RENAL EFFECTS OF HEMOGLOBIN INFUSIONS IN DOGS IN HEMORRHAGIC SHOCK*‡

By PAUL B. HAMILTON, M.D., ALMA HILLER, Ph.D., and DONALD D. VAN SLYKE, Ph.D.

(From the United States Navy Research Unit at the Hospital of The Rockefeller Institute for Medical Research)

(Received for publication, September 3, 1947)

The most serious deterrent to the intravenous use of hemoglobin solutions has been the fear of renal damage such as has been observed to occur following its use, as also after transfusion reactions. Chills and fever have also been noted in man, but these reactions have proved of minor significance. The untoward results have been variously attributed to red cell stroma, abnormal concentrations of potassium, bacterial contamination, chemical changes in the hemoglobin, and even referred to the hemoglobin itself.[1]

Attention was first directed by Baker and Dodds (3) to changes in kidney function resulting from the intravenous injection of hemoglobin solutions into rabbits. These authors concluded that hemoglobin, which readily passes through the glomerular membrane, is precipitated in the kidney tubules where concentration of the glomerular filtrate takes place. They believe that two factors, increase of acidity and increase of salt concentration, together caused precipitation of the hemoglobin, and that in turn this precipitated hemoglobin mechanically plugged the tubule lumens. As a consequence of this plugging, renal function was damaged; a rise in blood urea nitrogen was taken as evidence of this damage. De Nevasquez (4) disagreed with the conclusions of Baker and Dodds when he found that for rabbits, in spite of elevated blood urea nitrogen, renal function as judged by the excretion of phenol red, remained normal regardless of the acidity of the urine following the intravenous injection of hemoglobin. De Gowin, Osterhagen, and Andersch (5) injected laked dog red blood cells (stroma not removed) into dogs and appeared to confirm the findings of Baker and Dodds, but on examination of stained sections of kidneys from these animals, De Gowin, Warner, and Randall (6) were led to state that although plugging of the renal tubules was observed there was an unexplained nephrotoxic process which could cause renal

* The work described in this paper was done under contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and The Rockefeller Institute for Medical Research.

‡ The Bureau of Medicine and Surgery of the United States Navy does not necessarily undertake to endorse views or opinions expressed in this paper.

[1] For bibliography pertaining to the various methods of preparing solutions of hemoglobin see Hamilton, Farr, Hiller, and Van Slyke (1). For bibliography pertaining to the intravenous administration of hemoglobin solutions to animals and man see Farr, Hiller, and Van Slyke (2).

insufficiency, independent of and in the absence of mechanical obstruction of the tubules by precipitated hemoglobin. Bing (7) investigated renal function of normal and acidotic dogs receiving hemoglobin injections and concluded that hemoglobin and methemoglobin in the normal dog and hemoglobin in the acidotic dog gave no depression of renal function as measured by clearance of para-amino hippuric acid and creatinine. He did find, however, that methemoglobin caused marked depression of kidney function when injected into acidotic animals. It appears that in Bing's experiments with methemoglobin and acidosis the animals suffered other damage in addition to depression of kidney function, because the dogs were usually moribund by the 3rd day after receiving the injection. As a rule, dogs do not become moribund from renal failure alone till the 4th to 8th day, even after surgical removal of both kidneys (8, 9).

In an attempt to gain further insight into the renal effects of treating shock by intravenous injection of hemoglobin solution the writers have infused solutions of dog hemoglobin prepared by the method of Hamilton, Farr, Hiller, and Van Slyke (1) into dogs in hemorrhagic shock. Renal function was investigated before and after replacing 50 cc. of blood per kilo body weight by an equal volume of approximately 7 per cent hemoglobin solution.

Preparation of Plasma and Hemoglobin Solutions for Infusion

Blood was collected from normal dogs in sterile 100 cc. centrifuge bottles containing 50 mg. heparin and centrifuged. The plasma was siphoned off, pooled, sterilized by passing through a Seitz filter, and stored in sterile bottles at 4°C.

From the residual red blood cells, solutions of hemoglobin were prepared according to the procedure described in an accompanying paper (1). The hemoglobin of the solutions was 95 to 98 per cent in the active form capable of carrying oxygen. Methemoglobin solutions were prepared by adding 1.1 moles of sodium ferricyanide per mole of hemoglobin. Analysis showed that at least 99 per cent of the hemoglobin was in the inactive form, incapable of carrying oxygen; *i.e.*, as methemoglobin.

Methods of Analysis

Total hemoglobin content of the injected hemoglobin solutions and of plasma and urine of dogs after hemoglobin injection was determined by the carbon monoxide capacity method, with addition of $Na_2S_2O_4$ to reduce any ferrihemoglobin to ferrohemoglobin ("active Hb"), according to the method of Van Slyke and Hiller (10) as modified by Van Slyke, Hiller, Weisiger, and Cruz (11).

Methemoglobin content of injected solutions and of plasma and urine of dogs after injection was calculated as the difference between total hemoglobin and active hemoglobin (11).

Blood plasma urea nitrogen was determined by the hypobromite method of Van Slyke and Kugel (12).

Urine urea nitrogen in the control periods, before hemorrhage, and in the earlier experiments, Figs. 1, 2, and 5, was determined by the hypobromite method of Van Slyke (13) but using the hypobromite reagent described by Van Slyke and Kugel (12). In the later experiments, Fig. 7, urine urea nitrogen was determined by the urease-aeration method of Van Slyke and Cullen (14) and in Figs. 3 and 6 by the gasometric urease method of Van Slyke (15).

Plasma chlorides were determined by the method of Van Slyke and Hiller (16).

Plasma CO_2 was determined by the method of Van Slyke and Neill (17, 18). The corrected factors of Van Slyke and Sendroy (19) were used to calculate the values for plasma CO_2.

Experimental Procedures

In the initial experiments animals were used after an 18 hour period of fasting. In later experiments all animals were placed for periods of 7 to 10 days in metabolism cages. Daily morning fasting venous blood samples were drawn for determination of plasma urea nitrogen. Twenty-four hour urine excretions were also collected in large flasks containing thymol and completely immersed in crushed ice in order to keep bacterial growth minimal or absent. The 24 hour output of urine urea was determined, and from the values of blood and urine urea the 24 hour urea clearance values $\left(\frac{UV}{B}\right)$ (20) were calculated. This procedure was adopted because it eliminated the necessity of training animals to submit to catheterization. Female dogs were used in all experiments and were kept on stock laboratory diets.

Animals were last fed on the morning of the day prior to withdrawal of blood and infusion.

When the effect of pre-existing acidosis as a complicating factor was investigated, the animals were given 0.25 gm. of ammonium chloride per kilo body weight, administered as 0.9 per cent solution by stomach tube in three doses, *viz.* during the morning and evening of the day before bleeding and infusion and before anesthetization on the morning of withdrawal of blood.

On the day of hemorrhage and infusion the animals were anesthetized with 30 mg. of sodium pentobarbital per kilo body weight (0.44 cc. of 6.6 per cent solution) and shaved. A femoral cannula and urethral catheter were inserted. An initial sample of blood was taken and urine was collected for a 30 minute period for the determination of pre-hemorrhage urea clearance values. Each animal was then bled 50 cc. every 7 to 10 minutes until the total volume of blood drawn reached 50 cc. per kilo body weight. Plasma, or a solution of sodium chloride, of oxyhemoglobin, or of methemoglobin was then injected at the same rate at which the blood had been drawn and in the same volume. Ten minutes after the end of the infusion a sample of blood was drawn, urine was collected for a 30 minute period, and the urea clearance was determined. In some animals repeated urea clearances were measured during the ensuing 4 hour period during which the anesthesia was prolonged by additional sodium pentobarbital infusion. After the urea clearances were determined the femoral cannula was removed and the site of the operation sewn up, the catheter removed, and the animal returned to a metabolism cage.

As in the pre-hemorrhage period, fasting plasma urea nitrogen was determined daily each morning and the urine area nitrogen output determined for the preceding 24 hours; 24 hour clearance values were calculated from these data. Clearance values and plasma urea nitrogen were followed till they had returned to the pre-hemorrhage level.

RESULTS

A. Experiments on Dogs without Pre-Induced Acidosis

Effect of Sodium Pentobarbital Anesthesia on the Urea Clearance and Acid-Base Balance.—In eight normal dogs 24 hour urea clearances done for 5 consecutive days were averaged as the control clearance for each dog prior to the day of anesthesia, bleeding, and infusion. A urea clearance obtained in each of these dogs over a period of 30 to 60 minutes after injection of sodium pentobarbital showed no appreciable change in one animal, but in seven dogs the clearance fell to from 47 to 82 per cent of the control clearance and averaged 60 per cent of the control.

Sera of four normal dogs analyzed for CO_2 and chloride before and 1 hour after injection of sodium pentobarbital showed no appreciable changes. The average CO_2 was 24.5 mM per liter before and 25.5 one hour after anesthesia. The average chloride was 113.9 mM per liter before and 112.7 mM one hour after anesthesia.

Controls without Replacement of Drawn Blood.—Six dogs were bled without replacement. A period of about 12 hours' oliguria followed in each case, but all the dogs then resumed excretion, and all recovered. Blood urea nitrogen rose a few milligrams per 100 cc. during the oliguria, but did not usually exceed

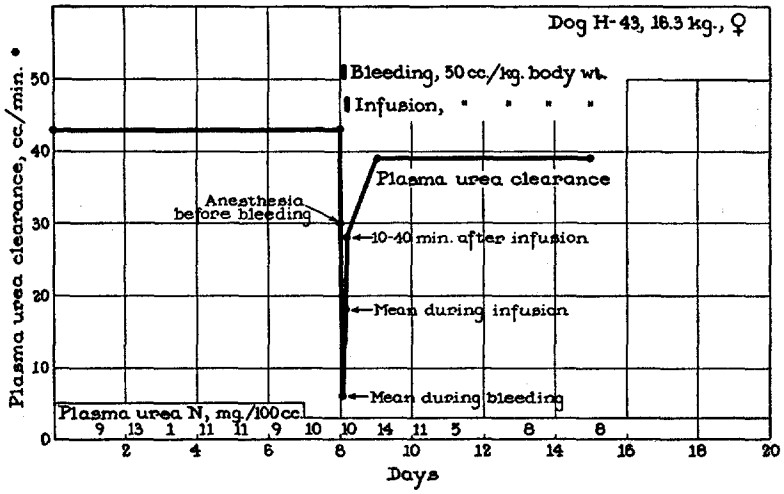

FIG. 1. Hemorrhage with plasma infusion.

20 mg. During the next day blood urea nitrogen and urea clearance had returned to approximately pre-hemorrhage level.

Replacement of Blood by 0.9 Per Cent NaCl Solution.—Two dogs were bled, and the withdrawn blood replaced with an equal volume of 0.9 per cent NaCl solution. The post-hemorrhagic oliguria was relieved by the injection. Both animals recovered and showed no significant elevation of blood urea nitrogen. Urea clearances were slightly depressed during the remainder of the day but returned to pre-hemorrhage levels on the following day.

Plasma CO_2 in these two dogs fell following hemorrhage and infusion to 80 per cent of the initial values, but returned to normal on the following day. The fall in CO_2 was balanced by an approximately equivalent rise in plasma chloride.

Replacement of Blood by Plasma.—Six dogs were used in this series. A typical result is shown in Fig. 1. The post-hemorrhagic oliguria is here shown by the low clearance before plasma infusion began. The oliguria was promptly re-

lieved by the plasma infusion. These animals all recovered without having shown any significant elevations of blood urea. Urea clearances during the hours after hemorrhage and plasma infusion were only slightly depressed, and on the following day were approximately the same as the pre-hemorrhage clearances.

It appears from these three sets of animals that the withdrawal of 50 cc. of blood per kilo body weight, whether replaced by plasma or NaCl solution, or not, does not affect kidney function for more than 24 hours.

An unexpected phenomenon following the plasma infusion was transitory edema, especially evident in swelling of the muzzle areas. The infusion increased the circulating plasma volume (although presumably not the total blood volume) to greater than pre-hemorrhage level; this increase may have been the cause of the edema.

Replacement of Blood by 7.6 Per Cent Oxyhemoglobin Solution.—

(*a*) *Effects on Renal Function.*—Four dogs were observed in this series. In each the post-hemorrhagic oliguria was at once relieved by the hemoglobin infusion. The urea clearance rose at once to pre-hemorrhage level (Figs. 2 and 3) and then fell again during subsequent hours. This secondary fall in clearance (which was not noted when the lost blood was replaced by plasma (Fig. 1)), appears to indicate specific, though temporary, damage to the kidney. In two of the four dogs there followed during the next day a rise of clearance to nearly pre-hemorrhage level (example, Fig. 2). Plasma urea nitrogen rose only a few milligrams. In the other two dogs, however, a number of days were required for complete recovery (Fig. 3), and plasma urea nitrogen rose higher (to 44 mg. in the dog of Fig. 3 and to 70 mg. in the other dog, not shown in the figures).

The edema noted after plasma infusions was not observed after the infusions of hemoglobin solution.

(*b*) *The Fate of Injected Oxyhemoglobin.*—The maximal concentration of hemoglobin reached in the plasma was approximately 5 gm. per 100 cc., observed immediately after finishing the infusion. The "half-life" of the oxyhemoglobin in the circulating plasma was about 8 hours; i.e., whatever hemoglobin concentration was present at a given moment in the plasma was reduced to about half in the next 8 hours. This is illustrated in Fig. 4, where the hemoglobin remaining in the plasma at various times after injection is expressed as a fraction of the maximal hemoglobin concentration obtained immediately after infusion. After 48 hours traces of pigment were still visible; in 72 hours the plasma was clear.

In the infused oxyhemoglobin solutions 3 or 4 per cent of the total pigment was methemoglobin, and this proportion was present in the plasma pigment at the end of the infusion, making a plasma concentration of about 0.2 gm. of

methemoglobin per 100 cc., total plasma hemoglobin being about 5 gm. per 100 cc. While the active hemoglobin disappeared rapidly, the methemoglobin

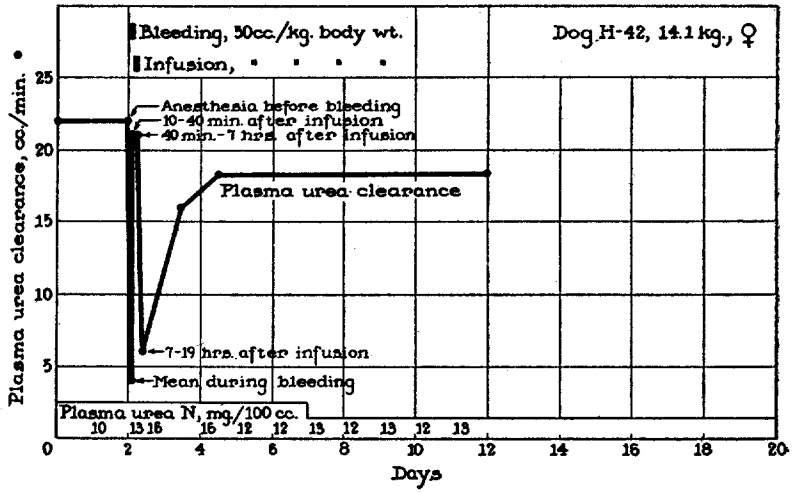

FIG. 2. Hemorrhage with oxyhemoglobin infusion.

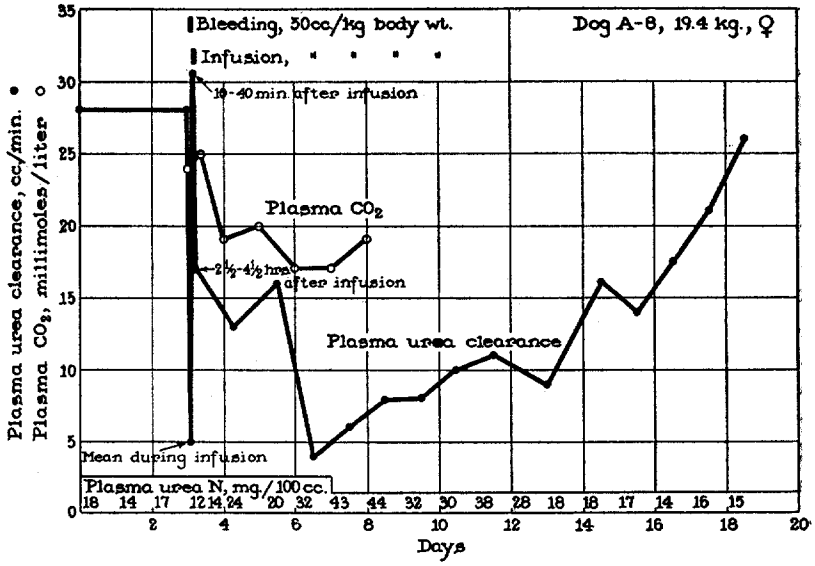

FIG. 3. Hemorrhage with oxyhemoglobin infusion.

concentration was approximately maintained for several hours, during which presumably a slow transformation of active hemoglobin into methemoglobin occurred, balanced by an equal rate of removal of methemoglobin from the

plasma. Later the methemoglobin diminished, and disappeared with the oxyhemoglobin.

Hemoglobin began to appear in the urine within about 15 minutes after the infusion was started. Altogether 30 to 40 per cent of the hemoglobin infused

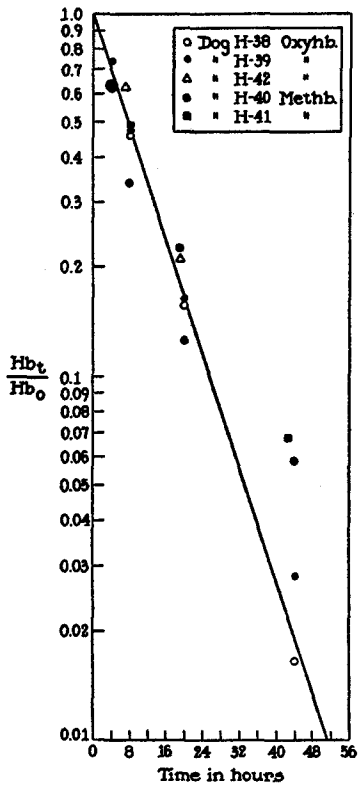

FIG. 4. Rate of disappearance of infused hemoglobin from plasma. Hb_0 = maximal Hb concentration observed in plasma immediately after infusion. Hb_t = Hb concentration t minutes after end of infusion. The points are from five different experiments, three with oxyhemoglobin, two with methemoglobin. Disappearance rate was about the same with both types of Hb.

was excreted in the urine. The remainder was presumably taken up by the animal's tissues.

Replacement of Blood by 7.6 Per Cent Methemoglobin Solution.—

(*a*) *Effects on Renal Function.*—Two dogs were tested. The effects on renal function were not markedly different from the effects of oxyhemoglobin infusions. As in the dogs receiving oxyhemoglobin, the post-hemorrhagic oliguria, observed in the dogs receiving no replacement fluid, was at once relieved. Some

elevation of blood urea nitrogen and some depression of clearance values were observed; the plasma urea nitrogen rose for the first 3 days to maximum values of 54 and 69 mg. per 100 cc. and thereafter dropped rapidly to normal levels within the next 3 days. The urea clearance values remained somewhat depressed during the time of elevated plasma urea nitrogen and thereafter rose to normal levels. The findings in one of these animals are illustrated in Fig. 5. The further course of these animals was uneventful.

(*b*) *Fate of Injected Methemoglobin.*—The rate of disappearance of the methemoglobin from the plasma was practically the same as that of oxyhemoglobin, the half-life being about 8 hours, as shown in Fig. 4.

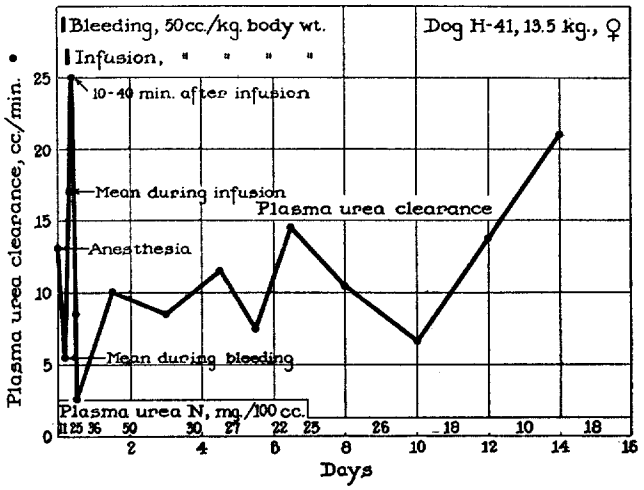

FIG. 5. Hemorrhage with methemoglobin infusion.

Transformation of methemoglobin to active hemoglobin (capable of binding O_2 and CO) occurred much more rapidly than the reverse reaction noted after oxyhemoglobin infusions. Forty minutes after the end of an infusion about 40 per cent of the pigment present in the plasma was in the form of active hemoglobin. As the concentration of total hemoglobin in the plasma fell, the proportion in the form of active hemoglobin continued to rise, reaching 60 to 65 per cent 8 hours after the end of the infusion, and remaining there as long as enough pigment remained to be accurately measured.

In the urine about the same proportion of the injected pigment was excreted (30 to 40 per cent), as after infusion of oxyhemoglobin. In a urine which was analyzed for methemoglobin and total hemoglobin the ratio was found to be practically the same as that in the plasma during the excretion.

B. Experiments on Dogs with Acidosis Induced by Administration of NH_4Cl before Hemorrhage

Controls without Replacement of Drawn Blood.—Hemorrhage itself always

caused a fall in plasma CO_2 (*e.g.* Fig. 3). The somewhat greater degree of acidosis reached when NH_4Cl had been previously administered appeared to be without significant influence on the renal effects of the hemorrhage. There was the same transitory oliguria and period of depression of urea clearance, followed by return to normal clearance within 24 hours, as shown in Fig. 6.

Replacement of Blood by Plasma.—One acidotic dog was observed in which the drawn blood was replaced by an equal volume of dog plasma. The degree of acidosis was similar to that of the animal in Fig. 6, plasma CO_2 being reduced

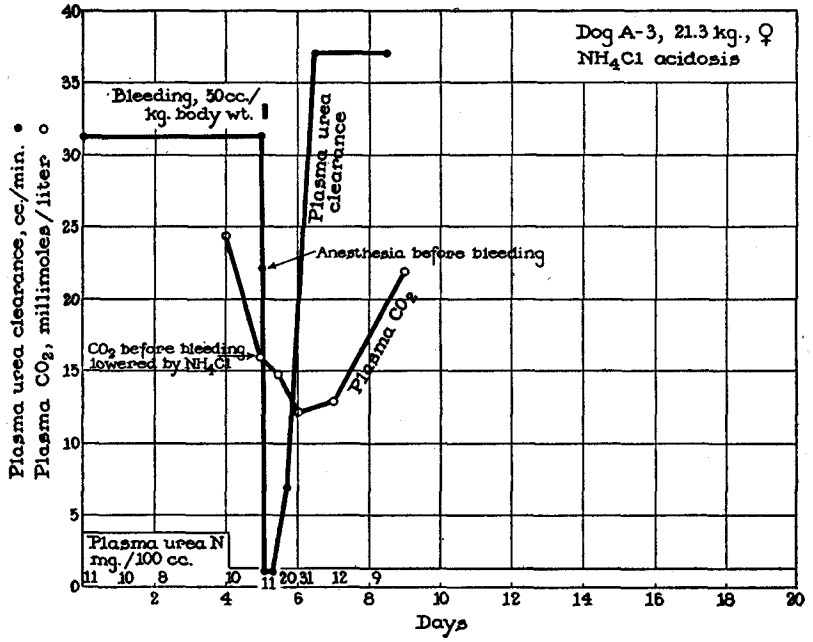

FIG. 6. Hemorrhage with no therapy.

from 25 to 17 millimoles per liter by the ammonium chloride, falling to 13 after hemorrhage, and returning to normal on the following day. The post-hemorrhagic oliguria was promptly relieved by the administration of plasma, urea clearance values returned to normal on the same day and remained normal thereafter. The plasma urea nitrogen never deviated from low normal values, 6 to 12 mg. per 100 cc.

Replacement of Blood by 7.1 Per Cent Oxyhemoglobin.—One animal, Fig. 7, was investigated. Comparison with Fig. 3 indicates but little influence of the acidosis on the renal effect of the replacement.

SUMMARY

Dogs were bled 50 cc. per kilo body weight and the blood withdrawn was re-

placed by equal volumes of 0.9 per cent NaCl solution, plasma, or 7 per cent oxyhemoglobin or methemoglobin solution.

Control dogs in which the withdrawn blood was not replaced by another fluid showed anuria or oliguria and depressed urea clearance for several hours after bleeding, but renal function returned to normal the next day.

Administration of 0.9 per cent NaCl solution or plasma promptly relieved the post-hemorrhagic oliguria and accelerated return of urea clearance values to normal.

Administration of oxyhemoglobin solution promptly relieved the post-hemorrhagic oliguria, but in some cases was followed by a period of 3 to 5 days in

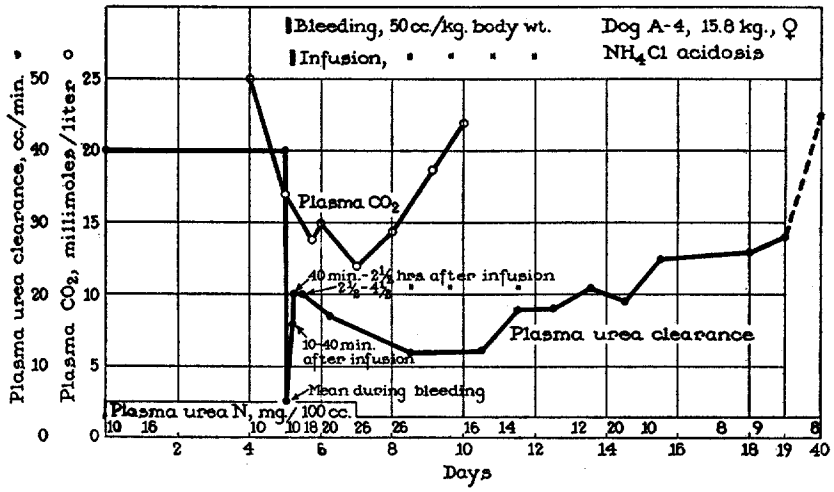

FIG. 7. Hemorrhage with oxyhemoglobin infusion.

which the urea clearance values were depressed to approximately 25 per cent of normal with an accompanying moderate elevation of plasma urea nitrogen. Return to normal clearance ensued during the next 5 or 6 days. The animals gave no signs of renal failure.

When pre-hemorrhagic acidosis was induced by the administration of ammonium chloride (plasma CO_2 reduced from the normal 25 to about 17 millimoles per liter) the renal behavior after replacement of blood by plasma or hemoglobin solution was essentially the same as in dogs not treated with ammonium chloride. However, since hemorrhage itself caused acidosis nearly as severe as that produced by NH_4Cl the results do not exclude acidosis as a factor in the effects of all the experiments.

After infusion of either hemoglobin or methemoglobin the concentration of total hemoglobin in the plasma fell off at such a rate that any given concentration was reduced by about 50 per cent in 8 hours. A small amount of pigment

remained at the end of 48 hours in the circulating plasma but none was detectable at the end of 72 hours.

When methemoglobin was introduced into the circulation, it was rapidly converted into active hemoglobin. After injecting a 7.6 per cent solution of methemoglobin (99 per cent methemoglobin), 41 per cent of the pigment in the circulating plasma was found to be active hemoglobin 35 minutes after the injection was completed.

No significant difference was noted between infused oxyhemoglobin and methemoglobin, either in effects on renal function or in rates of disappearance from circulation and excretion in the urine.

CONCLUSION

The immediate effects of treating hemorrhagic shock in dogs by replacing lost blood with 7 per cent hemoglobin solution were favorable, both on renal function and on general condition. However, subsequent transitory depression of the urea clearance for several days, shown by some of the treated animals, but not by untreated bled controls, indicates sufficient possibility of renal damage by the hemoglobin solution to prevent its recommendation at present as a blood substitute.

BIBLIOGRAPHY

1. Hamilton, P. B., Farr, L. E., Hiller, A., and Van Slyke, D. D., *J. Exp. Med.*, 1947, **86,** 455.
2. Farr, L. E., Hiller, A., and Van Slyke, D. D., *J. Exp. Med.*, 1947, **86,** 465.
3. Baker, S. L., and Dodds, E. C., *Brit. J. Exp. Path.*, 1925, **6,** 247.
4. De Nevasquez, S., *J. Path. and Bact.*, 1940, **51,** 413.
5. De Gowin, E. L., Osterhagen, H. F., and Andersch, M., *Arch. Int. Med.*, 1937, **59,** 432.
6. De Gowin, E. L., Warner, E. D., and Randall, W. L., *Arch. Int. Med.*, 1938, **61,** 609.
7. Bing, R. J., *Bull. Johns Hopkins Hosp.*, 1944, **74,** 161.
8. Hoff, H. E., Smith, P. K., and Winkler, A. W., *J. Clin. Inv.*, 1941, **20,** 607.
9. Hamilton, P. B., Phillips, R. A., and Hiller, A., unpublished data.
10. Van Slyke, D. D., and Hiller, A., *J. Biol. Chem.*, 1929, **84,** 205.
11. Van Slyke, D. D., Hiller, A., Weisiger, J. R., and Cruz, W. O., *J. Biol. Chem.*, 1946, **166,** 121.
12. Van Slyke, D. D., and Kugel, V. H., *J. Biol. Chem.*, 1933, **102,** 489.
13. Van Slyke, D. D., *J. Biol. Chem.*, 1929, **83,** 449.
14. Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, **19,** 211.
15. Van Slyke, D. D., *J. Biol. Chem.*, 1927, **73,** 695.
16. Van Slyke, D. D. and Hiller, A., *J. Biol. Chem.*, 1947, **167,** 107.
17. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61,** 523.
18. Van Slyke, D. D., *J. Biol. Chem.*, 1927, **73,** 121.
19. Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1927, **73,** 127.
20. Møller, E., McIntoch, J. F., and Van Slyke, D. D., *J. Clin. Inv.*, 1928, **6,** 427.