HEMOLYTIC REACTIONS PRODUCED IN DOGS BY TRANSFUSION OF INCOMPATIBLE DOG BLOOD AND PLASMA

II. Renal Aspects Following Whole Blood Transfusions

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DEATH FROM renal insufficiency with the postmortem findings of hemoglobinuric or lower nephron nephrosis frequently follows acute hemolytic reactions of various etiology including the transfusion of incompatible blood.[1–3] Extensive studies based largely upon the injection of solutions of hemoglobin, related pigments or laked blood as substitutes for hemolysis in vivo, have been carried out over a period of many years, but have failed to explain the exact mechanism of this type of renal failure.[3–5]

The hemolytic reactions in dogs produced by transfusion of incompatible dog blood described in the preceding paper[6] afford an ideal opportunity to study the effects of such reactions upon the kidney under a variety of conditions simulating those seen clinically.

It has been shown by several groups of investigators, using different animal species, that induced hemoglobinemia within the range encountered in most acute hemolytic disturbances in human subjects, produces only transient changes in normal animals with previously undisturbed renal function.[4, 7–10] On the other hand, particularly if the urine is acid, the injection of hemoglobin into an animal in a severe state of dehydration[11] or with kidneys previously injured[4] results in the formation of pigment casts in the renal tubules followed frequently by death in uremia. Similar results have been reported in dogs following the injection of very large amounts of hemoglobin[5] or laked red blood cells.[12]

This preliminary report is concerned with a controlled study of renal function carried out in conjunction with the experiments described in the preceding paper. The results indicate that in the *normal* dog with *either acid* or *alkaline* urine a combination of the intravascular hemolysis and other profound changes resulting from the transfusion of incompatible blood is not sufficient to produce renal failure.

Methods

Procedures used in the immunization of recipient dogs and the collection and transfusion of incompatible blood have been described in part one of this report.[6] All dogs were normal mongrels vaccinated against distemper. Female animals were used in all experiments involving quantitative renal function studies, urine being obtained through a curved metal catheter. For male dogs a ureteral catheter was used.

Water was given by stomach tube at intervals prior to each transfusion to insure adequate urine flow

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and sodium bicarbonate was added when alkaline urine was desired. In order to obtain the secretion of acid urine with a pH of about 6.0 or less at the time of transfusion, dogs were fed a diet of horsemeat, 200 Gm., lard 50 Gm. and ammonium chloride 3 Gm. for a period of three to four days.

Urinary hemoglobin concentration was determined by the cyanmethemoglobin method of Evelyn and Malloy.[13] Radioactive iron determinations[14] were made on the total hemoglobin excreted in those experiments in which the donated cells were labelled with this isotope.*

Effective renal plasma flow was measured by means of *para*-aminohippurate clearance (15).† Satisfactory blood and urine levels were obtained by injecting 0.5 to 0.8 ml. of the drug intraperitoneally 15 minutes before each 20 minute collection period. Glomerular filtration rates were determined by measuring mannitol clearance (16)† in some experiments and creatinine clearance[17] in others.

TABLE 1.—*Summary of Fourteen Hemolytic Transfusion Reactions in Dogs in which Renal Functions were Studied*

Dog number	Sex	Weight	Initial pH of urine	Blood transfused	Recipient's antibody titer	Maximal plasma Hb.	Approx. period of hemoglobinuria	Total Hb. excreted % of Hb. transfused	Maximal blood urea nitrogen
		Kg.		*ml.*		*mg. per 100 ml.*	*hours*		*mg. per 100 ml.*
43-31	Female	13.2	7.5–8.0	205	1–2	535	18	10	—
43-31	"	13.2	7.5–8.0	100	1–256	1130	20	17	20.5
*43-31	"	13.2	7.5–8.0	128	1–256	1180	20	27	11.5
*43-31	"	13.2	7.5–8.0	130	1–128	1110	24	37	14.5
*43-381	"	20.3	7.5–8.0	150	1–32	750	17	18	26.3
*43-381	"	20.0	7.58	75	1–16	543	8	25	16.5
1309	Male	7.6	7.5–8.0	35	1–32	534	12	26	—
47-79	"	13.6	?	150	?	1375	24	25	—
47-79	"	15.7	8.4	125	1–64	700	18	—	33.0
47-79	"	15.7	6.2	55	1–16	654	24	—	55.0
1182	"	11.9	5.87	40	1–16	1060	12	26	25.9
*43-326	Female	13.5	6.02	50	1–32	1039	16	40	42.6
*47-184	"	15.8	5.87	40	1–8	800	9	26	20.0
43-380	Male	13.0	5.55	150	1–128	1360	24	—	38.6

* Quantitative studies of renal function carried out before, during and after the transfusion reaction.

The aeration method of Van Slyke and Cullen[18] was used to determine the concentrations of blood urea nitrogen.

EXPERIMENTAL OBSERVATIONS

The renal aspects of hemolytic reactions produced by transfusion of incompatible whole blood were studied in fourteen transfusions given to 8 different dogs. Data of a general character relating to all experiments are summarized in table 1.

The results of single or multiple transfusions were essentially similar, with as many as four reactions having been produced in the same animal at varying inter-

* Drs. James A. Bush, John W. Hayden and Henry Tesluk assisted with the measurements of radioactivity.

† Determinations of *para*-aminohippurate and mannitol clearance were done by Mrs. Kathryn Y. Cusson and Dr. Christine Waterhouse.

vals of at least two weeks. The urine at the time of transfusion was alkaline, with a pH of over 7.5, in eight experiments, and acid, pH 5.5 to 6.2, in five experiments. In one instance the pH of the urine was not determined since the reaction occurred unexpectedly after a transfusion of mismatched blood given for another purpose.

Transfusions ranged in size from 35 to 205 ml. of whole blood. When compared with individual dog weights this represented from 2.5 to 15 ml. per kilogram or the approximate equivalent of from 200 to 1000 ml. of blood transfused into a 70 kilogram human being.

The maximum plasma hemoglobin concentration after each transfusion was apparently related both to the amount of blood injected and to the initial antibody titer, the height of which appears to determine to some extent the rapidity of red cell destruction.

Hemoglobin invariably appeared in the bladder urine within five or ten minutes after completion of the transfusion. Exact measurements of the duration of hemoglobinuria were not possible in all experiments, but the shortest period observed was eight hours and none extended beyond twenty-four hours. The variations encountered were unrelated to the pH of the urine, but maximal plasma hemoglobin concentration and body weight were apparently contributing factors. The total amount of hemoglobin excreted by the kidneys ranged from 10 to 40 per cent of that in the transfused blood and came only from this source since in the five experiments involving donor red cells labelled with radio-active iron the isotope content of the total hemoglobin excreted by the recipient's kidneys was identical with that in an equivalent amount of hemoglobin from the donor.

The concentration of urea nitrogen in the blood was determined at daily intervals for periods up to one week after each transfusion. Maximal values obtained are listed in column 9. A transient elevation was noted in most instances usually at 24 hours. This was slightly more marked in the group with acid urine but in all there was a prompt return to the pretransfusion level in from 48 to 72 hours.

Slight proteinuria was noted for a few days after the cessation of hemoglobinuria in some but not all animals with both alkaline and acid urine. There was no consistent alteration in the specific gravity of the urine at any time. Catheterized specimens of urine collected during the period of hemoglobinuria all contained variable amounts of brown granular material while the urinary sediment of dogs with initially acid urine also showed moderate numbers of pigmented casts.

Quantitative studies of renal function were carried out before, during and after the transfusion reaction in six experiments marked with an asterisk in table 1. In each of these a similar, clearly defined pattern was observed with respect to the renal excretion of hemoglobin, the rate of effective renal plasma flow and the glomerular filtration rate. The findings in two characteristic experiments are illustrated in figure 1. It is to be noted that there were no essential differences between the two experiments, in one of which alkaline urine and in the other acid urine was initially being excreted. Plasma hemoglobin concentrations are shown in the top graph and hemoglobin excretion rates are plotted in the second graph. The latter curves are roughly parallel to those of hemoglobinemia down to the

threshold level, and the calculated renal clearances of the pigment are found to be essentially similar to those observed after hemoglobin injection.[10]

Minor irregularities in the excretion rates of hemoglobin are related to the transient changes in effective renal plasma flow and glomerular filtration illustrated in the two lowest graphs of figure 1. The biphasic character of these curves

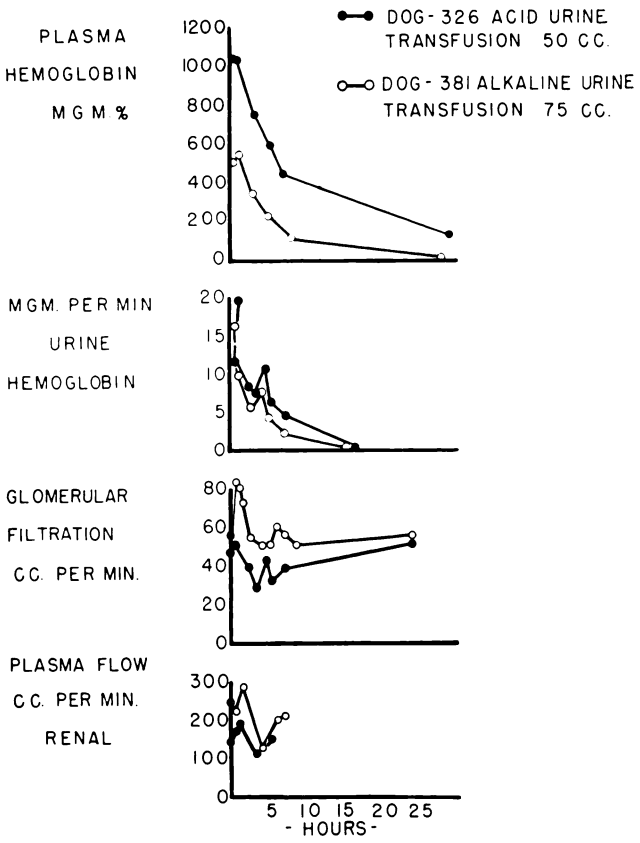

FIG. 1.—TRANSIENT CHANGES IN RENAL FUNCTION ASSOCIATED WITH HEMOGLOBINEMIA AND HEMOGLOBINURIA PRODUCED BY TRANSFUSION OF INCOMPATIBLE WHOLE BLOOD IN DOGS. Dog 326, Weight 13.5 kilo. Antibody titer 1–64. Dog 381, Weight 20.0 kilo. Antibody titer 1–16.

was a constant finding, with an early rise and a secondary fall below the baseline after several hours. While the degree of these changes was somewhat variable from experiment to experiment, both functions had returned to normal in from six to twenty-four hours in all instances. These transient alterations in renal hemodynamics appear to reflect the general vascular response to a transfusion reaction and indicate that a specific renal vasoconstrictor action of hemoglobin demonstrated some years ago[19, 20] is not an important factor in the development of renal insufficiency.

All animals were well hydrated at the start of each experiment and no oliguria developed, although urine flow was usually reduced for short periods when plasma flow and filtration were at low levels.

Figure 2 illustrates, in two typical experiments, the finding of a temporary alkalinization of the urine during the period of hemoglobinuria which occurred following transfusion of incompatible blood in all animals with initially acid urine. The mechanism of this change is not yet clear but may represent a compensatory effort on the part of the kidney to prevent the accumulation of large amounts of precipitated hemoglobin in the renal tubules. Maximal pH readings coincided with the highest concentrations of hemoglobin in the urine, suggesting that some neutralization results merely from the addition of hemoglobin, which

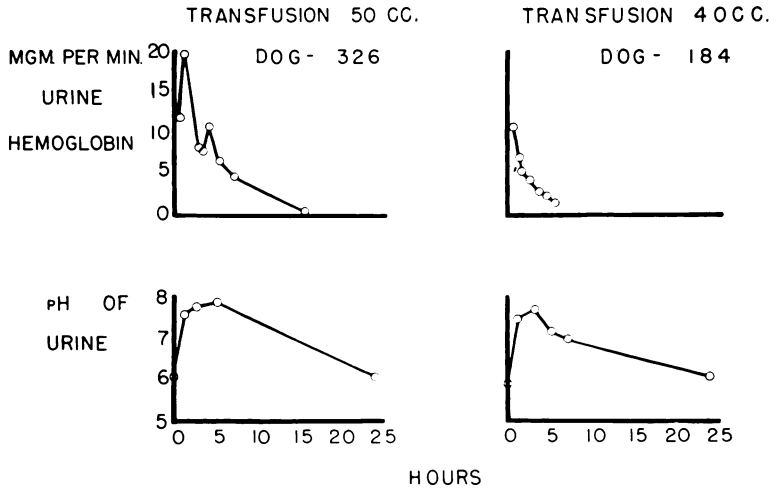

FIG. 2.—SPONTANEOUS ALKALINIZATION OF URINE DURING PERIOD OF HEMOGLOBINURIA INDUCED BY TRANSFUSION REACTIONS IN DOGS WITH INITIALLY ACID URINE AND NORMAL KIDNEY FUNCTION.

has been observed in vitro, but some interference with the excretion of acid by the lining cells of the distal convoluted tubules is also a possibility.

Two dogs, numbers 1309 and 1182, table 1, were killed twenty-four hours after transfusion reactions, similar to those after which the animals were followed throughout the recovery period. One other animal, 43-326, table 1, having apparently recovered from the transfusion reaction, died suddenly after forty-eight hours. Postmortem examination revealed an acute peritonitis which was attributed to contamination of the *para*-aminohippurate injected intraperitoneally. The kidneys of all three dogs were grossly normal. Histologically, the only finding of note was a small amount of brown, crystalline and granular pigment in occasional distal convoluted and collecting tubules in the two dogs with initially acid urine.

DISCUSSION

A critical analysis of the literature dealing with injection of hemoglobin solutions reveals that the mere production of levels of hemoglobinemia comparable

to those seen clinically in most acute hemolytic disorders has little or no damaging effect upon kidney structure or function in normal human subjects or animals.

Flink[5] stressed the importance of degree of hemoglobinemia in the development of renal damage, being unable to produce renal injury in dogs unless the initial plasma hemoglobin concentration was 3.7 Gm. per 100 ml. or the average of the initial and the 24 hour plasma concentrations was 2.2 Gm. per 100 ml. From the rather inadequate information available in the literature it is doubtful whether such concentrations ever occur following clinical transfusion reactions and the data presented in this and the preceding paper indicate clearly that the degree of maximal plasma hemoglobin concentration attained is not proportional to the amount of incompatible blood transfused. From the figures in table 1, the plasma concentrations which would have resulted from the sudden liberation of all the hemoglobin in the transfused blood can be calculated. In the experiments in which small transfusions were given the calculated and observed values correspond closely, whereas following larger transfusions the maximal plasma hemoglobin concentrations were only slightly higher than those following small transfusions. This indicates that the degree of hemoglobinemia induced was limited by the ability of the body to destroy incompatible cells. Dog 43-380 illustrates this point, since had all the transfused red blood cells been rapidly hemolyzed, the initial plasma hemoglobin concentration would have been 4.0 Gm. per 100 ml. instead of the observed concentration of 1.36 Gm. per 100 ml.

Although it is obvious from the work of Flink and others that excessive degrees of hemoglobinemia, directly or indirectly, can produce disturbances of renal function, this alone is probably an uncommon cause of hemoglobinuric nephrosis in man. On the other hand there are innumerable clinical and experimental examples of renal insufficiency which have resulted from the association of a moderate grade of hemoglobinemia and some nephrotoxic process.[1, 3, 4] This latter factor can be characterized in some instances as the general or local effect of such agents as shock, ischemia, a chemical poison, infection, or dehydration. However, in many acute hemolytic processes, notably those resulting in human subjects from the transfusion of incompatible blood, the cause of serious renal complications is not always clear. Since hemolysis during transfusion reactions is associated with profound changes of a generalized nature, it was considered possible that these might secondarily affect the kidney in a manner comparable to the more specific factors enumerated above. In the present study only normal dogs were used in order to determine whether the combination of these general effects with the concurrent hemoglobinemia was alone sufficient to produce renal insufficiency. Experimental conditions were varied with respect to size of transfusion, antibody titer of recipient, and hydrogen ion concentration of the urine. From the data presented it is apparent that within the range of these variables (table 1) only minor, transient changes in renal function were observed. A comparison of the findings in dogs with initially acid urine and those with alkaline urine reveals that, in the former, pigment casts occurred in the urine and persisted in a few nephrons for at least forty-eight hours in the kidneys studied histologically and

that nitrogen retention was slightly more marked. The final outcome, however, was the same in both groups of animals.

Hemolytic transfusion reactions in dogs suffering from shock, anemia, dehydration, and other conditions simulating those for which transfusions are frequently given clinically are being studied at the present time.

Conclusions

1. The normal dog's kidney reacts to the transfusion of incompatible dog blood in a manner similar to that observed after hemoglobin injection as far as the excretion of the pigment is concerned.

2. Lowering the pH of the urine to a level of 5.5 has no effect on the final outcome of the reaction nor on the mild, transient alterations in renal function which occur.

3. This type of hemolytic transfusion reaction, similar in most respects to that encountered in human subjects, does not of itself produce renal failure nor the pathological picture of hemoglobinuric or lower nephron nephrosis.

4. The findings in these experiments lend further support to the concept that the development of serious renal complications after a transfusion reaction results from a combination of the hemolytic process with some degree of previous or concomitant kidney damage related to the various clinical states for which transfusion therapy is indicated.

REFERENCES

[1] Lucké, B.: Lower nephron nephrosis. Mil. Surg. *99:* 371, 1946.

[2] Muirhead, E. E., Haley, A. E., Haberman, S., and Hill, J. M.: Acute renal insufficiency due to incompatible transfusion and other causes, with particular emphasis on management. Blood *3:* Special Issue No. 2 (The Rh Factor) p. 101, 1948.

[3] Ross, J. F.: Hemoglobinemia and the hemoglobinurias. New Eng. Med. J. *233:* 691, 732, 766, 1945.

[4] Yuile, C. L., Gold, M. A., and Hinds, E. G.: Hemoglobin precipitation in renal tubules. A study of its causes and effects. J. Exp. Med. *82:* 361, 1945.

[5] Flink, E. B.: Blood transfusion studies. III. The relationship of hemoglobinemia and of the pH of the urine to renal damage produced by injection of hemoglobin solutions into dogs. J. Lab. & Clin. Med. *32:* 223, 1947.

[6] Young, L. E., Ervin, D. M., and Yuile, C. L.: Hemolytic reactions produced in dogs by transfusion of incompatible dog blood and plasma. I. Serologic and hematologic aspects. Blood, this issue, p. 1218.

[7] Bing, R. J.: Effect of hemoglobin and related pigments on renal functions of normal and acidotic dogs. Bull. Johns Hopkins Hosp. *74:* 161, 1944.

[8] Corcoran, A. C., and Page, I. H.: Genesis of crush syndrome. J. Lab. & Clin. Med. *30:* 351, 1945.

[9] De Navasquez, S. J.: The excretion of haemoglobin, with special reference to the "transfusion kidney". J. Path. & Bact. *51:* 413, 1940.

[10] Monke, J. V., and Yuile, C. L.: The renal clearance of hemoglobin in the dog. J. Exp. Med. *72:* 149, 1940.

[11] (a) Lalich, J. J.: The influence of injections of homologous hemoglobin on the kidneys of normal and dehydrated animals. J. Exp. Med. *86:* 153, 1947.

(b) ——: The influence of available fluid on the production of experimental hemoglobinuric nephrosis in rabbits. J. Exp. Med. *87:* 157, 1948.

[12] De Gowin, E. L., Osterhagen, H. F., and Andersch, M.: Renal insufficiency from blood transfusion: relation to urine acidity. Arch. Int. Med. *59:* 432, 1937.

[13] EVELYN, K. A., AND MALLOY, H. T.: Microdetermination of oxyhemoglobin, methemoglobin, and sulfhemoglobin in a single sample of blood. J. Biol. Chem. *126:* 655, 1938.

[14] HAHN, P. F.: Radioactive iron procedures. Indust. & Engin. Chem. *17:* 45, 1945.

[15] CHASSIS, H., REDISH, J., GOLDRING, W., RANGES, A. J., AND SMITH, H. W.: The use of sodium p-aminohippurate for the functional evaluation of the human kidney. J. Clin. Investigation *24:* 583, 1945.

[16] SMITH, W. W., FINKELSTEIN, N., AND SMITH, H. W.: Renal excretion of hexitols and their derivatives and of endogenous creatinine-like chromogen in dog and man. J. Biol. Chem. *135:* 231, 1940.

[17] PETERS, J. H.: The determination of creatinine and creatine in blood and urine with the photoelectric colorimeter. J. Biol. Chem. *146:* 179, 1942.

[18] PETERS, J. P., AND VAN SLYKE, D. D.: Quantitative Clinical Chemistry, vol. 2, Methods. Baltimore, The Williams & Wilkins Co., 1932.

[19] HESSE, E., AND FILATOV, A.: Experimentelle Untersuchungen über das Wesen des hämolytischen Schocks bei der Bluttransfusion und die therapeutische Beeinflussung desselben: die Nierenfunktionsstörungen im akutem Experiment. Ztschr. f.d. ges. exper. Med. *86:* 211, 1933.

[20] MASON, J. B., AND MANN, F. C.: The effect of hemoglobin on volume of the kidney. Am. J. Physiol. *98:* 181, 1931.