OBSERVATIONS ON THE EFFECT OF IRRADIATION IN CHRONIC ACQUIRED HEMOLYTIC ANEMIA EXHIBITING HEMOLYTIC ACTIVITY FOR TRANSFUSED ERYTHROCYTES

By ROBERT S. EVANS, M.D., and ROSE T. DUANE, A.B.

EVIDENCE is accumulating that transfused cells are destroyed at an accelerated rate in one type of acquired hemolytic anemia. Since normal cells as well as the patient's are involved it is evident that a hemolysin is present which is active against all erythrocytes. In the few instances in which a hemolysin has been demonstrated in vitro it has exhibited the characteristics of an immune body requiring complement for activity. If an antigen, antibody reaction is the basis of hemolysis in the hemolytic anemias showing accelerated destruction of transfused cells it is not surprising that the agent has seldom been demonstrated in serum or plasma, since it is active at body temperature and would therefore be expected to become attached to the red cells as it is released.

Recent investigations on the formation of antibodies have re-emphasized the importance of lymphatic tissue and lymphocytes in antibody production. Previous observations by Hektoen and by Murphy and Sturm demonstrated that sufficient irradiation of lymphatic tissue will inhibit antibody formation in animals. It seemed worth while, therefore, to observe the effect of irradiation on the hemolytic anemia in a patient in whom it had been demonstrated that normal red cells as well as her own were destroyed in vivo at an accelerated rate. Because irradiation and transfusion were followed by a remission in the severity of the disease a second patient with chronic hemolytic anemia exhibiting similar features was also exposed to irradiation. The results of these observations are reported.

METHODS

The methods of study have been described previously. In addition, the technic of Ashby was used in following the rate of disappearance of the transfused Group O cells. The patient's blood was Group A Rh positive. Oxalated venous blood was drawn to the 0.5 mark in an erythrocyte pipet and diluted to the 10 mark with a 50 per cent dilution in saline of a high titered (1-10,000) Group B serum. After mixing by shaking 1 minute the sample was allowed to stand 1 hour at room temperature. It was then rotated on an Aloe pipet shaker for 15 minutes before filling the counting chamber. The unagglutinated cells were counted by the usual technic and the number per c.mm. computed. Prior to transfusion the counts of unagglutinated cells averaged 40,000 per c.mm. Following transfusion with Group O cells two counts were made with separate pipets and the average used. With this method variations in two counts were never greater than 10 per cent.

From the Department of Medicine, Stanford University School of Medicine, San Francisco 15, California.
CASE REPORT

Mrs. F. A. J., age 38, entered the clinic service of Stanford University Hospital on August 23, 1945, for study of a recurrence of a hemolytic anemia 18 months after splenectomy had apparently resulted in a cure. She was born in California of Portuguese parents. A kyphoscoliosis which had caused her little trouble had been present since birth. Measles and pertussis were the only childhood diseases recalled. She had been married 34 years and had had four pregnancies. Three children are living and well; one died in infancy of an unknown cause. Her father died of diabetes and her mother of tuberculosis. One sister died of uremia and heart failure and another of tuberculosis. Two siblings are living and well. There is no family history suggestive of hemolytic anemia.

She was well until 1939, when she began to have pain in the hips and lower back radiating down the posterior aspect of the right leg. The pain was intermittent but increased so she consulted a physician in November of 1941. The hemoglobin was 77 per cent and the spleen was palpable and tender. She received iron by mouth and intravenously during the next several months, along with injection of arthritis vaccines. In May 1942 the hemoglobin was 8 Gm. per 100 cc. and erythrocytes 2.38 million per c. mm. Moderate variation in size and shape of the red cells was noted. A reticulocyte count was not done, but polychromatophilia was said to be rare. Blood Wassermann, Kolmer and Kline were positive. She received several injections of arsenicals but no consistent antiluetic therapy was instituted. A series of injections of gold sodium thiosulfate was begun on June 8, 1942, for her arthritic symptoms. Because of continuation of her obscure anemia associated with splenomegaly she was referred to the private service of Stanford Hospital on July 20, 1942, for further study.

She was noted to be sallow but not icteric. The sclerae were clear. Pupillary reactions were normal. There was no generalized adenopathy. The heart was enlarged and there was a systolic murmur at the apex and in the pulmonary area. The edge of the spleen was felt 5 cm. below the costal margin and the liver edge was palpable and smooth.

The hemoglobin was 6.17 Gm. per 100 cc. and erythrocytes 1.75 million per c. mm. The leukocytes numbered 3,800 per c. mm. The differential count of leukocytes was normal except for a high banded count and 1 per cent myelocytes. There were 400 nucleated red cells per c. mm. and the reticulocyte count was 20 per cent. Blood platelets were 118,000 per c. mm. The icterus index was 11. Mean corpuscular values were within normal limits. Hypotonic fragility of the erythrocytes was close to normal with hemolysis beginning at .50 per cent salt solution and the control at .46 per cent. There were no microspherocytes in the stained smear, and the average cell thickness was 2.3 micra. The Wassermann reaction was positive although atypical and was reported as follows:

- Cholesterinized heart antigen (− − −)
- Acetone insoluble antigen (+ + +)
- Alcoholic extract antigen (+ + +)
- The Hinton flocculation was negative.

A diagnosis of hemolytic anemia was made and she was returned to her physician with the suggestion that transfusion therapy be tried for a while.

There was evident improvement for several months following repeated transfusions. However, the symptoms of weakness and fatigue recurred, and while the anemia did not become severe the hemoglobin was 10.64 Gm. per 100 cc. and the reticulocytes 10 per cent at the end of one year. Cell fragility of hypotonic saline was still close to normal. She was readmitted to the hospital on August 16, 1943, and because of the continued signs of hemolytic anemia a splenectomy was done by Dr. Frederick Reichert on August 23, 1943. A bone marrow biopsy taken from the left 11th rib at the time of splenectomy revealed hypertrophy of the marrow stroma and an increased proliferation of erythroblastic cells. The operation was followed by rapid improvement, and the hemoglobin level and erythrocyte count rose to a normal range in 2 weeks' time.

She was well and entirely free of anemia until March 1945, when there was a recurrence of arthritis with swelling of fingers, wrists, and knee joints. During the next 2 months she received gold sodium thiosulfate injections totaling 11 cc. About 3 months after beginning the gold therapy she noted weakness and "pounding of the heart" and shortness of breath on exertion. The above symptoms increased in severity until the time of her third admission on August 23, 1945.

Physical Examination.—She was cheerful and able to be up and around her room. The skin and mucous
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membranes were pale. The sclerae were not icteric. The pupils reacted well to light and accommodation. The tongue was normal in appearance. There was no generalized lymphadenopathy. The chest was clear to percussion and auscultation. The cardiac rhythm was regular and a systolic murmur was heard over the precordium. Blood pressure was 110 mm. systolic and 70 diastolic. The splenectomy scar was firmly healed. The liver edge could not be felt. Pelvic examination was normal. The extremities, including the joints, were not remarkable and the deep tendon reflexes were equal and active.

**Laboratory Work.**—Examination of the blood gave the following values: hematocrit 14, erythrocytes 2.1 million per c. mm., Hb. 8.6 Gm. per 100 cc., MCV 114 cubic micra, MCH 40 micro micrograms, MCHC 36 per cent, reticulocytes 17.6 per cent, 210 nucleated erythrocytes per c. mm., 60,000 platelets per c. mm., and 7,000 leukocytes. The differential count of leukocytes was polymorphonuclear neutrophils 70 per cent, eosinophils 5 per cent, lymphocytes 18 per cent, and monocytes 7 per cent.

Examination of the stained erythrocytes showed great variation in size with many large polychromatophilic cells and many small densely staining cells and microspherocytes.

The blood Wassermann reaction was negative on this and several subsequent occasions. Total proteins were 7.3 Gm. per 100 cc.

**Course.**—The salient features of her subsequent course until the time of death are shown in figure 1. Because she was comfortable in spite of her anemia it was decided not to give her transfusions until necessary. She was, therefore, allowed to return home after 6 days' hospitalization. Subsequent examinations during the following month showed a drop in hematocrit and an increase in reticulocyte percentage. There was a marked increase in weakness and pallor, and she was readmitted on September 15, 1945, with a hematocrit of 12. The erythrocyte count had dropped to 1.45 million per c. mm. and the hemoglobin to 7.2 Gm. per 100 cc. The reticulocytes had increased to 32 per cent and the icterus index to 30. The urobilinogen output had increased to 2500 mg. per day. The leukocyte count was 12,500 with 21 per cent lymphocytes. There was a slight increase in hypotonic fragility as compared to the previous examination. She exhibited a titer of cold agglutinins of 1:32, but no agglutination could be demonstrated at body temperature. No atypical isohemolysin could be demonstrated in her serum when suspensions of her own or normal cells were incubated in her serum at 37° for 4 to 10 hours.

With an increase in severity of her anemia she complained of more epigastric distress and nausea after taking food or liquids. The gastrointestinal symptoms improved or became more pronounced in relation to the severity of her anemia throughout the rest of her course.

The effect of repeated transfusions of citrated Group O cells is shown in figure 1 and in more detail in figure 2, where the number of unagglutinated Group O cells and the total erythrocyte count before and after transfusion are shown. The number of autologous erythrocytes was computed by subtracting the number of unagglutinated cells from the total number per c. mm. As shown in figure 2 the rise in hematocrit following transfusion was transitory and the drop toward the pretransfusion levels was rapid. As shown in figure 2 the drop in erythrocyte count was due to the rapid disappearance of the transfused cells.

The rapid restoration of the circulating hemoglobin to normal levels by multiple red cell transfusions had no immediate untoward results. Reactions were minimal and for several days following transfusions she felt in a more or less normal state of health. The rapid drop in hematocrit was associated with the recurrence of weakness, shortness of breath, and indigestion. Transfusions became increasingly difficult because of thrombosis of the superficial veins.

As a result of these observations on the accelerated destruction of transfused normal cells it was concluded that a hemolysin must be present which was active in vivo but could not be demonstrated in vitro. Repeated attempts to demonstrate the presence of a hemolysin in the serum by incubating cell serum suspensions all met with failure. Since it seemed likely that the hemolysin was an immune body type it was decided to see if any measures could be used to modify its production. Accordingly on October 29 she was given 71 cc. of thorotrast (24 to 26 per cent thorium dioxide) intravenously with two purposes in mind. It was thought that the absorption of a large amount of colloidal material by the reticuloendothelial cells might modify the production or elaboration of a hemolytic antibody. Thorotrast was chosen instead of some other colloidal material because of the possibility of demonstrating an accessory spleen which might be removed. There was no reaction to the injection of thorotrast. Films of the upper abdomen showed good visualization of the liver, but no shadow that could be interpreted as an accessory spleen was present.
FIG. 1. THE ESSENTIAL HEMATOLOGICAL DATA IN THE COURSE OF THE PATIENT FROM THE TIME SHE WAS FIRST SEEN IN RELAPSE UNTIL THE TIME OF DEATH

The first part covers the period of control observations as the anemia became more severe and the period of multiple red cell transfusions, and finally the injection of thorotrast which produced a transitory interruption in the fall in the hematocrit. The second part shows the effect of irradiation on the leukocyte count and the two periods of remission which were apparently induced by transfusion following irradiation. Determinations of the rate of excretion of fecal urobilinogen at intervals are shown in blocks as mg. per day.
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At the time the thorotrast was given the hematocrit was falling following transfusions and judging by previous experience could have been expected to drop further. As can be seen in figure 1, the fall in hematocrit was interrupted and there was a rise from 13 to 17 three days later before the gradual drop was continued. Ten days following injection of thorotrast the hematocrit had reached 20 and she was given 250 cc. of whole blood and 300 cc. of concentrated cells without producing a rise in the hematocrit or hemoglobin. Two injections of 100 cc. of Congo red were given on subsequent days without evidence that the hemolytic process was affected.

On November 12 the hematocrit was found to be 16 and the hemoglobin 4.2 Gm. per 100 cc.

![Graph](image)

**Fig. 2. The Transitory Effect of Multiple Red Cell Transfusions Preceding Irradiation**

This was due in part to the hemolysis of the transfused Group O cells which were counted as unagglutinated cells in the Group B serum. The number of patient's cells per cc. mm. was computed by subtracting the unagglutinated cell count from the total erythrocyte count. Approximately 50 per cent of the transfused cells disappeared from the circulation in 5 days' time, which is about ten times the normal rate.

Irradiation was begun by giving 100 r in air daily to the anterior mediastinum. The chest was 19 cm. thick. A 12 by 18 cm. portal centered over the mediastinum anteriorly and posteriorly was treated, using radiation of half value layer of 1 mm. of copper. The skin target distance was 70 cm. The same method and dose were used in irradiating the periaortic region of the abdomen in subsequent courses. On the fourth day of irradiation the hematocrit was 14 and the icterus index 40. Her clinical condition was very poor, with extreme weakness, air hunger, and persistent nausea. Oxygen therapy seemed to provide the necessary margin to sustain life. She was given a transfusion of 150 cc. of red cells by cannulating a vein in the ankle. During the next 4 days the improvement was gradual and sustained. Her color improved and she no longer exhibited air hunger. The nausea disappeared and she was able to eat normally. On the
fourth day following transfusion the hematocrit was 31 and the hemoglobin 8.5 Gm. per 100 cc. The icterus index, which had been 40 and 50, dropped to 10 and 15, where it remained during several weeks of remission. The urobilinogen output in the stool was measured as 730 mg. per day during the next 4 days.

The total leukocyte count, which had been 23,000 and 21,200 per c. mm. on two occasions prior to x-ray, dropped to the neighborhood of 8,000. The decrease in circulating lymphocytes and the total leukocyte count are presented in figure 1. Platelet counts varied between 100,000 and 200,000 per c. mm. throughout her course.

When the hematocrit was found to be 31 irradiation was discontinued for several days but was begun when the hematocrit fell to 26. Following the resumption of irradiation of the mediastinum and later of the abdomen the hematocrit rose again and the lymphocyte count, which had shown a temporary rise, fell to lower levels. She continued to feel a great deal better and regained sufficient strength during the period of partial remission to be ambulatory. The improvement in the anemia was accompanied by signs of diminished hemolysis. The icterus index remained at a level of 10 to 15 and the urobilinogen output in the stool was less than half the three previous determinations.

The curve of hypotonic fragility, which had shown a gradual increase since the first determination on August 27 along with increasing severity of the hemolytic process, showed a reversal of this trend and shifted back toward the normal stage. The curve of December 14 (figure 3) is a sample of those obtained during the period of partial remission.

Irradiation of the periaortic region of the abdomen was undertaken on two occasions using the same

![Graph of hypotonic fragility curves](image-url)
amount and size of portal as the mediastinum. It was during the second course of irradiation to the abdomen that she exhibited a rise in the number of lymphocytes in the circulation and a precipitous fall in the hematocrit and a return of her symptoms of weakness and nausea. With the drop in the hematocrit she exhibited a low grade fever and developed a mild productive cough which persisted several days. A chest x-ray on December 18 showed the lung fields to be clear. Urinalysis at the same time showed a transient pyuria, but there were no symptoms of urinary tract infection. She was given 30,000 units of penicillin every 3 hours for 7 days. This medication was accompanied by a subsidence of the cough and clearing of the urine sediment, but no improvement in the anemia. Irradiation of the mediastinum was resumed on January 2, 1946. By January 7 no significant improvement in the anemia had occurred, and she seemed unable to continue the struggle longer. A transfusion of 500 cc. of whole blood was followed again by a dramatic improvement. The hematocrit rose from 18 to 30 and then to 34 during the next 5 days, and the hemoglobin rose from 4.9 Gm. to 10.4 Gm. per 100 cc. She again felt greatly improved.

However, on January 16 she complained of pain in the right anterior chest and the following day a friction rub was heard. An x-ray of the chest failed to reveal any area of consolidation. Following this episode, however, the hematocrit fell rapidly and all the former symptoms of severe anemia returned. As can be seen in figure 1, this drop occurred along with continued irradiation. A transfusion of 500 cc. of whole blood was of temporary benefit only, and she died on January 29 with symptoms of marked air hunger.

Pathological Examination.—Spleen: The spleen, removed 29 months prior to death, weighed 955 Gm. The capsule was smooth and translucent. Two small infarcts were present. The malpighian bodies were large but not distinct and showed considerable hyaline homogeneous material in the centers. The reticulo-endothelial cells were laden with brownish pigment (hemosiderin) but the phagocytosis of red cells was not prominent.

Autopsy.—Gross: There was no free fluid in the serous cavities. The heart weighed 375 Gm. and showed normal values. There were no gross infarcts in the lungs but there were multiple small emboli in the pulmonary arteries, several of which were adherent to the vessel wall. Mediastinal lymph nodes appeared grossly normal. The liver weighed 1950 Gm. and appeared normal. Gallstones were not present. No accessory spleen was found. The adrenals were normal and the kidneys weighed 185 and 200 Gm. The uterus and adnexa were normal, as were the stomach and intestines. The femoral, sternal, and vertebral marrow was a deep red.

Histological Examination.—The multiple thrombi in the pulmonary arteries were in various stages of organization and recanalization. Others were more recent but none was found that could be considered of fresh occurrence. The lymph nodes of the anterior mediastinum showed some diffuse fibrosis but no typical radiation fibroblasts were found. The marrow showed hyperplasia of all elements and marked erythroblastic activity. Phagocytosis of red cells was prominent and many macrophages were filled with granules of thorium dioxide. There was a fine diffuse fibrosis of the sternal marrow and some evidence of an arrest of development of marrow elements.

The Kupffer cells of the liver were filled with refractile grayish granules of thorium dioxide. Iron stains showed a moderately heavy deposit of iron pigment in the liver cells and Kupffer cells. The kidneys showed hemosiderin pigmentation of the tubular epithelium.

The mucosa of the esophagus and cardiac end of the stomach was ulcerated in
patchy areas and replaced by young granulation tissue and infiltrated with inflammatory cells. The remainder of the gastrointestinal tract was normal.

**Irradiation of a Second Patient with Chronic Hemolytic Anemia.**—The second patient with chronic acquired hemolytic anemia and spherocytosis to be studied for the effect of irradiation has been reported previously.\(^4\) She exhibited a marked increase in cell fragility which made it possible to demonstrate that transfused cells were made abnormally fragile to hypotonic solution within 24 hours after injection.

Since the partial remissions in the first patient followed transfusions as well as irradiation, it was desirable to determine first what effect if any irradiation alone would have on the hemolytic process. During the 12 day period of observation the hematocrit varied between 24 and 27, and the output of bilirubin in the ileostomy excreta was measured as 835 and 1200 mg. per day. The effect of 200 r daily in air to the mediastinum given every other day on the total leukocyte and lymphocyte count is shown in figure 4. The reduction in circulating lymphocytes was marked, but there was no measured effect on the hematocrit or bilirubin output to indicate a slowing of the hemolytic process. If there was any significant effect it was in the direction of increasing the degree of anemia temporarily. Five months after irradiation the hematocrit was 26, the leukocytes 12,000 per c. mm., and the lymphocytes 4,000 per c. mm.
In a previous report evidence that injections of colloidal gold had precipitated the onset of the abnormal hemolytic process in one patient was presented. In the case history presented here the relationship of gold therapy to the onset of the hemolytic process is doubtful, since an anemia and palpable spleen were noted before gold therapy was begun. However, the relapse 20 months following splenectomy coincides closely with the resumption of gold therapy. Following splenectomy, the hemoglobin, erythrocyte count, and reticulocyte percentages were normal in all determinations up to the time gold therapy was resumed. The fall in hemoglobin thereafter was evidently gradual, since the onset of pallor and weakness was insidious. The anemia increased in severity until it finally became necessary to resort to transfusion therapy. It is evident that gold therapy must be considered capable of setting off an abnormal hemolytic process.

Histological examination of the spleen and the subsequent autopsy findings failed to throw any new light on the pathogenesis of the disease. The phagocytosis of the red cells by the macrophages of the marrow, although extensive, does not account for a process that damaged the majority of the mature red cells present in the circulation. On the contrary, the phagocytosis was probably stimulated by the presence of the damaged cells. It is likely that the multiple small pulmonary emboli resulted from the thrombosis of superficial veins following transfusions, since no other source was found. The acute ulcerations of the esophagus and stomach account for the epigastric symptoms which were pronounced when the anemia was severe, which suggests that anemia and anoxemia played a role in their production.

Kracke and Hoffman have previously reported the association of positive serology of atypical behavior and chronic acquired hemolytic anemia. In their patient, too, the serological reactions reverted to normal following splenectomy.

According to the method of differential agglutination the transfused Group O cells disappeared from the circulation at roughly ten times the normal rate, since approximately 50 per cent of the cells were eliminated in 4 to 5 days' time. This rate of hemolysis of transfused cells is consistent with the pyrrole pigment excretion, which was somewhat greater than ten times the normal rate. It is obvious that the transfused red cells must have participated in the increased hemolysis in that repeated multiple transfusions which in some instances nearly doubled the total cell volume continued to have a transitory effect. Also, there was evidence from studies of the quantitative fragility curves before and after transfusion that the cells introduced showed a gradually increasing susceptibility to hemolysis in hypotonic solutions during the 3 or 4 days following their introduction. All these observations which show that normal cells were damaged and hemolyzed at an accelerated rate indicate the presence of a hemolysin in the patient's circulation. It is of course only an assumption that the hemolysin was an immune body, but in the absence of other evidence that seems to be the most likely possibility. It is unlikely that the cold agglutinins played a significant role in the hemolytic process since the titer was relatively low and there was no activity at body temperature.
The temporary rise in hematocrit that followed the injection of thorium dioxide may be significant since it was the first interruption in the drop in hematocrit following transfusion that had been recorded. There was nothing to suggest that the temporary rise in hematocrit was due to hemo-concentration following the injection. The only direct evidence that the rise was due to a slowing of the rate was a fall in the icterus index from 50 to 30 and then an increase to 50 as the hematocrit began to drop again. There are several possible explanations which might account for a temporary slowing of hemolysis after the injection of a large amount of colloidal material. Filling the reticulo-endothelial cells with colloidal particles might interfere temporarily with phagocytosis of damaged cells and allow more to remain in the circulation. On the other hand the colloidal particles could interfere directly with the hemolysin or form a protective coating around the erythrocyte. Lastly, the absorption of colloidal material might modify temporarily the rate of production or elaboration of hemolytic antibody by the reticulo-endothelial cells. The evidence that the reticulo-endothelial system is a source of antibodies is controversial, but the possibility has not been excluded by recent emphasis on the importance of the lymphatic tissue.

The injection of Congo red was apparently without effect. Congo red has been shown by Richardson to protect erythrocytes against hemolysis by a variety of agents, but the concentration of the dye used in these in vitro experiments was much greater than would be achieved in intravenous injection of 200 mg. It is noteworthy that Congo red, unlike thorium dioxide, is not, according to observations in animals, absorbed by the reticulo-endothelial cells alone.

The prolonged partial remission which followed the single transfusion given after 4 days of irradiation showed that either the irradiation or some other completely unknown factor had modified in a radical way the severity if not the nature of the hemolytic process. The most obvious effect of the irradiation was the depression of the number of circulating leukocytes, particularly the lymphocytes, which is in agreement with the studies of Minot and Spurling on the effect of x-ray therapy. As can be seen in figure 1 there seemed to be a rough inverse relationship between the number of circulating lymphocytes and the degree of severity of the anemia until the terminal 2 weeks of her course. The leukocytosis and lymphocytosis that preceded the period of irradiation had no obvious clinical explanation but seemed to be associated with the multiple red cell transfusions and followed a period of increased blood breakdown.

It is possible theoretically that destruction of large numbers of circulating lymphocytes would increase temporarily the rate of red cell destruction by releasing hemolytic antibody. The evidence that that occurred is equivocal. As shown in figure 1, the hematocrit fell from 16 to 14 during the drop in lymphocyte count and the reticulocytes increased from 29 to 50 per cent, but the severity of the anemia was increasing before irradiation was begun. It was not possible to obtain accurate studies of pigment output during this period. The second patient showed a lowered hematocrit during the drop in circulating lymphocytes but here, too, this degree of variation has been observed to occur spontaneously.

The first partial remission ended with the onset of symptoms which suggested
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a respiratory infection and the second with an episode which seemed likely to have been a pulmonary embolus. The drop in hematocrit at the end of the first remission was preceded by a rise in lymphocyte count and a leukocytosis, which suggests that an infectious process may increase the severity of hemolysis by activating lymphatic tissue and increasing the production of a hemolysin.

It is obvious that both partial remissions observed in the patient were initiated by transfusions although in each case the hematocrit rose to a higher level than could have been accounted for by the transfusion alone. The failure to produce a remission in the second patient, who seems to have had a closely similar hemolytic process, with irradiation alone supports the importance of the transfusions in initiating the remissions. There is no good explanation as to why transfusions were necessary to initiate a sustained remission. It is conceivable that an antihemolytic substance in plasma became effective at this point or that the remaining hemolytic antibody was absorbed by the normal transfused cells. Future studies should, if possible, include observations on the effect of plasma and washed red cell transfusions following irradiation.

As already indicated, marked changes in erythrocyte morphology and fragility in hypotonic solutions were observed during the course of the hemolytic anemia. Prior to splenectomy, when the hemolytic anemia was relatively mild, hypotonic fragility was normal as measured by the usual technic. The mean cell thickness, computed by the formula $\frac{M.C.V}{M.C.D^2}$, was 2.3 micra and microspherocytes were absent in the stained smear. However, when first seen in relapse the blood smear showed a large percentage of spherocytes, the M.C.T. had increased to 3 micra, and the fragility in hypotonic solution was increased as shown in figure 3 (August 27). As the hemolytic process accelerated, the fragility curve became more abnormal (October 25 and November 7), while with each remission the curve shifted back toward normal (December 14 and January 14). The correlation of the hypotonic fragility, which reflects the degree of spherocytosis, with the severity of the hemolytic anemia agrees with findings of Dameshek and Schwartz, who demonstrated that the degree of spherocytosis and the severity of the hemolytic process in experimental hemolytic anemia were proportional to the amount of anti-erythrocyte serum injected.

SUMMARY

The history and course of a middle-aged woman with a chronic acquired hemolytic anemia have been presented. Splenectomy resulted in a complete remission of the disease for 18 months before a relapse occurred which led eventually to a fatal termination.

The original cause of the abnormal hemolytic process is not known, but the onset of the relapse was closely associated with the resumption of gold therapy.

The acceleration of the hemolytic process was associated with an increasing tendency toward spherocytosis and susceptibility of the erythrocytes to hemolysis in hypotonic solution.
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It is demonstrated that transfused normal erythrocytes shared in the hemolytic process and were eliminated from the circulation at an abnormal rate although no atypical isohemolysin was demonstrated by in vitro tests.

An attempt was made to alter the rate of hemolysis in several ways. The injection of thorium dioxide was followed by signs of transitory slowing of red cell destruction. Injections of Congo red were apparently without effect. Irradiation of the mediastinal and periaortic nodes was begun to ascertain if the production of a hemolytic antibody in lymphatic tissue could be altered. Irradiation was followed by a fall in leukocyte and lymphocyte counts in the peripheral blood. Transfusions were then followed by partial remissions in the hemolytic process for varying periods of time in two instances. Irradiation of a second patient with a chronic hemolytic anemia of similar character produced a fall in leukocyte count and lymphocyte count, but in the absence of transfusions no slowing of the hemolytic process occurred.

No definite conclusions can be drawn from these fragmentary observations on the effect of irradiation, but further investigation as to the character of the hemolytic substance and possible methods of modifying its production are indicated when the opportunity is afforded.

REFERENCES

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