Clinical Experience with Hemoglobin-Saline Solutions


The attention of this laboratory has for some years been directed to studies of the behavior of dissolved hemoglobin in the blood stream of man and mammals (1-6). In common with other investigators we have hoped to find therapeutic uses for hemoglobin and particularly for its saline solutions. During the war we made an effort to produce such solutions and we were able to secure a few clinical observations (6). These observations were fragmentary and did not certainly establish therapeutic value for our solutions. However, in some cases there were favorable indications and in a few patients results of physiological importance were obtained. The present communication covers such of these experiences as seem to us to be of interest to other workers in this field. Detailed case reports will not be given, but will be furnished to any reader interested in having more complete information about our patients.

Clinical experience with intravenous injections of hemoglobin solutions begins with the papers of Sellards and Minot (7). These authors were mainly interested in the ‘tolerance to hemoglobin’ defined as “the amount of hemoglobin required to produce hemoglobinuria”. In no case did they observe hematopoietic stimulation by the injected hemoglobin, prepared in sterile solutions, although they made the requisite blood cell studies. The more recent clinical literature has been reviewed by Cannan and Redish (8). They have prepared and injected crystalline human hemoglobin into a series of human subjects. Other important papers are those of Duesberg (9), Ottenberg and Fox (10), O'Shaughnessy et al. (11), Fairley (12), Gilligan et al. (13) and Kark and Meiklejohn (14). The effects of hemoglobin free in plasma may also be studied in the literature concerning m.arch hemoglobinuria, reviewed by Gilligan and Blumgart (15) and further described by Gilligan et al. (16).

None of these reports establishes a therapeutic value for intravenously injected hemo-

---

Received for publication September 3, 1948.

1 Aided by grants from the Penrose Fund of the American Philosophical Society, and from the Bresler Research Fund of the University of Maryland.
globin-saline solutions. The studies were intended to explore the feasibility of intravenous injections and to determine the maximum safe level (8, 11), to follow the intensity and duration of hemoglobinemia and hemoglobinuria (10, 13, 15) or to search for the appearance of hemoglobin derivatives such as hematin, methemalbumin, porphyrin and bilirubin (9, 12, 13, 14, 16). None of the authors has studied the blood cell picture, hence no observations are reported on hematopoietic stimulation. In nearly all cases only a single injection was made. The maximum amount of hemoglobin administered was about 50 gm. (8, 11); three of the groups did not exceed 10 gm. (10, 12, 13). Most of the authors comment upon the absence of jaundice in their cases. Even the maximum amounts did not produce clinical icterus, although moderate increases in plasma bilirubin were detected (9, 13, 15, 16). Most authors observed no reactions. Reactions occurred for some when the amount injected exceeded 10 gm. (8, 11, 12); nausea, vomiting, tightness of the chest, pains in back and loins and moderate fever are mentioned as symptoms.

It was our purpose to determine clinically the effect of repeated intravenous injections of hemoglobin-saline solutions prepared in this laboratory. We hoped to study the effect of such solutions upon hematopoiesis and in shock. Injections were made into a total of 14 patients. Seven patients were treated repeatedly. Five of these had secondary anemias due to hemorrhage or infection. Two had anemias caused by leukemia and agnogenic myeloid metaplasia respectively. In order to study other phenomena, single injections were given to 7 other patients whose blood pictures were within normal limits.

METHODS

Preparation of Solutions. In a previous communication (5) we have described our efforts to prepare desiccated hemoglobin in lyophile form. We were never able to produce a dry product which did not contain appreciable amounts of methemoglobin (5-15 %) upon resolution, and which did not show an insoluble residue. We early observed that the removal of oxygen before freezing and drying diminished the percentage of methemoglobin in our lyophile product, and later found that hemoglobin may be preserved in solution, without formation of methemoglobin or insoluble material, if the oxygen has been removed (17, 18).

We therefore came to work entirely with sterile solutions of human hemoglobin, either freshly prepared, most of the oxygen removed, and refrigerated until used, or sealed in completely oxygen-free ampoules and held at room temperature. In all but one of our clinical tests we have used relatively fresh refrigerated solutions. Many animal experiments have been successfully performed with oxygen-free solutions held for weeks or months. Further clinical experience with solutions of the latter type is needed.

Our solutions were obtained by the use of human red cells, thrice washed with 1.5 per cent NaCl and centrifuged to remove plasma. A sterile technic was followed throughout. To one volume of packed cells we added half a volume of sterile distilled water. To each 100 cc. of the resulting mixture 20
cc. of ether or toluene was added, producing complete hemolysis and formation of a thick gel. The gel was broken by vigorous stirring and centrifuged until it separated into an upper layer containing the precipitated stromata, and a lower layer consisting of clear aqueous hemoglobin-saline, in which pigment concentration ran from 12 to 18 per cent. This clear layer was siphoned off and filtered. The potassium and traces of the organic solvent were then removed by 2-stage dialysis against 1.0 per cent NaCl solution. As a final precaution the solutions were passed through sterilizing filtration. At the same time the oxygen was removed. The solutions were filtered into plasma-vacs, or, alternatively, were led into sterile evacuated ampoules and sealed in vacuo. Samples of each run were rabbit tested for pyrogens, a limit of tolerance of 1.5°F. rise/10 cc/kg. injected being accepted.

The blood group employed does not influence the clinical results, at least in so far as the major groups (A, B, AB and O) are concerned. In no case have the red cells of our patients been agglutinated by our solutions. Final hemoglobin concentrations were between 10 and 14 per cent. Methemoglobin formed 2 per cent of total pigment.

**Analytical Methods.** Hemoglobin and methemoglobin were determined by the method of Evelyn and Malloy (19). Hematocrits were read by the use of Wintrobe tubes. Reticulocytes were counted by the method of Wintrobe (20). NPN was read by the method of Folin and Wu (21). BUN and urea clearances were determined by the method of Peters and Van Slyke (22). Hippuric acid was determined by the method of Quick (23). Plasma proteins were read by the falling drop method of Barbour and Hamilton (24). Glomerular filtration rate was measured by the mannitol method of Smith, Finkelstein and Smith (25). Renal blood flow and Tm were read by the method of Goldring et al. (26), substituting sodium paramino-hippurate for diodrast. Blood and urinary pH was determined by the glass electrode.

**OBSERVATIONS**

**Changes in Blood Pressure, Heart Rate and Temperature after Hemoglobin-saline Injections.** In animals large injections of hemoglobin-saline tend to raise the blood pressure (2) even when the volumes of blood withdrawn, and of fluid injected, are exactly the same. This pressor action may be ascribed in part to the high colloidal osmotic pressure of hemoglobin-saline solutions, which draw fluid into the blood and so raise its volume. A chemical pressor principle is also present.

In our human patients we usually observed an elevation of blood pressure, even after the infusion of volumes of fluid so small that they could not significantly increase blood volume. There were wide individual differences, as shown in figure 1. Patients E. R. and K. K. showed rather small elevations in blood pressure. Patient E. P. was hypertensive. In this case the blood pressure fell after the infusion. The pressor response was well developed in patients H. C., C. H., L. T. and S. L. It was also prominent in M. B. (fig. 2) and M. S.
(fig. 6). Associated with the pressor rise the heart rate was always diminished, presumably as the result of carotid sinus reflex action.

![Fig. 1](http://jap.physiology.org/)

**Fig. 1. Blood pressures, heart rates and temperatures** after the infusion of hemoglobin-saline into seven patients.

Oral temperatures are shown in the figures. They showed no significant changes in patients H. C., L. T., S. L. and M. B., indicating an absence of pyrogens. In one case, M. B., the injection of a volume as large as 500 cc. gave no temperature rise (fig. 2). In other cases pyrogenic reactions were observed, usually moderate, but occasionally severe. The observations encourage the
belief that, with greater precautions in the preparations of the solutions, it may be possible to produce hemoglobin-saline which will give little or no pyrogenic reaction in any patient.

Relation between Injected and Excreted Hemoglobin. The appearance of hemoglobin in the urine, after its intravenous injection, has often been reported and used as an argument against its clinical application. In our cases the hemoglobin recovered in the urine rarely exceeded 25 per cent of that injected and was often much less. Figure 3 shows the relationship between injected and excreted hemoglobin in patient H. C. Of a total of 63 gm. injected in this test, only 7.4 gm. appeared in the urine during the next 30 hours, an excretion ratio of 12 per cent. Figure 3 also shows the time course of a moderate diuresis following the injection. In association with this diuresis urinary pH swung toward the alkaline side, then became more acid again as the urine flow diminished. No alkali was given to this patient. In other patients, similar alkaline tides in the urine were detected after hemoglobin-saline injections, even when no alkali was given.

In figure 4 is shown the relationship between injected and excreted hemoglobin in 6 patients. The data include 27 tests, in which a total of 935 gm. of
hemoglobin were injected. Of this amount 167 gm. appeared in the urine, an average urinary loss of 18 per cent. Only in patient M. B. did the excretion percentage depart markedly from this average, when the average urinary loss was 41 per cent.

No significant effect of the administration of alkali on the quantity of hemoglobin excreted through the kidney was observed.

Stimulation of Hematopoiesis. In three of our five cases of secondary anemia we obtained evidence that intravenously injected dissolved hemoglobin can stimulate hematopoiesis. Patient L. T. was a 37-year-old colored female with lymphogranuloma and rectal stricture associated with bleeding which had caused a moderate anemia. Patient H. C. was a 35-year-old white female admitted for a chronic osteomyelitis of the left foot, and cellulitis of the lower left leg. A moderately severe anemia was present. Patient E. R. was a 34-year-old white male with multiple pyogenic cutaneous ulcers. He had bled
severely as a result of wide excision of these ulcers, his hematocrit finally falling to 18.

In all three cases the repeated intravenous administration of hemoglobin-saline solutions was shortly followed by rises in hemoglobin and hematocrit values. The results of treatment are shown in figure 5. The solution volumes are marked above the arrows which indicate injection times. In patients H. C. and E. R. hemoglobin and hematocrit values rose together. There was no significant change in the mean corpuscular hemoglobin concentration. The hematopoietic activity consisted in an accelerated production of more red cells with hemoglobin content similar to those already circulating. Reticulocytes increased in patient H. C., but were very high initially in patient E. R. and fell as hematopoietic activity relieved the anemia.

In patient L. T. the data were complicated by continuing rectal bleeding, augmented by additional blood loss during and following the first stage of the Lahey operation. Nevertheless the rises in hemoglobin and hematocrit values prior to operation appear to be significantly related to the first series of infu-

![Fig. 4. Relationship between injected and excreted hemoglobin in 6 patients.](http://jap.physiology.org/ Downloaded from http://jap.physiology.org/ by 10.220.33.4 on August 14, 2017)
sions. Less clearly the later rise in these values may be associated with the smaller second series of infusions. In both series reticulocytes significantly increased. However, an increase in the hematocrit did not keep pace with the rise in hemoglobin. As a result, mean corpuscular hemoglobin concentration,

originally low, showed a significant rise. In patient H. C. faint icterus was present after the infusions. In all other cases icterus was not observed.

_Treatment of Shock._ It was our original intention to develop hemoglobin-saline solution for use as a blood substitute in lieu of the conventional plasma preparations. The ability of these solutions to transport oxygen seemed to confer upon them an advantage not found in any other blood substitute and to
fit them uniquely for the treatment of shock and hemorrhage. We found it difficult to obtain suitable cases for the study and have treated only a single patient.

Patient M. S. was a 22-year-old white female. In her second pregnancy she was admitted on February 2, 1943, for hypertension, but was discharged on February 10, undelivered and improved. She was readmitted on February 17 in active labor. Her blood pressure was 170/110; otherwise her condition was satisfactory. Labor progressed under nembutal and paraldehyde sedation. At 6:50 A.M., February 19, the patient delivered spontaneously a full-term child. The secundines were apparently fully expressed, followed by several blood clots. There was evidence of considerable retro-placental hemorrhage prior to delivery.

The patient was placed in charge of a nurse who was instructed to massage the uterus. The nurse was inexperienced, misunderstood her instructions and left the patient. When next seen by the doctor at 7:45 A.M. the patient had suffered massive hemorrhage and was in a state of collapse. The pulse was not palpable. She was still under the influence of sedation. She was immediately given 500 cc. of plasma, which restored blood pressure sufficiently to permit a systolic reading of 80 mm. Hg. Another 500 cc. of plasma were given at 8:15 A.M. At 9:00 A.M. she received 200 cc. of 25 per cent glucose. Between 9:15 and 10:15 A.M. she received 300 cc. of whole blood—all of her type then available in the hospital bank. These later infusions failed to raise the blood pressure further. The pulse remained rapid (150 to 140) and very weak. Bleeding continued and her condition was critical.

Since no more compatible whole blood was available, the resident obstetrician called one of us (C. & R.) into consultation and asked that hemoglobin-saline be administered. The cardio-vascular data secured before and after treatment are shown in figure 6. A blood sample, hereafter referred to as the 'control sample', was secured just before infusion began. Beginning at 10:33 A.M. hemoglobin-saline was given into the external jugular vein at the rate of 30 cc/min. until 300 cc. had passed. The blood pressure rose to 106/80 and the pulse dropped to 100 at 10:45 A.M. Consciousness returned at about the same time.

Administration of hemoglobin-saline continued at a slower rate until 500 cc. had been given at 11:00 A.M. At this time the blood pressure was 140/80, pulse 96. Since bleeding continued, the patient was returned to the delivery room for inspection. A small vaginal laceration was repaired and relaxed uterus packed. The uterine cavity was not inspected at this time. In spite of continuing hemorrhage the patient's color, previously ashen, was now good, the pulse volume was greatly improved and the veins were fairly full.

Further infusions of hemoglobin-saline were now given, as shown in figure 6. The final total administered was approximately 2300 cc., containing 250 gm. of hemoglobin. Bleeding continued until mid-afternoon when uterine examination revealed a fragment of retained secundine which was removed. Many clots and much fluid blood were lost at this time, but bleeding then ceased. A severe pyrogenic reaction occurred whose time course is shown in figure 6.

During and soon after the hemoglobin-saline injections a total of 15 gm. NaHCO₃ were administered. The urine, originally acid at pH 6.15, turned alkaline about midnight. The pH rose progressively throughout the whole course of the case, until a terminal value of 8.9 was obtained although no more alkali was given. To combat both the reactions and the anemia, oxygen was administered almost constantly.

The time course of the changes in plasma and red cell hemoglobin is shown in figure 7. Plasma hemoglobin reached a value of 2.7 gm. per cent immediately after the fourth injection. Plasma proteins were 4.28 gm. per cent in the control blood sample, then rose to values
Fig. 6. Effect of hemoglobin-saline in shock patient M. S.

Fig. 7. Blood pigments in patient M. S.

(including hemoglobin) between 4.6 and 5.6 gm. per cent during the whole course of the hemoglobinemia. The hematocrit value observed in the control sample was 17. As hemoglobin-saline was infused the hematocrit progressively fell, until values as low as 5.5 were obtained. For 30 hours the values were between 5.5 and 7. This patient lived because of
the hemoglobin dissolved in the plasma, which supplemented the oxygen capacity of the intracorpuscular hemoglobin.

The reticulocyte count increased rapidly in this patient, reaching a peak of 12 per cent after 30 hours. The values are shown in figure 7. Plasma methemoglobin was also determined and is shown in figure 7. Its concentration rose only slightly during the whole course of the hemoglobinemia.

Kidney block became evident very early. Urine volume declined to less than 5 cc/hr. and remained at this low level for four days. The block failed to respond to all treatment, including whole blood transfusions. Nerve block on the 5th day was followed by an increase in urine volume to 10 cc/hr. Clinical signs of uremia appeared on the 6th day. The patient died at 9:10 A.M. on the 9th day after delivery, showing a terminal 2:1 heart block. Terminal NPN was 220; BUN was 190.

Renal failure in such a case is to be expected, even when no hemoglobin has been given. Young (27) has described similar obstetrical patients, and Adam (28) has reviewed the literature. In this case there were no less than five factors, recognized in the literature as able to cause oliguria: pre-eclampsia, retro-placental hemorrhage, retained placenta, massive post-partem hemorrhage with shock, and putrid endometritis of mild form.

This case has done more than any other in our series to demonstrate both the possibilities and limitations of intravenous injections of hemoglobin-saline. It justified our expectation that our solution might be effective clinically in raising blood pressure and restoring blood volume after extensive hemorrhage, confirming our earlier animal experience (2). It showed that hemoglobin in solution in the plasma is able to transport oxygen, in man as in animals. It established a new high level for volume of fluid and weight of hemoglobin injected.

Kidney Damage after Hemoglobin-saline Injections. In our series of 14 cases we have injected hemoglobin-saline 77 times. Seven cases—those most fully and carefully studied—have received multiple injections, numbering 3, 5, 9, 10, 12, 13 and 18 times, respectively, and totaling 70 times. In only one of these cases did oliguria occur, namely in the case of shock (5 injections) where the circumstances were sufficiently complicated to make a judgment as to the causative factor impossible. In seven other cases only single injections were made. Oliguria was observed in one of these, as the result of the injection of a toxic solution, the patient later recovering. These figures suggest that impairment of urine flow by hemoglobin-saline is exceptional, contrary to the fears of some critics.

In addition to the kidney damage observed in the case of shock, 3 of the 6 other multiply injected patients have shown transient rises in NPN and decreased urea clearances, without oliguria. Indications of this character emphasized again the desirability of securing more extensive and detailed information concerning kidney function, by methods more precise than those routinely used in clinical laboratories. We attempted therefore to determine glomerular fil-
tration rate, renal plasma flow and tubular Tm values, using the clearances of mannitol and paramino-hippuric acid (PAH) according to the procedures of Homer Smith and his colleagues (25, 26). By these methods we have studied 2 patients, one in only a preliminary fashion, the other more thoroughly.

### Table 1. Renal clearances in case II

<table>
<thead>
<tr>
<th>DATE</th>
<th>FILTRATION RATE</th>
<th>BLOOD FLOW</th>
<th>FILTRATION FRACTION</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/27/43</td>
<td>85.9 cc/min.</td>
<td>539.9 cc/min.</td>
<td>10.2</td>
<td>70.4 mg. PAH/min.</td>
</tr>
<tr>
<td>10/4/43</td>
<td>84.6 cc/min.</td>
<td>542.6 cc/min.</td>
<td>15.6</td>
<td>59.5 mg. PAH/min.</td>
</tr>
<tr>
<td>10/25/43</td>
<td>73.1 cc/min.</td>
<td>419.6 cc/min.</td>
<td>17.4</td>
<td>56.0 mg. PAH/min.</td>
</tr>
<tr>
<td>11/7/43</td>
<td>72.2 cc/min.</td>
<td>439.9 cc/min.</td>
<td>16.4</td>
<td>57.9 mg. PAH/min.</td>
</tr>
</tbody>
</table>

200 cc. sterile hemoglobin-saline solution

<table>
<thead>
<tr>
<th>DATE</th>
<th>FILTRATION RATE</th>
<th>BLOOD FLOW</th>
<th>FILTRATION FRACTION</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/8/43</td>
<td>26.9 cc/min.</td>
<td>174.4 cc/min.</td>
<td>15.4</td>
<td>21.3 mg. PAH/min.</td>
</tr>
<tr>
<td>11/11/43</td>
<td>31.3 cc/min.</td>
<td>167.7 cc/min.</td>
<td>18.7</td>
<td>20.2 mg. PAH/min.</td>
</tr>
<tr>
<td>11/15/43</td>
<td>34.0 cc/min.</td>
<td>208.6 cc/min.</td>
<td>16.3</td>
<td>18.0 mg. PAH/min.</td>
</tr>
<tr>
<td>11/22/43</td>
<td>47.3 cc/min.</td>
<td>252.1 cc/min.</td>
<td>18.8</td>
<td>32.8 mg. PAH/min.</td>
</tr>
<tr>
<td>11/30/43</td>
<td>57.8 cc/min.</td>
<td>290.8 cc/min.</td>
<td>19.9</td>
<td>32.2 mg. PAH/min.</td>
</tr>
<tr>
<td>12/13/43</td>
<td>67.0 cc/min.</td>
<td>319.9 cc/min.</td>
<td>20.9</td>
<td>38.6 mg. PAH/min.</td>
</tr>
<tr>
<td>12/28/43</td>
<td>72.9 cc/min.</td>
<td>373.0 cc/min.</td>
<td>19.5</td>
<td>49.9 mg. PAH/min.</td>
</tr>
<tr>
<td>1/11/44</td>
<td>75.1 cc/min.</td>
<td>486.2 cc/min.</td>
<td>15.4</td>
<td>58.6 mg. PAH/min.</td>
</tr>
</tbody>
</table>

In the first case we secured only a reading on renal plasma flow by PAH. The pressor rise and bradycardia in this case, S. L., after infusion of 150 cc. of hemoglobin-saline, are shown in figure 1. Four readings on renal plasma flow before infusion gave 1054, 993, 1208 and 955 cc. On the day after infusion values of 1222 and 1030 cc. were secured. There was no evidence of any impairment in this function.
The second case, E. P., was that of a 60-year old white female, long a patient in the Baltimore City Hospitals, suffering from rheumatoid arthritis and hypertension. After preliminary studies of renal function a single injection of 200 cc. hemoglobin-saline (24 gm. hemoglobin) was made. Cardiovascular data immediately after injection are shown in figure 1. Twelve determinations of mannitol and PAH clearances were made, three before and nine after infusion. The data are shown in table 1.

It will be seen that the infusion of our solution led to very appreciable diminutions in glomerular filtration rate, renal plasma flow and Tm, to about one third of the original levels. The effect persisted for many days, recovery to original levels occurring only after two months. Urine volume was well maintained throughout, rising somewhat in the first day, and continuing thereafter within normal limits.

Our experience with this last case forced us to recognize that the more precise modern renal methods may disclose impaired function which the routine tests, such as the NPN, the BUN and the urea clearance, either fail to detect or indicate very inadequately. In many laboratories the routine clinical tests are considered sufficient to determine significant renal damage. After this experience we have not been content to consider them. We feel that more clinical cases must be studied, with careful attention to renal function by the modern methods. We believe that an acceptable hemoglobin-saline solution must not cause such change in glomerular filtration rate, renal plasma flow and Tm values as we observed in this last case.

In three cases liver function was tested by Quick's hippuric acid method (23). Values were within normal limits before and after hemoglobin-saline injection.

Hemoglobin Derivatives before and after Injection. Critics of the use of hemoglobin-saline solutions have feared the presence in them of toxic breakdown products, derived from the pigment molecule. We have not made a thorough search for such toxic substances, nor have we been led to accept the thesis that some such factor is sure to be present. However, the possibility of the occasional appearance of such toxins must be recognized.

Porphyrin at once occurs to mind as a possible toxic material. Dr. Frank H. J. Figge, of our Department of Anatomy, determined the concentration and type of porphyrin present in two of our solutions and found them to contain 66 γ and 124 γ per 100 cc.—amounts not greater than those normally present in blood. Spectroscopic examination disclosed only the relatively non-toxic protoporphyrin.

Methemoglobin is also routinely present in our solutions to the extent of 2 per cent of the total pigment. Similar percentages are found in normal blood. Some years ago we described (2) a slow increase of methemoglobin in the plasma of cats after the intravenous injection of hemoglobin-saline. At death it often
constituted half of the pigment remaining in the plasma. These older experiments were made with solutions containing hemoglobin derived from beef cells.

We had feared a similar rise in the concentration of methemoglobin in our clinical cases. Fortunately human hemoglobin does not behave like beef hemoglobin. We have been able to study its behavior in two of our human cases, in which hemoglobinemia endured for many hours. The data obtained in patients H. C. and M. S. are shown in figures 3 and 7. We have made similar observations in many animal experiments when human hemoglobin was injected. Very little change occurs in the absolute concentration of methemoglobin, which remains low throughout. In all multiply-treated cases in our clinical series and in all similar animal experiments, there has never been any evidence of anaphylactic reaction to the infused hemoglobin-saline solution.

**DISCUSSION**

In spite of the hazards undoubtedly present in the preparation and clinical use of hemoglobin-saline solutions, our data suggest the following favorable indications: 1) Such solutions have been given repeatedly to the same human subject, by intravenous injection, in volumes and weights of hemoglobin far exceeding any previously reported in the literature, without evidence of anaphylaxis. 2) These solutions do not agglutinate cells of the four main blood groups. 3) Injected into uncomplicated cases of secondary anemia, they stimulate hematopoiesis. 4) They exert a colloidal osmotic pressure which enables them to restore lost blood volume and raise blood pressure. 5) Dissolved hemoglobin transports oxygen much as it does when confined within the red cell and so augments the oxygen capacity of the blood. 6) Methemoglobin does not accumulate in the plasma even after large injections of these solutions.

The best hope for the therapeutic use of hemoglobin-saline solutions may lie in the direction of the stimulation of hematopoiesis by multiple infusions of small volumes over many days. Such stimulation has repeatedly been reported in animal experimentation (29). It was to be expected in man, but has not previously received clinical demonstration. In our series five cases of secondary anemia due to hemorrhage or infections were treated. In three of these definite improvement was seen. The other two cases had concomitant hemorrhage at the time of infusions so that the beneficial effect of hemoglobin-saline, if present, could not be evaluated. In two other cases of anemia, one with lymphatic leukemia and the other with agnogenic myeloid metaplasia, no improvement was observed from repeated injections.

The value of these solutions in the treatment of shock is more uncertain. Our experience with shock is limited to a single case and is obviously insufficient to establish the solutions as a therapeutic aid for this condition in man. The observations on this case can be considered as suggestive only, although they appear to establish several fundamental points, particularly 4, 5 and 6 above.
We consider that solutions containing 10 to 12 per cent of hemoglobin are best for such work, although their colloidal osmotic pressure is somewhat higher than that of normal plasma.

The only experimental study of the efficacy of hemoglobin-saline solutions in the treatment of shock in animals is the recent report of Lamson et al. (30). These workers were able to save dogs after very severe hemorrhage if the solutions were given quickly. Their results confirm our own animal experience in demonstrating that very large substitutions of whole blood by hemoglobin-saline are possible (in our series up to 90%) without causing shock or renal impairment. These favorable results call into question such adverse findings as those of Flink (31), who injected large quantities of hemoglobin solutions into normal dogs without removing blood and followed renal changes by serial biopsies. By his technic he induced extreme hyperemia, sufficient in itself to cause damage to many tissues.

In spite of favorable indications in some of our human cases, we are forced to recognize that we have not yet succeeded in producing benign solutions routinely. We have made solutions which passed all animal tests but which nevertheless gave reactions when used clinically. At the present time we do not possess an animal test which will surely protect the human subjects. The reasons for these difficulties remain in doubt, but the following possibilities may be recognized:

1) Bacterial contamination of hemoglobin-saline solutions is an ever present hazard. The original red cells are not always absolutely sterile. Every step in the process of preparation must be carefully guarded, since the solutions furnish a very favorable culture medium for many organisms.

2) Even in sterile solutions, or later, after injection into the blood stream, the hemoglobin molecule may be modified or transformed and may then give rise to toxic derivatives. We know very little about the conditions which permit such products to form, but their appearance in the circulating blood, and their connection with definite pathology, has certainly been established in some cases reported in the literature.

3) Constituents of the red cell other than the hemoglobin may pass into the solutions and exercise a deleterious influence. Among these are the remnants of the stroma proteins. The enzymes of the red cells must also in part enter our solutions and may there become modified or denatured.

A considerable literature deals with such hemoglobin derivatives as those mentioned in possibility 2. In a long series of papers Barkan and his collaborators (32, 33) claimed the existence of a type of hemoglobin from which the iron may be more readily removed than from ordinary oxyhemoglobin. The hemoglobin moiety containing the 'leicht abspaltbare Bluteisen', or labile iron, is no more than 5 per cent of the total pigment. This modified hemoglobin molecule, known as 'pseudo-hemoglobin', seems to be a first step toward the
formation of bilirubin. The existence of such a modified hemoglobin, even within the normal red cell, has been confirmed by Lemberg and his colleagues (34), who have secured spectrophotometric evidence for the presence of a ‘bile-pigment hemoglobin’ which they name ‘choleglobin’. In this derivative the porphyrin ring of the prosthetic group has broken by oxidative scission, but remains attached to its globin. Later, within cells of the reticulo-endothelial system, the modified prosthetic group of choleglobin breaks from its protein, loses its iron and is transformed through biliverdin to bilirubin. This sequence of chemical change appears to be the normal and preferred route for hemoglobin degradation. No evidence is given in this literature that choleglobin or any of its breakdown products are toxic. Certainly bilirubin is not. According to Bomford (35) intravenous injections of bilirubin stimulate hematopoiesis in anemic dogs. Weech, Vann and Grillo (36) have injected considerable amounts of bilirubin into human cases with no indications of toxicity.

Hemoglobin may, however, break down along a second chemical pathway. Methemoglobin, containing iron in the ferric form, is normally present in small amounts within the red cells (37). From its molecules hematin (ferrihemate) may split off. Hematin, losing its iron, may become protoporphyrin. Various agents, such as the sulfonamides which produce methemoglobinemia, may also increase the blood concentration of these derivatives without the appearance of bilirubin (38).

In our studies of the nearly complete replacement of blood in cats by beef hemoglobin-saline, death was definitely anoxic in character and was related to the terminal concentration of dissolved hemoglobin in the blood stream (about 3 gm. %), not to the amount of methemoglobin then present. The terminal values for methemoglobin varied considerably. In the longer survivals methemoglobin sometimes rose to a value of 3 gm. per cent. In our clinical studies human methemoglobin dissolved in the plasma has never risen to such heights. The concentration remained nearly constant at the low level originally present (0.2-0.3 gm. %). A similar behavior of human methemoglobin has been observed after injection into animals. It is therefore very unlikely that the reactions so observed in some of our cases are due to methemoglobin.

Such evidence as we can discover in the literature suggests that methemoglobin is not an actively toxic agent. Its physiological effects arise from the oxygen deficit associated with its presence (39). Not only is the oxygen capacity of the blood reduced, but the oxygen dissociation curve of hemoglobin is shifted to the left (40), both in solution and within the red cells, so that oxygen unloading in the tissues is retarded. Bing (41) shows that methemoglobin does not create a kidney block in dogs except when an experimental acidosis has been induced by oral administration of ammonium chloride, to give a urine more acid than pH 5.8.

When methemoglobin breaks down to form globin and hematin, a number
of new possibilities confront us. Schumm (42) found hematin regularly present in the blood of pernicious anemia patients and it has even been considered a diagnostic test for this disease. Hematin is formed within the cells of the malarial parasite (43) and thence liberated into the blood stream. Duesberg (9), using rather small injections of hemoglobin, was unable to detect hematin formation in normal men, although bilirubin increases were easily seen. He argued that the two substances cannot be on the same pathway of breakdown of the hemoglobin molecule; i.e. that bilirubin cannot arise from hematin. In all cases of liver disease involving destruction of the parenchyma, however, Duesberg detected hematin formation after hemoglobin injections. Fairley (44) claims that in primate blood the Schumm test demonstrates the presence, not of free hematin but of a new pigment, methemalbumin (first detected in cases of backwater fever), which forms when hematin joins with plasma albumin. It does not appear in the urine. The existence of such a pigment, differing from hematin, has been fully confirmed by the spectroscopic studies of Foy and Kondi (45) and others.

In animals hematin may be very toxic. Anderson et al. (46) report that hematin produces acute and chronic changes in the kidneys and in the reticulo-endothelial and vascular systems. The observed lesions can be accounted for by multiple small thrombi, without invoking any intrinsic toxicity of ferrihemate. These authors cannot detect any combination of hematin with plasma proteins, in agreement with Fairley, who found such a union only in primates. Nevertheless, hematin does not appear in the urine, but is deposited in cells of the reticulo-endothelial system.

The rôle of the liver must be kept in mind in all discussions of the fate of intravenously injected hemoglobin-saline solutions. Scheff (47) has found that the formation of methemoglobin in vivo by aniline is diminished by hepatectomy. The rapid increase of ferritin iron in these organs in dogs, after partial hemolysis of red cells by phenylhydrazine, has been observed by Hahn et al. (48).

It seems fair to conclude, from this literature, that when, and if, hematin appears in the human blood stream it will be quickly engulfed by cells of the reticulo-endothelial system, particularly in the liver, bound as methemalbumin and so rendered innocuous. In the absence of disease of the liver, or when it is overwhelmed by massive hemoglobin injections, free hematin may appear in the plasma and produce lesions. Hematin, losing its iron, becomes porphyrin, some types of which are toxic. Our solutions contain only traces of relatively non-toxic protoporphyrin. The observations of Kark and Meiklejohn (14) suggest that hemoglobin does not readily degrade to porphyrin within the blood stream; the porphyrinuria of plumbism does not arise from hemoglobin breakdown.

An important condition controlling the breakdown of the hemoglobin
molecule is the acid-base equilibrium in blood and urine. Baker and Dodds (49) on the basis of experimentation with rabbits concluded that hemoglobin and its derivatives, particularly methemoglobin and hematin, mechanically block the renal tubules when precipitated from an acid urine. They advocated the administration of alkali to prevent such a result. De Gowin and his associates (50), using dogs, reported that they could substantially confirm Baker and Dodds, but recognized that mechanical obstruction of renal tubules was not sufficient to explain all of their deaths. They inferred the presence of a nephrotoxic factor, able to cause necrosis of tubular epithelium. They have, however, been opposed by Bing (41) and by De Navasquez (51), who could not confirm Baker and Dodds in animal experiments.

The ideas of Baker and Dodds have been accepted by many clinicians, most recently by Bywaters (52), who urges administration of alkali in treatment of the crush syndrome, and by Shen, Han and Fleming (53) in management of burn cases. They have been opposed by other clinicians (45, 51, 54). Our own patients have generally received alkali. We have given a number of injections without alkali and have seen no difference in the results. In some of our cases the urine turns alkaline even without alkali administration. Bing (41) observed similar alkaline urines in his acidic dogs, after induction of oliguria. The phenomenon may be a sign of renal pathology. The proper procedure in management of the cases remains in doubt. Such an alkaline shift in the urine is certainly not typical of all clinical conditions accompanied by hemoglobinemia. Ross (55), for instance, in his classical study of black-water fever, could detect no acidosis in the blood, but found the urine to be acid in every case.

It must be emphasized that almost every investigator in this field has used a different method for the preparation of his hemoglobin-saline solutions. Conflicting results and claims have therefore inevitably arisen. A standard hemoglobin solution is needed, prepared in such a way that its stability is insured. The field is a difficult one, beset with many hazards. More clinical studies are needed. This record and argument are set down for the benefit of those who may attempt them, as guidance and warning.

SUMMARY

A method is described for the preparation of hemoglobin-saline solutions for intravenous injections in clinical cases. The results of such injections into 14 patients are described. Seven patients received more than one injection, 7 other patients only one.

In the multiply injected group there were 5 cases of secondary anemia due to hemorrhage or infection. Of these, 3 patients showed definite improvement after treatment, exhibiting reticulocytosis and an increase in blood hemoglobin and hematocrit values (fig. 5). In a 4th patient the effect of the injections could not be evaluated, since hemorrhage continued. In none of these four
cases did oliguria develop. In a 5th patient (figs. 6 and 7) the secondary anemia developed from a severe hemorrhage post partum, which led to a state of shock. Administration of hemoglobin-saline (2300 cc. in 5 injections) restored blood pressure to normal. The patient appeared to be recovering, but developed oliguria and uremia and died on the 9th day.

In one case of lymphatic leukemia and one of agnogenic myeloid metaplasia no improvement was observed after repeated injections. Urine flows remained normal. In the singly injected group no beneficial effects were observed. One patient showed oliguria, with recovery.

Although definite oliguria was observed in only two cases out of the 14 treated, indications of renal impairment (by NPN or clearance values) were observed in three other cases of the multiply injected group and one of the singly injected group. In the latter case, after injection of 200 cc. of hemoglobin-saline, glomerular filtration rate, renal plasma flow and Tm were reduced to about one third of the original values, with later recovery to normal. Liver function remained normal in the three cases tested. In some cases injections up to a volume of 500 cc. (= 50 to 60 gm. hemoglobin) have given no rise in temperature or other reactions. In other cases pyrogenic reactions have been observed, usually mild, but occasionally severe, complicated by other reactions. The effect of single injections upon temperature, for 7 patients, are shown in figure 1. In 6 patients the average amount of hemoglobin which appeared in the urine (27 tests) was 18 per cent of that injected (fig. 4).

The following favorable indications have also been observed: 1) Such solutions have been given repeatedly to the same human subject, by intravenous injection, in volumes and weights of hemoglobin far exceeding any previously reported in the literature, without evidence of anaphylaxis. 2) These solutions do not agglutinate cells of the four main blood groups. 3) They exert a colloidal osmotic pressure which enables them to restore lost blood volume and raise blood pressure. A chemical pressor principle is also present. The pressor effect endures for several hours. The rise in blood pressure is accompanied by decrease in heart rate (figs. 1 and 2). 4) Dissolved hemoglobin transports oxygen much as it does when confined within the red cell and so augments the oxygen capacity of the blood. 5) Methemoglobin does not accumulate in the plasma even after large injections of these solutions (figs. 3 and 7).

REFERENCES


55. Ross, G. R. *Memoir Series of the London School of Hygiene and Tropical Medicine*, 1932, no. 6.