The Effects of Hemoglobin Solutions on Renal Functions in Man

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THE USE of hemoglobin solutions as a possible plasma substitute in shock, hypoproteinemia and other diseases has been suggested. Normally this material in the form of red cells is discarded in the preparation of plasma and its fractions. That hemoglobin may be administered in large quantities without untoward results has been reported, despite the familiar sequelae of hemoglobinuria in diseased states.[1] There are numerous reports on the circulatory effects of infusions of saline, plasma, gelatin and whole blood.[2–4] Very few of these reports give any extensive data on the renal hemodynamic alterations incident to the administration of the particular material infused. Because of the well known renal and general vascular alterations accompanying shock[5] and incompatible blood transfusion[6] it was thought advisable to study the renal dynamic and some general vascular effects of infusions of hemoglobin solutions in man. Studies of the effects on renal hemodynamics of hemoglobin solutions administered to animals have been reported.[7, 8]

The hemoglobin solution used, and its method of processing from human red blood cells has been described by Pennell and Smith.[9] It differs from other hemoglobin solutions[10, 11] in that conversion to methemoglobin on standing is prevented by the use of dextrose, nicotinic acid amide, ammonia and traces of hexose diphosphate.

Methods and Procedure

In the present study, one group of 8 patients with no evidence or history of renal disease were used, and another group of 6 patients with renal disease (nephrotic syndrome) were used. All subjects were tested at least ten hours after their last meal and hydrated with 500 to 1000 cc. of water by mouth at least one to two hours before the start of the procedure. The usual continuous infusion procedure for renal clearances was followed. Urine collections during control and hemoglobin periods varied from 8 to 30 minutes depending on urine flow through an indwelling urethral catheter. Bladder evacuation was assured by a water washout with air injection and expression. Blank determinations of urine and blood were drawn prior to the test. Control periods, prior to infusing hemoglobin, were obtained. Primary and sustaining infusions of inulin and para-aminohippurate were administered during control periods using 5 per cent glucose in water as the diluent. The infusion of a 6 per cent hemoglobin solution was started immediately following the control periods and contained the same concentrations of inulin and PAH as those in the control. The rate of sustaining infusions was regulated at 4 cc. per minute at all times by the use of a Harvard tunnel clamp.

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Aided by grants from the National Institutes of Health, U.S.P.H.S. and the New York Heart Association.

Submitted April 1, 1951; accepted for publication May 21, 1951.

The authors wish to express their thanks to Sharp and Dohme, Inc. for the generous supplies of hemoglobin solution used in this study.

The glomerular filtration rate was measured by the clearance of inulin using Dische's method as modified[12]; the effective renal plasma flow by the clearance of sodium para-aminohippurate (PAH).[13] Renal extraction of PAH was determined by renal vein catheterization using the technic of Warren, Brannon, Merrill,[14] and Bradley and Bradley.[15] Urinary sodium output was measured employing the flame photometer with lithium as the internal standard. Determinations of hemoglobin in plasma and urine were done by a macro modification of the method of Turner.[16] Hematocrit determinations were made on heparinized blood in Wintrobe tubes centrifuged at 2500 r.p.m. for 30 minutes. Blood pressure and pulse rate were noted at regular intervals.

Results

Table 1 lists the consecutive clearance periods in the patients tested. Not all procedures were carried out in all patients. All data are converted to the standard 1.73 square meters for surface area.

Listed in the chart is the time relationship between the administration of the hemoglobin solution and the development of hemoglobinuria. This varied from patient to patient in both the normal and "renal" group from about thirty minutes to two and one-half hours and seemed to bear no constant relationship to anything else we measured. In 2 patients (E. H. and L. C.) studies were done during this time interval (during the infusion of hemoglobin with no hemoglobinuria).

A. *Effects on Urine Output*

In all but 1 patient (M. W.) there was a very definite and sharp drop in the minute output of urine immediately upon administering the hemoglobin solution. This fall in urinary output amounted to approximately 60 per cent of the control urine flows.

B. *Effects on Glomerular Filtration Rate*

In the normal of group patients we were struck by the variability of the results from patient to patient since out of 6 subjects 4 showed a fall in filtration rate to 34 per cent below the controls and 2 subjects a rise to 29 per cent above controls. These variations in measurement of the filtration rate appear to be more than a reflection of inherent variabilities from patient to patient.[12] The trend in a patient was consistent from period to period.

C. *Effect on Effective Renal Plasma Flow*

The most striking effect on renal dynamics resulting from hemoglobin infusion is reflected in a precipitous fall in the effective renal plasma flow, up to 63 per cent below control levels in the normal group and to a maximum of 57 per cent below control levels in the "renal" group. The average percent fall for all subjects was 41.4 per cent below their control levels. This fall is almost instantaneous as evidenced in patient T. J. That the data reflect plasma flow is borne out by the apparently constant and high extraction for PAH in 3 patients in whom these data were obtained on at least two occasions, during control periods and during the infusion of hemoglobin.

D. *Effect on Sodium Excretion*

In all but 1 subject (S. K.) there was a striking fall in sodium excretion during hemoglobin infusion.

TABLE 1—*Renal Function and Other Data with Hemoglobins on Cases Studied*

Patient	Remarks*	Urine flow cc./min.	G.F.R.	R.P.F.	P.A.H. ext.†	C HGB mg/min.	Plasma HGB. concentration in mg/100 cc.	Sodium excr. mM/min.	Total HGB. inf.‡	Hematocrit	Blood pressure	Elapsed time HGB. inf. and hemoglobinuria
T. J.—34 M	C	8.0		557				0.13		46	130/90	
Bronchiectasis	C	7.6		502				0.11				
	C	9.6		527				0.15				
	HP	1.65	111.2	238		3.0	389	0.03		45	120/88	
	HP	1.7	98.6	165		2.9	399	0.03	18 Gm.			50 min.
	HP	1.8	101.8	202		2.75	421	0.03				
	HP	1.1	73.1	170		2.1	435	0.02		47	130/90	
L. C.—26 M	C	4.9		472						43	120/80	
Convalescent	HI	4.7		514								
pneumonia	HI	13.7	148.5	498								
	HI	5.0	97.1	411					20 Gm.			153 min.
	HI	10.3	123.0	395								
	HP	0.8	109.3	331		1.9	629					
	HP	0.7	103.2	336		2.0	678			43	120/80	
M. W.—25 M	C	1.3	119.5	689	98					52	134/76	
Schizophrenia	C	1.9	101.5	321.5								
	HI				92.7		369					
	HP	1.9	83.6	287	91.6	1.8	440		20 Gm.		130/80	60 min.
	HP	2.85	112.5	407		2.5	504					
	HP	2.0	96.6	380	93.9	2.7	511			51	130/80	
A. S.—29 M	C	8.2	113.5	429	93.2					52	150/80	
Convalescent	C	5.4	111.4	468								
spont. pneumo-	HI				92.3							
thorax	HP	1.4	170.5	212	97.2	4.8	313		20 Gm.		130/85	24 min.
	HP	0.4	120.5	127.5	95.0	3.0	360			51		
G. G.—35 M	C	8.2	91.3	409	93.7					56	115/90	
Peptic ulcer	C	9.9	98.2	416								
	C	10.2	83.8	436								
	HP	2.2	60.2	229		4.45	276					
	HP	2.0	60.8	192		4.25	274		8 Gm.			24 min.
	HP	1.4	58.8	229	96.9	4.15	270			56	120/80	
E. H.—46 M	C	9.8	153.0	487.5				0.08		52	122/84	
Peptic ulcer	C	7.3	103.6	466				0.07				
	C	6.9	146.4	486				0.18				
	HI	2.4	92.6	205				0.13				
	HI	2.3	95.2	298				0.13				
	HP	2.4	109.7	321		2.7	269	0.02	20 Gm.	59		71 min.
	HP	2.5	149.0	358		2.9	290	0.04				
	HP	1.7	123.1	272.5		3.3	307	0.10			120/80	
J. K.—45 M	C	13.5	100.6	603						53	122/84	
Peptic ulcer	C	7.4	94.0	548								
	HP	5.0	98.7	424.5		4.2	192					
	HP	4.9	102.5	308.5		3.9	216		15 Gm.		128/90	31 min.
	HP	4.9	130.0	338		4.7	275					
	HP	3.6	126.3	309		4.4	310			60	130/84	
W. W.—46 M	C	4.6	144.0	732				0.29		34	130/80	
Convalescent	C	8.3	118.5	641				0.29				
pneumonia	C	11.9	127.5	664				0.32				
	HP	1.4	87.4	295.5		1.7	265	0.08		34	130/80	
	HP	2.1	85.7	350		2.2	293	0.09				
	HP	2.3	104.4	424.5		2.9	327	0.13	18 Gm.			33 min.
	HP	1.6	72.3	351		2.6	364	0.12				
	HP	2.4	93.4	397		3.2	401	0.15		34	130/80	

Patient	Remarks*	Urine flow cc./min.	G.F.R.	R.P.F	P.A.H. ext.†	C HGB mg/min.	Plasma HGB. concentration in mg/100 cc.	Sodium excr. mM/min.	Total HGB. inf.‡	Hematocrit	Blood pressure	Elapsed time HGB. inf. and hemoglobinuria
C. McD.—54 F	C	0.92	65.6	238.5				0.086		25	120/40	
Multiple myeloma	C	0.76	57.9	204.0				0.072				
(proteinuria)	C	0.86	59.0	212.0				0.069				
	HP	0.59	54.0	71.0		0.47	222	0.024				
	HP	1.0	71.2	75.4		0.71	249	0.027				
	HP	0.63	39.1	72.9		0.46	295	0.025				
	HP	0.60	40.4	92.9		1.065	332	0.038	20 Gm.			29 min.
	HP	0.62	39.0	96.8		0.99	375	0.033				
	HP	0.65	44.9	111.6		1.242	409	0.034				
	HP	0.69	51.0	125.5		1.184	444	0.037		24	124/38	
H. L.—65 M	C	1.23	23.7	20.4				0.022		20	115/75	
Nephrotic syndrome	C	1.13	23.9	16.5				0.022				
	C	1.64	33.8	20.2				0.031				
	HP	0.48	10.3	12.2		0.34	184	0.007				
	HP	0.52	12.7	12.9		0.22	212	0.007				
	HP	0.81	17.7	18.8		0.35	252	0.010			118/80	
	HP	0.42	18.1	15.1		0.34	295	0.006	30 Gm.			35 min.
	HP	0.46	17.2	20.5		0.39	341	0.008				
	HP	0.51	20.2	31.3		0.61	349	0.010		22	115/70	
S. K.—60 M	C	4.3	18.8	205				0.055		39	220/115	
Kimmelstiel-Wilson disease	C	3.6	20.5	184				0.040				
	C	3.3	25.5	186				0.034				
	HP	1.6	20.3	93.4		0.30	285	0.036				
	HP	2.1	22.4	113.4		0.52	303	0.044				
	HP	2.2	21.8	119.7		0.50	324	0.046				
	HP	2.3	18.8	110.2		0.59	358	0.045	18 Gm.			35 min.
	HP	1.9	20.5	111.3		0.56	395	0.046				
	HP	1.2	22.5	120.1		0.58	439	0.048				
	HP	1.5	25.9	114.5		0.84	485	0.080		35	210/110	
A. W.—24 M	C	1.7	22.6	268				0.003		35	120/85	
Nephrotic syndrome	C	0.9	23.7	255				0.005				
	HP	0.2	19.2	162		2.8	150	0.006				
	HP	0.3	20.8	94		2.2	273	0.002	22 Gm.			43 min.
	HP	0.2	17.2	85		3.4	321	0.006		35	118/80	
M. O.—19 F	C	3.9	54.6	362				0.055		23	140/90	
Nephrotic syndrome	C	2.2	41.9	289				0.042				
	C	2.0	53.2	261				0.044				
	HP	0.4	37.5	164		0.64	275	0.018				
	HP	0.3	28.8	118		0.74	269	0.011				
	HP	0.5	43.4	228		1.36	344	0.020	20 Gm.			35 min.
	HP	0.6	39.4	240		1.92	443	0.026		23	138/95	
	HP	0.6	36.6	230		1.98	548	0.032				
H. M.—38 M	C	2.4	41.9	542				0.013		38	130/80	
Nephrotic syndrome	C	1.2	29.6	348				0.012				
	C	1.7	35.4	383				0.015				
	HP	2.0	40.9	400		0.38	426	0.020				
	HP	1.1	37.2	318		0.54	456	0.010	22 Gm.			108 min.
	HP	0.7	29.2	249		0.53	484	0.009				
	HP	0.5	26.6	212		0.59	521	0.009		38	128/80	

* HP: hemoglobinuria present, as evidenced by a bright red urine. HI: hemoglobin infusion running, but hemoglobinuria not present. C: control.

† P.A.H. Extraction—expressed as percent.

‡ Total hemoglobin infused in grams during the experiment.

The authors wish to express their thanks to Dr. L. Katz of the Boroklyn V. A. Hospital for patient A. W. and to Commander Harold Lyons (U.S.N.M.C.) for patient H. M.

E. *Effect on Hematocrit and Blood Pressure and Pulse Rate*

No striking effect on hematocrit, blood pressure, or pulse rate was noted during or as a result of the hemoglobin infusion.

F. *The Renal Clearances of Hemoglobin*

The renal clearances of hemoglobin apparently followed the same type of curve as those obtained with glucose when the blood level is constantly increased. It would seem in the group of normals that the kidney can clear approximately 4 mg. of hemoglobin per minute, and the average renal threshold is approximately 250 mg. per cent.

Casual urine specimens collected hours after hemoglobin infusion was stopped indicated that hemoglobinuria may continue even when blood levels of hemoglobin are below the threshold for onset of hemoglobinuria.

Discussion

Our first interpretation of the marked fall in PAH clearance was that the data reflected not a true fall, but rather that reabsorbed hemoglobin in the renal tubules was interfering with the tubular transport mechanism for PAH. Since the renal extractions of PAH are constant throughout the experiments presented above one must accept the fact that hemoglobin solutions have a severely vasoconstrictor effect upon the vascular bed of the kidney.

The immediate effects of shock on the kidney are circulatory, and renal blood flow is diminished. It is not necessary at this time to discuss the complications due to adding a further vasconstrictive substance such as hemoglobin to an already ischemic organ. The effects of renal ischemia whether mechanical or secondary to shock have been adequately covered in the literature,[17] and hemoglobin would accentuate the renal ischemia regularly occurring in shock. This appears to be an important factor in causing tubular injury and necrosis in cases of shock with spontaneous hemoglobinemia, as in crush syndrome.[18]

The variable effects of hemoglobin solutions upon the dynamics of the glomerular filtration rate deserve comment. Rise in filtrate with fall in plasma flow can only be due to efferent vasoconstriction, apparent fall in filtration rate might be due to backward diffusion of inulin through damaged tubules or to vasoconstriction of afferent arterioles. Richards[19] has shown, in the nephrons of frogs poisoned by various substances, that the filtration went on with normal rapidity, but the entire filtrate was reabsorbed from the tubules, so that no urine entered the bladder. In such a condition, the cells of the tubular epithelium lose their power of selective reabsorption. One must admit the possibility of hemoglobin acting as a severe nephrotoxic substance and producing the fall in inulin excretion together with marked reabsorption of water and sodium. Even though filtrate rates are high, after injecting hemoglobin the renal tubule cells engorged with hemoglobin may encroach on the lumen and a fall in the cross section circumference ratio of the lumen increases the surface area for absorption, and thus causes the fall in urine output and sodium excretion. This concept is in accord with the observation of changes in kidney volume as an effect of hemoglobin.[20] These facts all make one hesitate to use hemoglobin solutions in any condition where the kidney shows depression of function.

Of interest is the observation of Hampton and Mayerson[21] who suggest that when large amounts of hemoglobin iron are available, iron is present in the kidney in the form of ferritin. That ferritin in minute traces has a marked antidiuretic effect is known[22] and it may be this substance which is responsible for the renal hemodynamic alterations noted in the present study. Indeed, minute amounts of ferritin have been detected in red blood cells by Agner,[23] and may be present in hemoglobin solutions.

Conclusions

1. The effects of infusions of a solution of hemoglobin upon renal function were observed. The solution contained traces of dextrose, nicotinic acid amide, ammonia and hexose diphosphate to stabilize it against methemoglobin formation. Control injections of 5 per cent dextrose containing the stabilizing agents in the same strength showed no effects on renal functions. The effects of hemoglobin are: (a) in one-third of the cases there was a rise in the glomerular filtration rate over their respective control levels, and in two-thirds of the cases there was a fall. (b) Marked fall in effective renal plasma flow, urinary output and sodium excretion in all cases. No effect upon renal extraction of PAH, hematocrit, pulse or blood pressure was noted.

2. Hemoglobin solution is apparently "nephrotoxic" and theoretically should not be used therapeutically in shock, or any condition where the kidney function already is impaired acutely.

3. No irreversible change or subjective evidence of renal injury was observed after doses of 8 to 30 Gm. of hemoglobin were given intravenously in 14 subjects with normal or elevated diastolic pressures and urine flows. Six of these subjects already had marked proteinuria and elevated urea levels in the blood.

REFERENCES

[1] Ross, J. F.: Hemoglobinemia and hemoglobinurias. New England J. Med. *233:* 691, 732, 766, 1945.

[2] Altschule, M. D. and Gilligan, D. R.: Effects on cardiovascular system of fluids administered intravenously in man. Dynamics of circulation. J. Clin. Investigation *17:* 401, 1938.

[3] Holt, J. P. and Knoepfel, P. K.: Changes in plasma volume and cardiac output following the intravenous injection of gelatin, serum and physiological solution. J. Clin. Investigation *23:* 657, 1944.

[4] Fletcher, A. G., Hardy, J. D., Riegel, C. and Koop, C. E.: Gelatin as a plasma substitute: The effects of intravenous infusion of gelatin on cardiac output and other aspects of the circulation of normal persons, of chronically ill patients, and of normal volunteers subjected to large hemorrhage. J. Clin. Investigation *24:* 405, 1945.

[5] Lauson, H. D., Bradley, S. E. and Cournaud, A.: The renal circulation in shock. J. Clin. Investigation *23:* 381, 1944.

[6] Weiner, A. S.: Blood Groups and Transfusions, ed. 3, Springfield, Charles C Thomas, 1945.

[7] Hamilton, P. B., Hiller, A. and Van Slyke, D. D.: Renal effects of hemoglobin infusions in dogs in hemorrhagic shock. J. Exper. Med. *86:* 477, 1947.

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

[9] Pennell, R. B. and Smith, W. E.: Preparation of stabilized solutions of hemoglobin. Blood *4:* 380, 1949.

[10] FARR, L. E., HILLER, A. AND VAN SLYKE, D. D.: Preparation of dried hemoglobin without loss of activity. J. Exper. Med. *86:* 465, 1947.

[11] AMBERSON, W. R., JACOBS, J. E. AND HISEY, A.: Human hemoglobin solutions as a blood substitute. Federation Proc. *1:* 3, 1942.

[12] BRANDT, J. L. AND BAKER, K.: A study of methods for determining the glomerular filtration rate in man and animals by the use of inulin, mannitol, and creatinine; with a presentation of a method for measuring the inulin clearance. To be published.

[13] SMITH, H. W., FINKELSTEIN, H., ALIMINOSA, L., CRAWFORD, B. AND GRABER, M.: The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dogs and man. J. Clin. Investigation *24:* 388, 1945.

[14] WARREN, J. V., BRANNON, E. S. AND MERRILL, A. J.: Method of obtaining renal venous blood in unanesthetized persons with observations on extraction of oxygen and sodium para-aminohippurate. Science *100:* 108, 1944.

[15] BRADLEY, S. E. AND BRADLEY, G. P.: The effect of increased intra-abdominal pressure on renal function in man. J. Clin. Investigation *26:* 1010, 1947.

[16] TURNER, A.: A micro-method for the determination of hemoglobin. Bull. U. S. Army Med. Dept. *5:* 605, 1946.

[17] PHILLIPS, R. A. AND HAMILTON, P. B.: Effects of renal ischemia in dogs. II. Effects of 20, 60, and 120 minutes of renal ischemia on glomerular and tubular function. Am. J. Physiol. *152:* 523, 1948.

[18] CORCORAN, A. C. AND PAGE, I. H.: Genesis of crush syndrome. J. Lab. & Clin. Med. *30:* 351, 1945.

[19] RICHARDS, A. N.: Direct observations of change in function of the renal tubule caused by certain poisons. Tr. A. Am. Physicians *44:* 64, 1929.

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

[21] HAMPTON, J. K. AND MAYERSON, H. S.: Hemoglobin iron as a stimulus for the production of ferritin by the kidney. Am. J. Physiol. *160:* 1, 1950.

[22] BAEZ, S., MAZUR, A. AND SHORR, E.: Hepatorenal factors in circulatory homeostasis. XX: Antidiuretic action of vasodepressor VDM (ferritin). Am. J. Physiol. *162:* 198, 1950.

[23] GRANICK, S.: Personal communication.