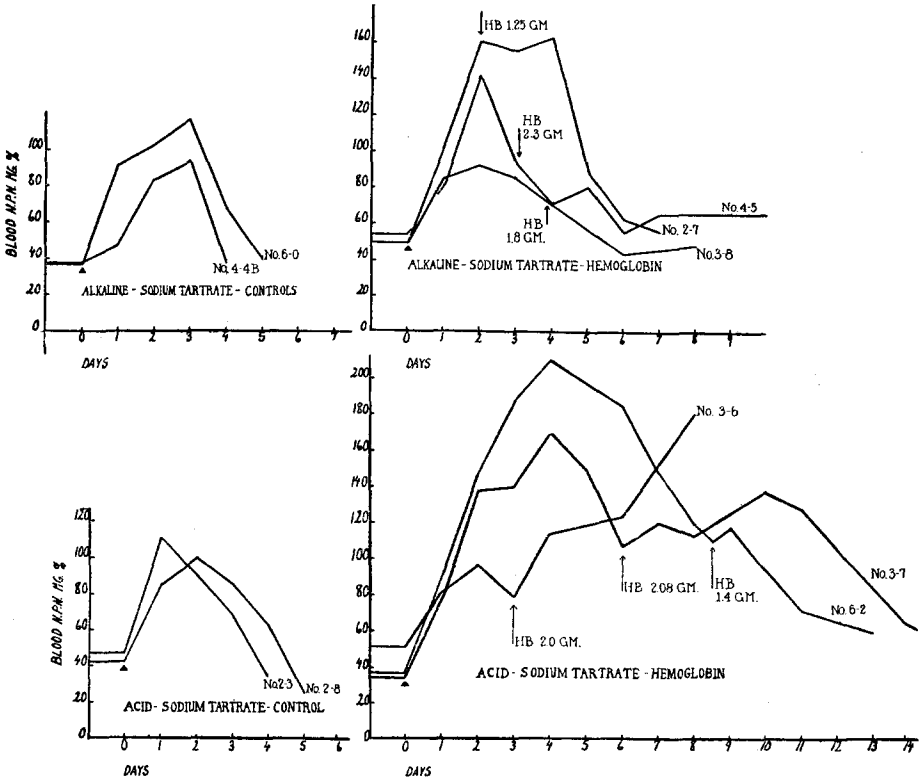
Summary of Sodium Tartrate Experiments

Rabbit No.	Weight	Reaction of urine	Sodium tartrate injected	Hemoglobin injected	Blood N.P.N.								Effect on urine volume	Remarks
					Initial level	Following sodium tartrate		At hemoglobin injection		Following hemoglobin injection		Final determination		
						Max. conc.	Day of exp.	Conc.	Day of exp.	Max. conc.	Day of exp.	Conc.	Day of exp.	
	kg.		*gm./kg.*	*gm.*	*mg. per cent*	*mg. per cent*		*mg. per cent*		*mg. per cent*		*mg. per cent*		
3-5	3.15	Alk.	0.95	0	63.0	93	1	—	—	—	—	63.0	3	Not determined	Recovered
4-4	3.20	Alk.	0.95	0	38.0	94	3	—	—	—	—	38.0	4	Slight oliguria 3–4 days	Recovered
6-0	2.23	Alk.	0.90	0	37.0	117	3	—	—	—	—	40.0	5	No change	Recovered
2-7	2.85	Alk.	0.85	1.25	49.3	160	2	160	2	163	4	48.5	7	Not determined	Recovered
3-8	2.70	Alk.	0.85	1.81	49.1	93	2	70	4	57	5	43.0	6	No change	Killed on 8th day Occasional Hb casts
4-5	2.96	Alk.	0.85	2.32	54.5	142	2	93	3	80.6	5	66.0	7	Oliguria marked 3rd–7th day	Recovered
5-6	2.80	Alk.	0.95	1.75	49.0	216	3	132	6	201	9	150.0	15	Anuria days 2 and 3, days 7 and 10	Died 15th day. No Hb casts
2-3	2.07	Acid	0.80	0	47.8	110	1	—	—	—	—	36.0	4	Not determined	Recovered
2-8	2.42	Acid	0.85	0	43.3	99	2	—	—	—	—	30.0	5	Not determined	Recovered
5-5	2.71	Acid	0.90	0	57.0	92	1	—	—	—	—	61.0	6	No change	Recovered
2-9	2.75	Acid	0.85	1.25	38.0	140	2	140	2	140	3	55.0	9	Not determined	Recovered
3-6	2.77	Acid	0.85	2.00	52.0	97	2	79	3	180	8	180.0	8	50 per cent oliguria after Hb	Died 9th day Hb casts ++++
3-7	2.65	Acid	0.85	2.08	34.0	170	4	107	6	138	10	61.0	15	Not determined	Died 15th day Hb casts ++++
6-2	2.83	Acid	0.85	1.35	38.0	210	4	119	7	117	8	60.0	12	Oliguria days 1 and 3	Recovered. Killed 12th day. Occasional Hb casts

on the 8th day, contained a very few hemoglobin casts, but appeared otherwise normal (Fig. 3). Rabbit 5-6, however, with the greatest degree of nitrogen retention following sodium tartrate (maximum concentration 216 mg. per 100 cc. on the 3rd day) showed a secondary rise in non-protein nitrogen after the

injection of hemoglobin which persisted until the animal died on the 15th day of the experiment. Autopsy revealed a marked degree of tubular necrosis with calcification, but no hemoglobin casts.

Two animals with acid urine (Nos. 2-9 and 6-2) received relatively small doses of hemoglobin (1.25 to 1.35 gm. respectively). This was given to No. 2-9



TEXT-FIG. 3. Non-protein nitrogen curves in experiments in which sodium tartrate was used to produce renal injury. Sodium tartrate was injected in each instance on day 0, as indicated by the black arrow-heads. Other details of the experiments are to be seen on the chart.

at the peak of the tartrate effect, while No. 6-2 was injected well along in the recovery period. In neither case was there any appreciable effect attributable to the hemoglobin. Histological examination of the kidney of rabbit 6-2, killed on the 12th day, showed a relatively normal structure and only occasional hemoglobin casts.

Results in the other two rabbits with acid urine (Nos. 3-6 and 3-7) were more striking. No. 3-6, whose tartrate injury was mild as indicated by the initial

maximum rise in non-protein nitrogen to 97 mg. per 100 cc., received 2.00 gm. of hemoglobin on the 3rd day of the experiment. Following this there was progressive nitrogen retention and a persistent oliguria, until the animal died 5 days later. Rabbit 3-7 received 2.08 gm. of hemoglobin while recovering from a moderately severe grade of tartrate injury. Subsequently, the non-protein nitrogen rose gradually for 4 days and then declined slowly until death occurred 9 days after the injection of hemoglobin. The kidneys of both these animals showed the presence of very numerous hemoglobin casts associated with marked tubular dilatation and moderate necrotic and degenerative changes in the tubular epithelium (Fig. 4).

Histological examinations were also carried out on the kidneys of rabbits used for functional studies and which died or were sacrificed 5 days or more after injection of hemoglobin. In comparing these kidneys with those of the purely histopathological experiments, certain differences were noted. When tubular damage was mild to moderate, irrespective of the manner of its production, numerous hemoglobin casts invariably persisted in the kidneys of the animals with acid urine, which died or were sacrificed shortly after recovery. In contrast, no more than an occasional cast was ever observed in the kidneys of rabbits with alkaline urine when these were examined after a comparable lapse of time. The persistent casts were denser than the initially precipitated hemoglobin casts, and were associated with greater degrees of tubular dilatation and epithelial compression.

DISCUSSION

From a survey of the foregoing experimental data certain clear cut factors emerge which appear to be integral parts of the mechanism by which renal failure develops following many transfusion reactions and in other fatal hemolytic conditions. In the preliminary histopathological studies it soon became apparent that precipitation of hemoglobin in renal tubules was not primarily dependent on the acidity or alkalinity of the urine, nor upon the quantity of circulating hemoglobin, but upon some functional abnormality of individual nephrons. Such abnormalities are indicated by lesions, ranging from barely perceptible changes to frank alterations in the appearance of the convoluted tubular epithelium. That such alterations may be etiologically non-specific is indicated by the rather similar end results obtained with such diverse agents as transient, complete ischemia and a chemical poison having a particular affinity for the convoluted tubules of the kidney.

Given a combination of renal tubular injury and hemoglobinemia, however, the importance of urinary pH in determining the final outcome must not be underestimated. In the acid state, the injection of hemoglobin results in the formation of persistent pigmented casts and leads to an elevation of non-protein nitrogen which under certain circumstances is progressive until death ensues.

In animals with alkaline urine on the other hand, hemoglobin casts which are less marked initially tend to disappear almost completely within a few days and cause little or no nitrogen retention. Whereas all acidotic rabbits dying with evidences of renal failure after the injection of hemoglobin showed extensive cast formation indicative of tubular blockage, the one fatality in a rabbit with alkaline urine was not associated with hemoglobin casts in the renal tubules and was shown by postmortem examination to have resulted from a very severe initial tartrate injury.

With reference to the primary renal lesion referred to above, there appears to be an optimum degree of tubular damage, which at present can only be characterized as moderate, in conjunction with which hemoglobin cast formation is at a maximum for any given levels of plasma hemoglobin concentration and urinary pH. The finding of scattered casts in unclamped kidneys of acidotic rabbits subjected merely to anesthesia and the temporary clamping of the opposite renal pedicle indicates that hemoglobin precipitation may follow minimal kidney damage in the acid state and suggests that minor degrees of cast formation can occur without causing any appreciable disturbances in renal function.

The outcome of the experiments in which the renal circulation was occluded for different periods of time also clearly demonstrates a relationship between the degree of kidney damage and the degree of cast formation and functional disturbance, which results from the subsequent injection of hemoglobin in acidotic animals. The number of casts formed was moderate and nitrogen retention was transient after application of the clamp for 15 minutes; while after a 25 minute period of clamping the non-protein nitrogen levels increased progressively until death occurred and cast formation was massive.

The results of the experiments in which tubular damage was produced by sodium tartrate were somewhat more variable than those in which the renal pedicle was clamped, but the finding of numerous hemoglobin casts in the two acidotic rabbits dying with evidences of renal failure after moderate tartrate injury implies that the same general factors were operative in both groups.

Up to this point of moderate damage, a direct relationship apparently exists between the degree of tubular injury and the quantity of hemoglobin precipitated in the kidney. However, when the damage is severe enough to produce widespread necrosis of convoluted tubular epithelium little, if any, precipitated hemoglobin can be detected, even with a strongly acid urine and a high level of hemoglobinemia. Evidence that hemoglobin casts are scanty or absent when initial tubular injury is very severe has been derived exclusively from the experiments in which sodium tartrate was used. Nevertheless, the analogous appearance of the kidneys, following a large dose of sodium tartrate and a prolonged period of ischemia (22) respectively, makes it not improbable that the hemoglobin inhibition is similar under both conditions. The faiulre of hemoglobin precipitation in the presence of very severe damage is most likely

due to interference with the excretory mechanism to such an extent that hemoglobin does not enter the tubular lumina and, therefore, cannot be precipitated.

Finally, the recovery of two animals given tartrate and with acid urine, after small doses of hemoglobin suggests that, in conjunction with the factors already discussed, the degree of hemoglobinemia is a quantitative factor in determining the amount of cast formation and associated functional disturbance.

It is thus apparent that the ultimate outcome in any given instance, both anatomically and functionally, is dependent upon a fine balance being struck between the degree of renal injury and the level of hemoglobinemia, as well as upon the pH of the urine. This concept offers a possible explanation for the extremely variable number of casts found in the kidneys of human beings dying in uremia as the result of fatal hemolytic conditions, in many of which tubular blockage is not a prominent feature.

SUMMARY AND CONCLUSIONS

1. A readily reproducible pathological lesion closely resembling that typical of the "transfusion kidney" has been obtained by the injection of hemoglobin into rabbits having acid urine, whose renal tubules had previously been damaged to a moderate degree by (*a*) a short period of complete renal ischemia, or (*b*) the administration of a specific chemical poison—sodium tartrate.

2. It has been found that hemoglobin is precipitated in the tubules of damaged kidneys excreting either acid or alkaline urine, in contrast to the absence of hemoglobin precipitation in normal kidneys.

3. In the acid state hemoglobin casts are more numerous and more persistent than in the alkaline, and are associated with renal functional disturbances, in contrast to the lack of such disturbances when the urine is alkaline.

4. The ultimate outcome, both anatomically and functionally, in any given instance is determined by variations in the degree of tubular damage, the level of hemoglobinemia, and the urinary pH.

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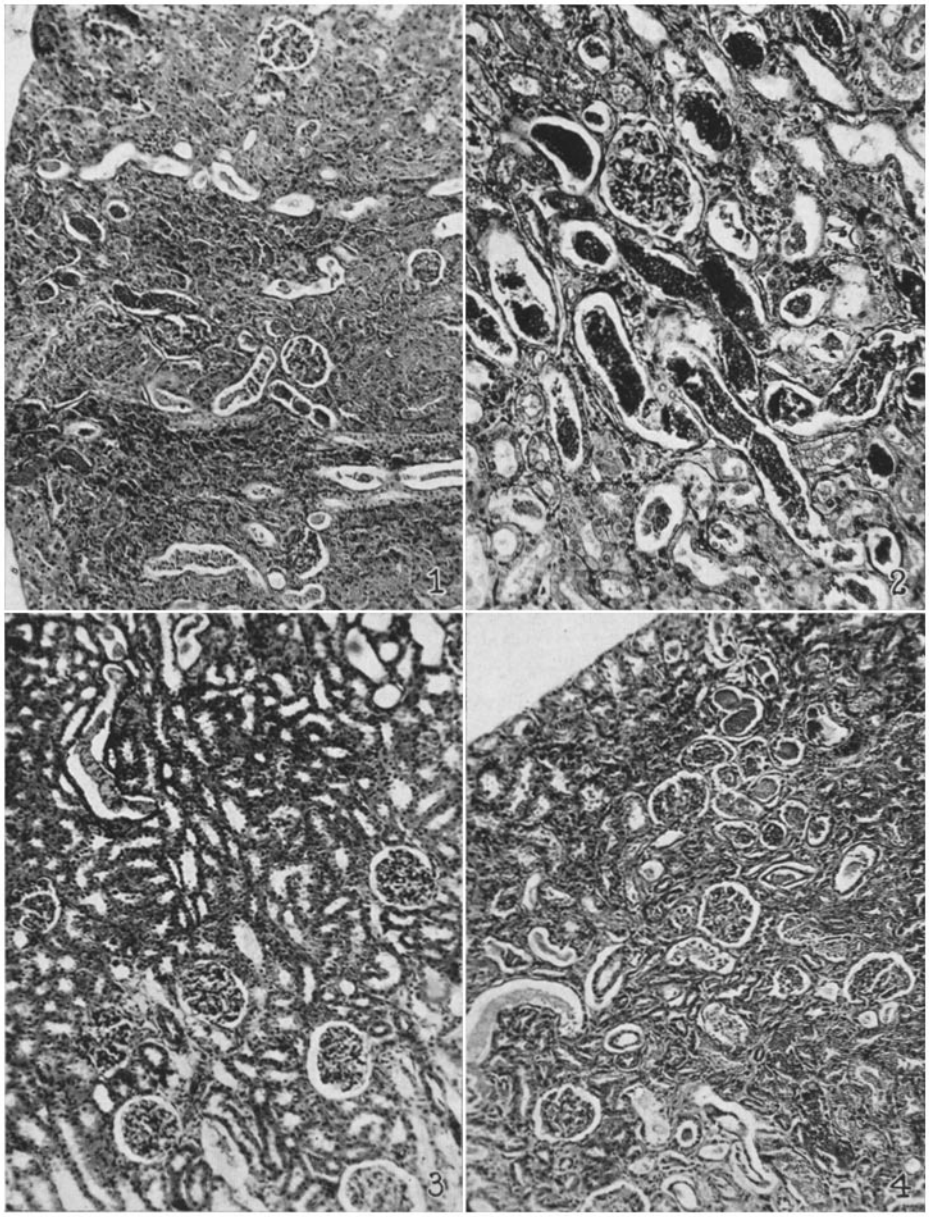
EXPLANATION OF PLATE 24

FIG. 1. Kidney, rabbit 4-3; acid urine. Previous right nephrectomy. Left renal pedicle clamped for 15 minutes and hemoglobin injected. There are many hemoglobin casts in the distal convoluted and collecting tubules. These tubules also show dilatation and compression of the epithelium. Mild degenerative changes are seen in proximal convoluted tubules. Hematoxylin and eosin. × 75.

FIG. 2. Kidney, rabbit 5-7; acid urine. Previous right nephrectomy. Left renal pedicle clamped for 25 minutes and hemoglobin injected. Died on 5th day. There are great numbers of hemoglobin casts in the distal convoluted and collecting tubules. The granular nature of these casts is demonstrated and the tubular dilatation evident. Proximal convoluted tubules show hydropic changes, but no necrosis. Hematoxylin and eosin. × 115.

FIG. 3. Kidney, rabbit 3-8; alkaline urine. Treated with sodium tartrate subcutaneously and with hemoglobin intravenously when non-protein nitrogen was falling. Animal recovered. Occasional hemoglobin casts are seen in distal convoluted and collecting tubules. The rest of the kidney is normal. Hematoxylin and eosin. × 75.

FIG. 4. Kidney, rabbit 3-6; acid urine. Treated with sodium tartrate subcutaneously and hemoglobin intravenously when the non-protein nitrogen was falling. Died 6 days after hemoglobin injections. There are many hemoglobin casts in dilated tubules. Degenerative changes are mild. Hematoxylin and eosin. × 75.



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