FACTORS INDUCING RENAL SHUT-DOWN FROM LYSED ERYTHROCYTES: AN EXPERIMENTAL STUDY*

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I. INTRODUCTION

PATIENTS TO WHOM TRANSFUSIONS of blood are given are usually in shock. In acute cases, the arterial pressure is often subnormal and the cardiac output typically diminished. The renal blood flow, in shock, does not remain a constant fraction of the cardiac output but falls actually more than the cardiac output. This is true not only in shock from hemorrhage (Cournand, *et al.*,[12]; Lauson, *et al.*,[27]) and chronic blood loss (Bradley and Bradley[8]) but also when the cardiac output is subnormal in spite of hypervolemia, as may occur in myocardial failure (Merrill[34]; Mokotoff, *et al.*[35]).

Individuals with chronic severe anemia may have a normal arterial pressure, an actually elevated cardiac output, an elevated venous pressure, but a remarkably low blood volume (Sharpey-Schafer[40]; McMichael[29]). Cardiac output, then, is maintained by the rise in venous pressure probably caused by a generalized constriction of capillaries and venules including renal vessels. In short, patients for whom blood transfusion is indicated have a contracted renal vascular bed and a diminished rate of glomerular filtration. A decreased effective renal blood flow in the absence of intrinsic renal damage is probably our most sensitive index of shock, especially of "chronic shock."

Intravenous administration of moderate amounts of hemoglobin or of lysed homologous or autologous red cells to normal man (O'Shaughnessy, *et al.*[36]) and normal dog may not be as nocuous as is generally believed. This suggests that patients who have undergone renal failure from a pint or less of incompatible blood probably had initial renal ischemia from a diminished blood volume.

Thus we set out to find factors which promote renal shut-down from lysed red cells and to locate the mechanism of the shut-down.

II. METHODS

1. *Exteriorization of the vesical trigone* and collection of urine has been described in the preceding paper (Maluf[30]).

2. *Renal Denervation.* The kidney was delivered through a subcostal incision. The perinephric tissue was dissected away until the kidney was attached to the body solely by its artery, vein and ureter. The renal artery and vein were carefully freed from their adventitia, by a fine watchmaker's forceps, for a distance of about 1.5 cm. from the hilum. The ureter was freed from connective tissue for a like distance. The stripped vessels and ureter

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were painted with 50 per cent phenol in ethanol. As soon as the phenol tarnished the vessels it was washed off with alcohol and aqueous zephiran.

To find whether the above method produces effective denervation, one kidney was so treated and the other left intact. A few days later renal clearances were measured simultaneously from each kidney before and after a large intravenous dose of epinephrine hydrochloride (0.22 mg./Kg.). There resulted a marked reduction of effective renal blood flow and glomerular filtration in the denervated but not in the normal kidney (for details see Maluf, 1949[31]). This potentiation to epinephrine has been described by Schneider and Wildbolz[41] and Kubicek and others.[23] Thanks to the exteriorized vesical trigone it was possible, in our work, to use the normal kidney as control for the contralateral denervated organ in the unanesthetized animal.

3. *Renal Function.* (a) *Maximal concentration and dilution.* Water was withdrawn from the cage at about 6 P.M. Next morning the antidiuretic urine was collected in one scoop after it had collected in the groin of the supine animal, hence there could be no increase in concentration by application of prolonged suction. Diuresis was produced by the administration of 50 cc. tepid tap water per Kg. by stomach tube. The specific gravity was measured pyknometrically in a small thin-walled pipette with pointed tip and internal constriction so as to contain from 0.3 to 1.0 cc. of liquid.

(b) *Glomerular filtration, effective renal blood flow and tubular maximum for para-aminohippurate* have been measured as described elsewhere (Maluf, 1949b[31]). All measurements were made on unanesthetized trained animals.

4. *Lysed Erythrocytes.* Blood was collected aseptically from the femoral artery, which was exposed and painted with formalin to kill organisms which may have entered with the cutaneous incision (Pope, *et al.*,[38]). The erythrocytes were allowed to sediment in a refrigerator; if they did not sedimen adequately they were centrifuged. The blood was used within a few days of collection. Within half an hour before infusion, the plasma was aspirated and the cells lysed by adding one and one-half times their volume of apyrogenic distilled water. The hemolysate was transferred through a gauze filter to an Upjohn transfusion bottle containing sodium chloride for isotonicity. The drip was at about 12 cc. per minute into an external jugular vein. If the dog became too dyspneic after about 600 cc. had entered, it was allowed up for a few minutes and then the infusion was completed. At the end of the infusion the hemolysate showed only a trace of methemoglobin spectrophotometrically. Since free hemoglobin in solution steadily changes into methemoglobin (Amberson, *et al.*[2]), the hemolysate was used immediately. Cultures of the hemolysate on blood agar and in enriched broth showed no growth.

5. *Histamine shock* lasting over one and one-half hours was produced by subcutaneous injection of histamine dihydrochloride suspended in cottonseed oil and lanolin (Hueper and Ichniowski[22]).

6. *Intra-aortic Injection with India Ink.* Higgins' India ink was diluted 1:1 with 1.8 per cent sodium chloride. Under nembutal (30 mg./Kg.), a

midline laparotomy was performed carefully and almost bloodlessly. Twenty cc. of the diluted ink were injected in retrograde fashion through a bent needle into the aorta immediately caudal to the renal arteries. The renal pedicles were tightly clamped within half a minute of the injection. Care was taken to avoid pressure on the kidneys and any mobilization of the kidneys until the injection was over and the pedicles clamped. A No. 20 needle was usually used and this caused severe bleeding after its withdrawal unless the aorta was then clamped proximally. When the pedicle clamps were applied too late, as occurred occasionally in the earlier trials on normal dogs, the cortex sometimes stained less than the medulla. This was presumably due to a washing away of ink by the inflow of unstained blood into the cortex on its way to the medulla: a phenomenon noted by Trueta, *et al.*[43] by roentgenography. When digital pressure was applied to a portion of the renal cortex during the injection, that local portion failed to be injected.

III. RESULTS

1. Effects of Intravascularly Injected Lysed Erythrocytes on Renal Function. Four normally hydrated dogs received relatively large doses of freshly lysed canine erythrocytes in isotonic sodium chloride intravenously. The dog in the sample protocol below received about 5.8 Gm. of free hemoglobin per Kg. A dog has 1.2 times as much renal mass per unit of body weight as a man; furthermore, a dog's kidney is about twice as active as a man's per unit of renal weight. Thus, relative to the magnitudes of glomerular filtration and effective renal blood flow, this would be equivalent to giving about 169 Gm. of hemoglobin to a 70-Kg. man $\left(\frac{5.8 \times 70}{1.2 \times 2} = 169\right)$. This is the amount of hemoglobin contained in about 1.4 liters of blood at 12 Gm./100 cc.

These dogs had received 50 cc. of tap water by stomach tube every few days to flush their kidneys and prevent retrograde infection from their exteriorized trigone. Urinary pH during diuresis was between 5.0 and 6.0. Their diet was a mixture of horse meat and cereal. The pH at the exteriorized bladder during antidiuresis was alkaline, doubtless because of the ammonia produced by urea-splitting bacteria.

Measurements of renal function (glomerular filtration, effective renal blood flow, tubular maximum for para-aminohippurate, and maximal concentrating and diluting power of the kidneys) showed practically no change from one day to several days after the infusion.

PROTOCOL

28 July, 1947 Female mongrel; 15.6 Kg. Vesical trigone exteriorized.
18 August Measurements of antidiuretic and diuretic urinary flow and specific gravity (Fig. 1).

29 August — Renal function as follows:

Glomerular filtration	= 40 cc./min. (left kidney).
Glomerular filtration	= 42.6 cc./min. (right kidney).
Effective renal blood flow	= 186 cc./min. (left kidney).
Effective renal blood flow	= 193 cc./min. (right kidney).
Tubular maximum for PAH	= 11.6 mg./min. (left kidney).
Tubular maximum for PAH	= 10.1 mg./min. (right kidney).
Diuretic urinary flows	= 3.34–4.28 cc./min. (left kidney).
Diuretic urinary flows	= 3.14–3.70 cc./min. (right kidney).
pH of urine in diuresis	= 6.0
Hematocrit	= 40.4 vol. %
Blood urea	= 38 mg./100 cc.

8 September — 270 mg. nembutal, s.c., for sedation

Minutes:

0-75 — *1,070 cc. of freshly lysed red cells in isotonic saline through external jugular vein* (= 73.8 cc./Kg. and 5.8 Gm. Hgb./Kg.) The urine was brick-red within 5 minutes after beginning the infusion. Diuresis set in.

No shivering.

80-85 — Rates of urinary flow:

2.18 cc./minutes from left ureter.

2.80 cc./minutes from right ureter.

90-94 — Rates of urinary flow:

2.2 cc./minutes from left ureter.

2.9 cc./minutes from right ureter.

No casts in urine.

94 — Vomits food eaten 3 hours before infusion. (The dogs which were not fed on the day of infusion and which received red cells lysed by apyrogenic distilled water did not vomit or retch on the same day after the infusion; see protocols below).

95 — Walks to cage in good condition.

135 — Vomits more of food eaten before the infusion. Drinks water.

345 — Retches occasionally. Active and alert. Red stream of urine from cage to gutter about 3 meters away; still urinating amply. Water removed from cage. Has drunk 500 cc. water in 165 minutes.

Feces normal in color and consistency; sclerae normal in color. No further vomiting.

Next Morning — About 260 cc. dark-red, thick urine in pan below cage. Hemochromogen still prominent in urine. Holds 800 cc. tap water given by stomach tube to evoke diuresis.

Renal function as follows:

Glomerular filtration	= 37.6 cc./min. (left kidney).
Glomerular filtration	= 42.4 cc./min. (right kidney).
Effective renal blood flow	= 208 cc./min. (left kidney).
Effective renal blood flow	= 222 cc./min. (right kidney).
Tubular maximum for PAH	= 12 mg./min. (left kidney).
Tubular maximum for PAH	= 14.1 mg./min. (right kidney).
Diuretic urinary flows	= 1.61–2.1 cc./min. (left kidney).
Diuretic urinary flows	= 1.7 –2.5 cc./min. (right kidney).
pH of diuretic urine	= 6 to 6.5

No casts in urine

13 Sept. No water since evening before in preparation for concentration and dilution tests:
Sp. Gr. = 1.004 to 1.047 (left kidney).
Sp. Gr. = 1.002 to 1.053 (right kidney).
Can concentrate and dilute as before the infusion (also Fig. 1).

21 Sept. Both kidneys again show normal concentrating and diluting power. Dog remains healthy.

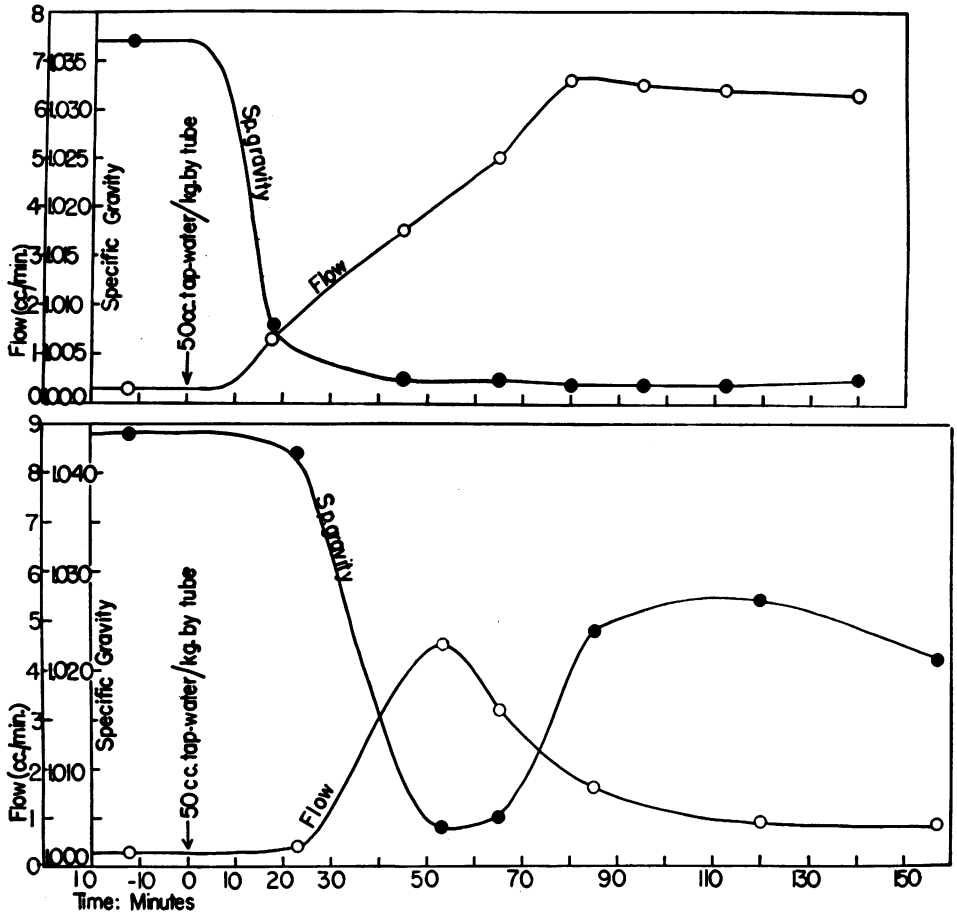

FIG. 1.—Diuretic and antidiuretic urinary flows and specific gravities before and after ingestion of a large quantity of water in a normally hydrated dog before (upper graph) and one day after (lower graph) the infusion of lysed red cells. Free hemoglobin received: 6 Gm./Kg. Maximal and minimal concentrating power is still present after the infusion.

2. Effects of Intravascularly Injected Lysed Erythrocytes Plus Dehydration on Renal Function. There was a marked difference between the effects of intravascularly injected lysed red cells into well hydrated and into severely dehydrated dogs. The former lived without obvious renal injury. The latter became anuric or oliguric and underwent severe renal damage. Below is one

exemplary protocol out of six, all of which had a uniform outcome. To study their renal circulation in the anuric or oliguric phase, the dogs were sacrificed before a probable uremic death.

Dehydration was continued until the urinary flow was 0.006 cc./Kg./min., this being the value found by Harrison and others[20] to foster renal shut-down from 4 Gm. of hemoglobin per Kg. or from 1 Gm. of methemoglobin per Kg.

Any fall in blood volume, which probably occurred during dehydration, was more than fully compensated by the intravenous injection of the hemolysate. The volume infused was about two-thirds of the theoretical normal blood volume and the external jugular veins became engorged. The arterial pressure was always normal, as indicated by the prominently visible femoral pulse. Consequently, the postinfusion renal shut-down could not have been due to hypopiesia.

One dog underwent *renal denervation* about two weeks before receiving the infusion. This conspicuously failed to prevent renal failure.

PROTOCOL

27 Mar. 48	Female mongrel; 12 Kg. Vesical trigone exteriorized.
12 May	Renal function as follows:
	Glomerular filtration = 35.1 cc./min.
	Effective renal blood flow = 226 cc./min.
	Tubular maximum for PAH = 8.1 mg./min.
	Diuretic urinary flow = 1.76–2.88 cc./min.
	NPN. = 57 mg./100 cc.
	Hct. = 32 vol. per cent.
13 May	*Water out of cage in* A.M.*; commencement of dehydration.*
	Weight = 11.8 Kg.
16 May	Refuses food for first time
18 May	Mouth and nostrils parched; temples, cheeks and eyes sunken
A. M.	Urinary flow = 0.005 cc./Kg./min.
	Weight = 10.4 Kg.
	Hct. = 38.9 vol. per cent.
	NPN. = 31 mg./100 cc. plasma
Early P. M.	100 mg. nembutal S.C. for sedation.
Minutes:	
0	*Infusion of lysed red cells in isotonic NaCl begun.*
10	Reddish-brown pigment in urine.
40	Diuresis begins; about 420 cc. of hemolysate in; dog dyspneic, struggles and whimpers; contralateral jugular vein is distended. Infusion discontinued. Dog allowed about.
55	Dyspnea ceases; *infusion recommenced.*
	Mild diuresis continues with dark brown-red pigment.
75	Total amount infused = 555 cc.; = 53.3 cc./Kg. and 2 Gm. Hgb./Kg.
115	Animal drowsy; sleeping in cage; external jugular veins distended; no vomiting.
265	About 50 cc. "black water" in pan below cage. Less drowsy; allowed 50 cc. water by mouth.
325	No vomiting as yet; allowed another 50 cc. water by mouth.
Next morning	No fluid in pan under cage; thighs quite dry.
	Markedly oliguric; less than 0.004 cc. urine per min.; urine dark brown and turbid. Alert but weak.

11: 30–40 A. M.	Drank 950 cc. milk.
12:50 P. M.	Allowed 300 cc. water, which was soon vomited together with some of the milk drunk two hours ago.
1:20–1:42 P. M.	No urine from ureteral orifices, even on dilating orifices. T = 101.7°F (normal); R = 19/min. External jugular veins are prominent when lying down but dog still appears strikingly dehydrated and emaciated. Drowsy but readily awakened. Iris tinged red.
2:00 P. M.	Weight = 11.3 Kg. NPN. = 156 mg. Hct. = 39.5 vol. per cent Hgb. in plasma is almost as intense as in infusion solution.
2:10–2:20	*Still anuric.*
2:25 P. M.	Nembutal: 30 mg./Kg. I. V. for anesthesia. Laparotomy (midline without blood loss): liver not enlarged; viscera grossly normal; stomach distended with about 400 cc. curdled milk and some gas. Gross appearance of kidney normal. 20 cc. of India ink, which had been diluted with an equal volume of 1.8 per cent NaCl, were injected rapidly and in retrograde fashion into the abdominal aorta somewhat caudal to the right renal artery. The renal pedicle was then clamped immediately. The renal cortex was as well injected as the medulla (Fig. 6). The renal capsule was not under tension.

The kidney was fixed in 10 per cent formalin. Frozen sections (8μ and 25μ) were immersed in water to uncurl. Temporary preparations were covered with a droplet of water and a coverslip. The loops of the glomerular tuft were well filled with India ink (Figs. 2 and 3), which was restricted entirely to the blood vessels. There were numerous eosinophilic casts filling practically every collecting tubule in the field (Fig. 4). Elongate, light-brown *crystals,* probably of methemoglobin, were numerous in the tubular lumina (Fig. 5). The tubules were not dilated. There was intracellular pigment and, as shown by H. and E. mounts, no "lower nephron nephrosis."

In another dog, a 16-Kg. pointer which had received 3.2 Gm. of hemoglobin per Kg. as lysed red cells, there was an appreciable urinary flow of 0.187 cc. per minute by the second postinfusion day, this animal never having become anuric. As a gauge of renal function, however, this flow would be misleading since the plasma NPN was 182 mg./100 cc.; there was an almost hundred-fold reduction of glomerular filtration (98 cc. per minute initlally, down to 1.14 cc. per minute on the second postinfusion day), and of *effective* renal blood flow (761 cc. per minute initially, down to 6.4 cc. per minute on the second postinfusion day); and there was a failure to concentrate urine as shown by a low specific gravity and a urine-plasma-creatinine ratio of only 6. These values contrast strikingly with the oliguria of normal kidneys, in which there is maximal concentration during severe dehydration. Thus the urine-plasma-creatinine ratio of dogs subjected to comparable dehydration alone exceeds 200 (see below). Injections of India ink into the abdominal aorta during life showed that, in these dehydrated postinfusion dogs, the *absolute* renal blood flow was ample and the anuria or oliguria could not be explained by renal ischemia. Any hypovolemia which may have

resulted from dehydration was more than fully compensated for by the infusion. The injections were retrograde to avoid the objection that intrarenal vessels may be forced open by the small force of injection. Equal deposition of India-ink particles in blood vessels of the renal cortex and medulla showed that there was no shunting of blood from the cortex. These facts, together with the large number of pigmented and crystalline casts in the tubules and the

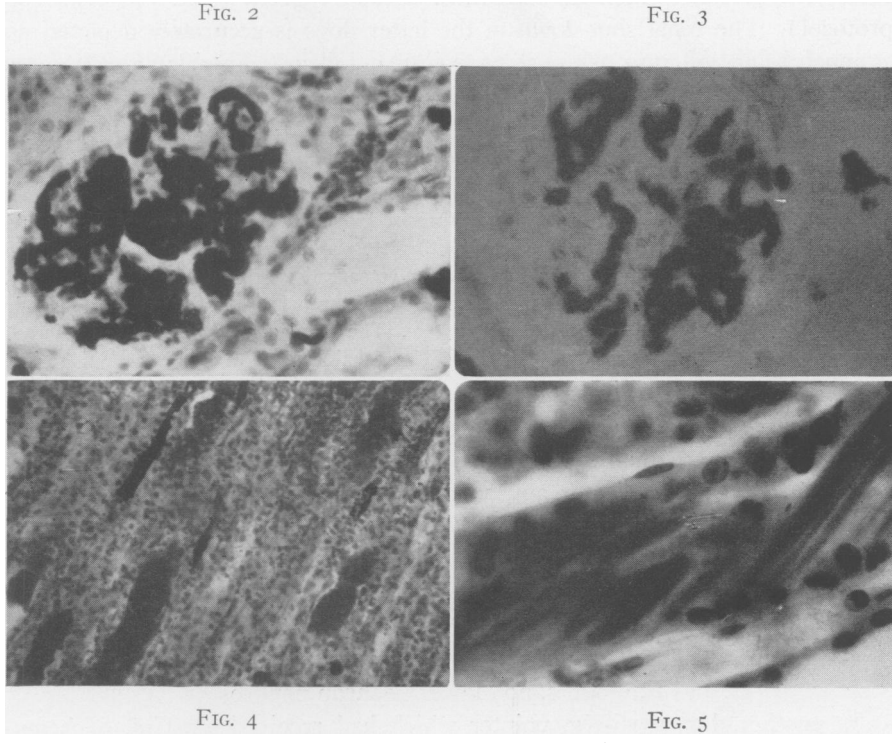

FIG. 2.—Glomerular tuft filled with particles of India ink injected during life into the aorta while the animal was anuric from intravenous administration of lysed erythrocytes superimposed on dehydration. Kidney fixed in formalin, section while frozen, and stained with hematoxylin and eosin.

FIG. 3.—Glomerular tuft containing particles of India ink; from same kidney as shown in Figure 2; fresh, frozen preparation; not stained.

FIG. 4.—Pigmented amorphous casts filling collecting tubules of same dog as is seen in Figure 2. Kidney fixed in formalin; sectioned while frozen; stained with hematoxylin and eosin.

FIG. 5.—Elongate crystals, probably of methemoglobin, in the collecting tubules of the same dog as is seen in Figure 2. Same preparation as in Figure 4.

absence of histologic evidence of renal cellular damage, indicate that the anuria or oliguria are, initially, at least, caused by tubular obstruction. The fact that the animals were not allowed to progress and die in uremia may explain the absence of increased intracapsular tension, absence of histologically obvious renal cellular damage and absence of peritubular edema.

3. Effects of Dehydration Alone on Renal Function. It was essential to study the effects of dehydration alone on renal function. The following protocol

shows that severe dehydration alone actually can reduce, reversibly, glomerular filtration and effective renal blood flow by about half. The specific gravity of the urine, however, becomes high. This contrasts with the low fixed specific gravity in oliguria from intravascular administration of lysed red cells plus dehydration. Parallel with the high specific gravity is the high urine-plasma ratio for creatinine, which contrasts with a low ratio in oliguria from dehydration combined with intravascularly given lysed red cells (see protocol). The renal shut-down in the latter dogs is accurately depicted not so much by the oliguria *per se* as by the great fall in glomerular filtration and effective renal blood flow. Unilateral renal denervation in a dog, the protocol of which is not presented here, did not alter the extent of reduction in renal function by dehydration alone.

PROTOCOL

28 November, 1947 Female mongrel. 9.3 Kg.
Exteriorization of vesical trigone. NPN = 32 mg./100 cc.

28 May, 1948 Renal function as follows:
Glomerular filtration = 58.9 cc./min.
Effective renal blood flow = 438 cc./min.
Diuretic urinary flow = 3.2–3.89 cc./min.
Urine-plasma ratio for creatinine = 18.4
Hct. = 49.2 vol. per cent.

9 June *Water out of cage; commencement of dehydration.*

14 June Rejects food for first time.

16 June Weight = 7.9 Kg. NPN = 36 mg./100 cc.
Urinary flow = 0.0061 cc./Kg./min.
Sp. Gr. = 1.042.
Renal function as follows:
Glomerular filtration = 23.5 cc./min.
Effective renal blood flow = 187 cc./min.
Urinary flow = 0.10 cc./min.
Urine-plasma ratio for creatinine = 235.0
Hct. = 54.3 vol. per cent
Shortly after the above measurements the dog was allowed to drink water as desired (= about 1,000 cc.), a large part of which was promptly ejected. Alert and active.
No further vomiting. One and one-half hours later it ate 1 pound of canned canine food. Six hours later it ate another pound.

19 June Weight 9.2 Kg. Hct. = 49.2 vol. per cent.
Renal function as follows:
Glomerular filtration rate = 45.2 cc./min.
Effective renal blood flow = 270 cc./min.
Diuretic urinary flow = 1.8 cc./min.

4. Effect of Intravascularly Injected Lysed Erythrocytes Plus Shock on Renal Function. It was important to find whether renal shut-down would result from the injection of lysed red cells into normally hydrated dogs if shock were superimposed.

Reversible shock was produced by injecting histamine dihydrochloride in oil subcutaneously into normally hydrated dogs. The infusion of lysed red

cells was performed when the dog was in prostrate shock. Even though the volume of solution infused was almost as great as the theoretical circulating blood volume, the effect of the infusion on the shock picture was not striking. The anuria of shock changed only to a scant urinary flow during the infusion. A few hours later, even after complete recovery from shock, there was absolute anuria or marked oliguria.

The oliguria caused by intravascularly injected lysed red cells plus shock is a genuine renal shut-down with at least a hundred-fold decrease in glomerular filtration and effective renal blood flow, a low urinary specific gravity, a

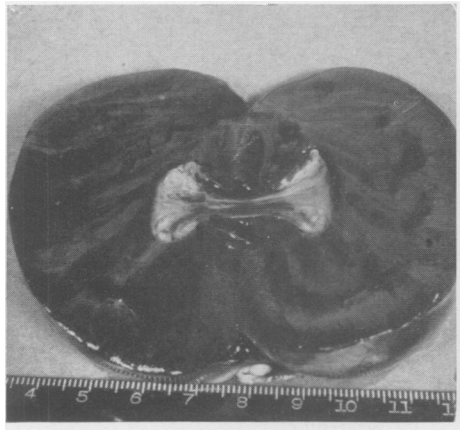

FIG. 6

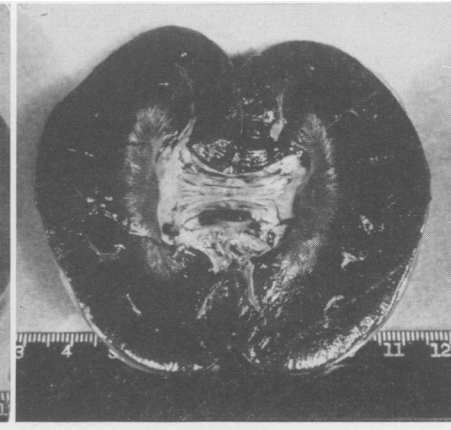

FIG. 7

FIG. 6.—Divided kidney of the dog which was sacrificed in anuria from intravenous administration of lysed red cells superimposed on dehydration (same dog as in Figs. 2 to 5). India ink was injected during life into the aorta and the renal pedicle was clamped immediately after. Both the cortex and the medulla are well injected.

FIG. 7.—Divided kidney of the dog which was sacrificed in anuria from intravenous administration of lysed red cells superimposed on histamine shock. The arterial pressure was normal at this time. Intra-aortic injection of India ink with immediate subsequent clamping of the renal pedicle resulted in equal and normal injection of both cortex and medulla.

low urine-plasma ratio for creatinine, and a marked uremia. This type of oliguria is identical with that from lysed red cells plus severe dehydration, and contrasts with the oliguria of severe dehydration alone (see above).

Injections with India ink, as described above, during the phase of *post-shock* renal shut-down, resulted in equal and adequate injection of the renal cortex and medulla. This indicates a normal absolute renal blood flow and shows that the renal shut-down cannot be due to a shunting of blood from the renal cortex to the medulla. The casts in the tubular lumina indicate extensive tubular obstruction as the primary cause of the shut-down. Here again the marked fall in effective renal blood flow, as measured by the para-aminohippurate clearance, is obviously due to inability of the tubules to allow passage of the hippurate because of their being obstructed.

Animals which were put into shock with the above dose of histamine, but which did not receive lysed red cells, recovered completely although they were

anuric for more than an hour. India ink injections during the anuria from shock showed merely a diminished injection of the kidneys with more ink in the renal cortex than in the medulla. Thus the anuria during histamine shock is the result of general renal ischemia and not the outcome of a shunting of blood from the renal cortex to the medulla.

The experiment of injecting lysed red cells into normally hydrated dogs in shock was done on three animals.

PROTOCOL

	Female mongrel; 13 Kg. Vesical trigone had been exteriorized.
6 June, 1948	Renal function as follows:
	Glomerular filtration = 50 cc./min.
	Effective renal blood flow = 290 cc./min.
7 June	Weight = 13.0 Kg. Food and water withdrawn from cage at 6 P. M.
8 June	Urinary flow = 0.16 cc./min.
	Sp. Gr. of urine = 1.041
	pH of urine = 6.0
Minutes:	
0	Femoral arterial pulse strikingly visible through skin; exteriorized bladder pink; R = 20/min.
	70 mg. histamine dihydrochloride (= 5 mg./Kg.) in 3 cc. cottonseed oil plus lanolin, s.c. through a No. 18 needle; tape was applied over the site.
23	Lies prone.
33	Stands and ejects bile-stained vomitus.
58	Around and walking; defecates.
68	Lying down with eyes half closed; femoral arterial pulse no longer visible but thready to palpation.
77–82	Urinary output not measurable; 1 drop; bladder a mottled purple-red. Dog markedly drowsy.
	Blood draw now showed: Hct. = 43.7 vol. per cent; NPN = 29 mg./100 cc.
78	*Infusion of lysed canine red cells in isotonic NaCl begun.*
78–133	No shivering; 1 to 2 drops of urine; normal in color.
134	Urine appears; not a copious flow; dark brown in color.
153	*Infusion discontinued.* Volume infused = 650 cc. (= 50 cc./Kg. and 3.76 Gm. Hgb./Kg.).
158	No diuresis; urinary flow still scant; dog licks lips; drowsy; lies prone.
163	Blood sample drawn now shows: Hct. = 41.7 vol. per cent; Plasma Hgb. = 2.8 Gm./100 cc.
168–173	Dark-brown urine; less than 0.02 cc./min.
273	Urinary flow still less than at 168–173 min.
	Femoral arterial pulse still not visible but feels more forcible; dog can stand but prefers to lie down. Bladder no longer markedly cyanotic. T = 101°.6F.
296	Vomits.
396–404	Femoral arterial pulse now distinctly visible although not as prominent as initially.
	Urinary flow less than 0.01 cc./min.; urine dark brown.
	Jaundice apparent on exposed areas of skin, such as inner portions of earflaps and belly.
660	Anuric. Alert and active. Ate almost 1 pound canned canine food; refused the rest. Allowed about 200 cc. water.

Next morning — Urinary flow = 0.01 cc./min., urine dark brown; jaundice increased; no "black water" in pan under cage during night but about 300 cc. of what looks like chiefly vomitus. Dog moderately alert and active.
Blood showed:
NPN. = 1122 mg./100 cc.
Hct. = 40.3 vol. per cent
Plasma Hgb. = 2 Gm./100 cc.
Drank, as desired, 140 cc. water, which was vomited after 20 minutes.

Evening — Is listless; retches when offered food.

Second postinfusion day — Listless but can walk. T = 99.°8F. Urinary flow is less than 0.006 cc./min.; urine a light brown; stools not tarry; no hyperpnea.

Third postinfusion day — Weight = 11.7 Kg. T = 98°6F. R = 48.
Femoral arterial pulsations visible; alert but listless; neuromuscular hyperirritability; Chvostek. Practically no fluid in pan at bottom of cage; a small, loose, deep-brown stool; urine a dark orange. Bleeding time elevated.
Urinary flow = 0.079 cc./min.
Sp. Gr. of urine = 1.020; Hct. = 47.3 vol. per cent
NPN. = 312 mg./100 cc.
Plasma Hgb. still appreciable (less than 1 Gm./100 cc.); plasma stained with bile pigment
Renal function:
Glomerular filtration = 0.40 cc./min.
Effective renal blood flow = 1.28 cc./min.
Urine-plasma ratio for creatinine = 5.1
Laparotomy under nembutal anesthesia: Subcutaneous tissues and viscera jaundiced; gastro-intestinal tract empty; renal capsule incised in situ and found not to be under tension; extensive oozing of blood from renal cortex when this was nicked slightly. Kidney excised; deeply stained with brown pigment; pigment in medulla darker than in cortex. When the kidney was divided and squeezed gently, more blood seemed to ooze from the cortex than from the medulla. There was thus no evidence of renal cortical ischemia. Intra-aortic India ink showed equal and normal injection of cortex and medulla (Fig. 7).

5. Effects of Intravascular Hemolysis by Distilled Water on Renal Function. Large amounts of apyrogenic distilled water were injected rapidly intravenously into normal hydrated dogs. Only mild, transient systemic effects resulted. The four dogs so treated received an average of 90 cc. of distilled water per Kg. in 18 minutes, which, relative to renal activity (see above), is equivalent to injecting 2,600 cc. into a 70-Kg. man. Renal function, as indicated by concentration and dilution tests and by the plasma—NPN, was not reduced. Landsteiner & Finch[26] injected 200 cc. of distilled water in from one to two minutes into patients who were not in shock and found no rise in blood urea. Voris[44] gave 1,000 cc. of distilled water intravenously to patients and noted no ill effects and only mild hemoglobinuria.

PROTOCOL

Female mongrel; 11.6 Kg.; vesical trigone had been exteriorized; normally hydrated; no food since night before. Initial NPN = 54 mg./100 cc.

14 March, 1948	Left renal artery and vein divided.
17 March	Evaluation of renal function:
	Glomerular filtration = 33.7 cc./min.
	Effective renal blood flow = 174 cc./min.
	Tubular maximum for para-aminohippurate = 13.5 mg./min.
	Diuretic urinary flows = 1.46–2.86 cc./min.
10 June	
2:20–2:38 P. M.	*1,025 cc. apyrogenic distilled water were injected* through a three-way metal cock and No. 15 needle *into an external jugular vein* using a 20-cc. syringe; *i.e.*, 88.5 cc. water/Kg. in 18 min. The exteriorized trigone showed no immediate diuresis or obvious pigmentation of the urine. The dog was not manifestly uncomfortable. It lay down comfortably, shook itself occasionally and belched gas.
	Hct.-before I. V. = 35.7 vol. per cent
	Hct.-after I. V. = 34.6 vol. per cent
	Plasma hemoglobin immediately after I. V. = 200 mg./100 cc.
3:19–3:24 P. M.	Marked diuresis. 4.4 cc./minute of light reddish-brown urine. Dog returned to cage in good condition. Extensive diuresis continues.
5:30 P. M.	Ate 1 pound canned canine food
12 June	NPN = 41 mg./100 cc. Plasma normal in color. Dog concentrates and dilutes urine normally.

6. Effects of Intravascular Hemolysis by Distilled Water Plus Shock on Renal Function. Human beings who are not in shock have been shown to tolerate, without renal damage, rapid intravenous injection of at least 900 cc. of distilled water. It has been suggested that the renal shut-down and uremia which may follow irrigation of the bladder with tap water during transurethral operations on the prostate gland may be due to a combination of shock with intravascular hemolysis (Creevy & Webb,[4] Landsteiner and Finch,[26] McLaughlin, and others[28]). It has not been possible, however, to produce renal failure in dogs by a combination of shock plus injection of distilled water even in quantities which one might not venture in man.

The dog of the following protocol had only one kidney; underwent 90 minutes of absolute anuria from severe shock lasting two hours; was rapidly infused with 95 cc./Kg. of distilled water during the anuria; and did not recover from anuria until 70 minutes after the infusion. This combination of apparently near-maximal adverse circumstances nevertheless failed to produce renal failure. Similar results occurred in the two other dogs so tested. It is possible that overhydration and reversible shock without blood loss prevented uremia. Creevy (1947) has pointed out that aggravating factors in man may be renal vasoconstriction from rapid blood loss, arteriosclerosis and pyelonephritis. This deserves further investigation.

PROTOCOL

	Female mongrel; weight = 8.85 Kg.; vesical trigone had been exteriorized.
7 May	Right nephrectomy.
18 May	Renal function as follows:
	Glomerular filtration = 48.9 cc./min.

	Effective renal blood flow = 250 cc./min.
	Tubular maximum for para-aminohippurate = 17.2 mg./min.
18 June	No food or water since evening before.
Minutes:	
0	Femoral arterial pulse visible through skin; exteriorized bladder pink; R = 22 min.
	5 mg. histamine dihydrochloride per Kg. in cottonseed oil and lanolin, s.c. over chest.
7	Dog lies prone and licks lips.
12	Gets up and defecates.
22	Again lies prone.
42	Retches.
63	R = 40/min. Femoral arterial pulse no longer visible and faint to palpation; exteriorized bladder cyanotic.
63 to 70	*Anuric.*
74 to 90	*850 cc. apyrogenic dist. water I. V., i.e.,* 95 cc./Kg. in 16 minutes. Vomits once during procedure. Defecates.
	Still anuric.
93	Uncomfortable; abdomen distended and rather tympanitic; grunts; stands voluntarily but unsteadily.
95	Blood drawn now shows: NPN = 31 mg./100 cc.: Hct. = 40.3 vol. per cent; Plasma Hgb. = 300 mg./100 cc.
	Dog stands but with head lowered.
125	*Dog still anuric;* lying down.
160	Urinary flow sets in; brownish-red urine; dog *has had about 90 minutes of absolute anuria.* Femoral pulse now forcible to palpation but still not visible through skin; dog lying down; no diuresis as yet.
520	Only 300 cc. of brownish fluid in pan at bottom of cage; urine a light reddish-brown; is distinctly lighter than six hours ago. Dog obviously recovered from shock but refuses food or water.
Next morning	Weight = 8.2 Kg. 750 cc. dark brown liquid in pan at bottom of cage. Urine is light reddish-brown.
	Hct. = 44.2 vol. per cent
	NPN. = 39 mg./100 cc.
	Urinary flow = 0.55 cc./min.
	Plasma hemoglobin somewhat below yesterday. Eats ¼ pound of canned canine food.
Second postinfusion day	Urine straw-colored and output copious; appetite normal. Food and water out of cage at 5 P. M. for concentration and dilution test tomorrow.
Third postinfusion day	Weight = 7.8 Kg.; Hct. = 47 vol. per cent; NPN = 31 mg./100 cc.
	Sp. Gr. of concentrated urine (at 0.16 cc./min.) = 1.029.
	Sp. Gr. of dilute urine (at 2.4 cc./min.) = 1.004.

IV. DISCUSSION

We have shown that normally hydrated dogs may withstand intravenous injection of lysed canine red cells which, so far as relative degrees of renal activity are considered, would be equivalent to the infusion of more than a liter of incompatible blood into a 70-Kg. man. This tolerance is true in spite of aciduria. Earlier studies which indicated a greater tolerance to hemoglobinemia in alkalinuria (Baker and Dodds,[3] for rabbits; De Gowin *et al.*,[15] for dogs) were inadequately controlled especially as to dehydration. Thus, De

Gowin and others made their dogs acidotic by mixing 8 Gm. of ammonium chloride with one-half pound of beef per day. The dogs occasionally refused this food "for several days at a time." Furthermore, while 7 of the 28 dogs died of renal failure, 6 died of intercurrent infection. As for rabbits, Yorke and Nauss[46] have noted the importance of dehydration in posthemoglobinemic anuria. Anuria and death occurred in the rabbits fed dry diets but not in those eating green food. They postulated a low glomerular filtration pressure due to dehydration. De Navasquez[16] and Yuile and others[47] failed to reproduce the results of Baker and Dodds in rabbits. Flink[17] injected from four to six Gm. of hemoglobin per Kg. into dogs and found just as severe renal damage in dogs with alkalinuria as in those with aciduria. When the initial concentration of hemoglobin in the plasma exceeded 3.7 Gm./100 cc., renal insufficiency always developed. He found no difference between the effects of solutions of hemoglobin crystals and of lysed red cells. Bing[6, 7] found from 0.5 to 1 Gm. of hemoglobin per Kg. practically innocuous to dogs, whether in aciduria or alkalinuria, and that methemoglobin was relatively nocuous in aciduric but not in alkalinuric dogs. Bing's anuric dogs, however, may have been dehydrated as a result of large ammonium chloride feedings. Furthermore, ammonium chloride itself, even in doses considerably smaller than Bing's, is somewhat nephrotoxic to man (Markert[32]), and especially to rabbits (Govan and Parkes[19]). Webster and others (1935) have pointed out that in the presence of physiologic salt concentrations, the state of aggregation of hemoglobin is not influenced by pH.

O'Shaughnessy and others[36] injected intravenously into a patient with cancer 50 Gm. of human hemoglobin in 30 minutes. This is equivalent, at least in terms of hemoglobin, to over 300 cc. of incompatible blood. No obvious renal damage resulted. Gilligan and others[18] and Ottenberg and Fox[37] obtained similar results with smaller quantities of hemoglobin; however, Gilligan and others noted chills, fever and severe abdominal pains in the patient receiving the largest quantity of stroma-free human hemoglobin, 16.4 Gm., *i.e.,* only 0.25 Gm. per Kg.

The importance of dehydration in causing renal shut-down from homologous hemoglobin has been shown by Yorke and Nauss[46] and Lalich[24, 25] for rabbits and by Harrison and others[20] for dogs. The present work in part confirms their findings. We have further found that severe dehydration will reduce the effective renal blood flow and rate of glomerular filtration in the dog. This may be true in man, as is indicated by the falling urea clearance and the rising NPN in man's blood during progressive dehydration (Adolph and others.[1]).

That shock plus hemoglobinemia or myoglobinemia can result in renal failure has been shown by Corcoran and Page[10] (1945). The present study in part confirms this.

The cause of renal shut-down from hemoglobinemia has been hypothesized on the basis of renal vasoconstriction, mechanical tubular blockage or

renal tubular damage. Evidence for renal vasoconstriction by free hemoglobin in the blood stream was produced by Reid[39] and Mason and Mann.[33] Hesse and Filatov[21] (1933) found a fall in arterial pressure and renal volume upon infusion of lysed autologous blood into dogs. Subsequent workers, who have performed the infusions aseptically, have not been able to confirm these findings (Bing,[7] 1944; Flink,[17] 1945; Corcoran and Page,[11] 1947). The diuresis which directly follows an infusion of hemoglobin solution into a normal dog and which tends to occur even in a dehydrated or hypopiesic dog contraindicates vasoconstriction as a primary factor in the renal shut-down.

Our studies with India ink indicate no obvious decrease in *absolute* renal blood flow during the anuria or oliguria which follows infusion of lysed red cells superimposed on reversible shock or dehydration. Our measurements of the renal clearance of para-aminohippurate, however, show a very marked fall in *effective* renal blood flow. These findings imply that damaged or obstructed tubules fail to extract the hippurate from the blood circulating through the renal parenchyma. It is significant that these were postinfusion studies and that the animals had recovered completely from shock or had their blood volume fully replenished from any loss incurred during dehydration. They nevertheless had marked renal shut-down. These India-ink studies also show that this renal failure cannot be ascribed to a shunting of blood from the renal cortex to the medulla. Such a shunt has been described by Trueta and others[42, 43] in a certain percentage of rabbits upon stimulation of the sciatic nerve or after injection of staphylococcal toxin.

Renal failure occurred when intravascular injection of lysed red cells was combined with dehydration or shock even in kidneys which had been denervated.

Corcoran and Page[9] incidentally noted that dogs in hemorrhagic shock with denervated kidneys responded to blood transfusion by a disproportionately slow and incomplete return toward normal of renal clearances. They therefore suggested that spinal anesthesia may actually interfere with the recovery of renal circulation in shock treated by transfusion.

Our dogs were sacrificed after a postinfusion period of from two to three days, when they were quite uremic. At this stage, the renal capsule was not under tension and there was no histologically obvious renal cellular damage. Many of the tubules in a field were occluded by brown casts or crystals. The casts were best preserved by fixing the kidney in 10 per cent formalin shortly after the animals were sacrificed and sectioned while frozen a few days later. Most of the casts were lost when the kidneys were sectioned while frozen without prior fixation. The loss probably occurred when the frozen sections were immersed in water to unroll. Although there was considerable loss of casts in specimens carried through paraffin in Flink's[17] studies, the majority of tubules were occluded by casts in dogs with serious or fatal renal damage. This has been confirmed by Lalich[24] and Harrison and others.[20] Harrison and others teased out casts by microdissection and found that they dissolved rapidly in buffers below pH 5.2 and above pH 7.6 and that they had the

spectral properties of methemoglobin. The studies of several investigators, including Bell,[5] indicate that tubular obstruction by pigmented casts is a prominent feature in the renal shut-down of the "transfusion-kidney." By showing the nontenability of the postulate that lysed red cells produce renal cortical ischemia, by studying the kidneys histologically within the first two days after infusion, and by showing that dehydration and shock are predisposing factors, we are left, as our apparent sole gross explanation, the following: *The mechanism of renal failure from the intravascular introduction of a moderate quantity of lysed red cells is primarily due to tubular obstruction from casts of hemochromogen combined with a low rate of glomerular filtration.*

V. SUMMARY

1. As much as 5.8 Gm. per Kg. of free homologous hemoglobin, as lysed red cells, may be given intravenously to a normal, well-hydrated dog without producing any striking sign of renal damage. In terms of lysed red cells and relative to the degree of renal activity, this is equivalent to giving at least one liter of incompatible blood to a 70-Kg. man. The initial urinary pH of the dogs was from 5.5 to 6.0.

2. When a dog is dehydrated until its urinary flow is only 0.006 cc./Kg./min., intravenous infusion of from 2 to 3 Gm. per Kg. of free hemoglobin as lysed red cells will lead to immediate anuria or oliguria and then severe uremia. The resultant oliguria is not of a concentrated but of a dilute urine. A nearly normal urinary output may mask a severe renal shut-down, as indicated by a hundred-fold decrease in the rate of glomerular filtration and *effective* renal blood flow.

3. The oliguria of severe dehydration alone, on the other hand, is of a urine with high specific gravity. Severe dehydration of the above extent results in an approximate halving of glomerular filtration and effective renal blood flow. This is reversible on rehydration and the animal promptly recovers.

4. Denervation of a kidney several days prior to dehydration does not influence the changes mentioned in 2 and 3.

5. Intravenous injection of from 3 to 4 Gm. per Kg. of free hemoglobin as lysed red cells into a dog in profound reversible shock and anuria from histamine will lead to postshock anuria or oliguria and then to severe uremia. As in the combination of dehydration with intravascular injection of lysed red cells, there results an immense fall in glomerular filtration and *effective* renal blood flow and a low urine-plasma ratio for creatinine.

6. Intra-aortic India-ink injections during life show that there is normal intraglomerular and peritubular circulation during the anuria or oliguria of the renal shut-down which follows intravenous injection of lysed red cells during dehydration or shock. There is no shunting of renal blood flow from the renal cortex. The tubules are filled with pigmented amorphous and crystalline casts.

7. The renal capsule is not under tension and there is no histologic evidence of renal cellular damage during the first two or three days of uremia.

8. A dog can stand rapid intravenous injection of 100 cc. of distilled water per Kg. without striking reduction in renal function and with complete recovery. Relative to the degree of renal activity, this is equivalent to rapid injection of 2.6 liters into a 70-Kg. man.

9. A dog can stand rapid intravenous injection of at least 95 cc. of distilled water per Kg., even during profound histamine-shock with its resulting anuria, without undergoing marked reduction in renal function and with complete recovery.

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