Hemolytic Anemia, a Host Response to Malignancy*

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Latent hemolytic anemias have been discovered in many systemic diseases by measuring the in vitro survival of red blood cells (5, 6, 8-10, 11, 16, 23, 38). A shortened lifespan of erythrocytes has been demonstrated in some patients with advanced carcinoma (20, 21, 22, 28, 34), but not in others (36). Since latent hemolysis may or may not be present and since it is not unique for advanced carcinoma (20, 21, 22, 28, 34), but not in others (36), it is not always associated with malignancy, no particular significance for tumor-host relationship has been attached to this interesting phenomenon, except by Hyman et al. (20).

The purpose of this study is not to confirm the fact that latent hemolysis may occur in advanced malignancy, but rather to show by repeated determinations of red cell survival how the onset of this hemolysis correlates closely with the beginning of rapid host deterioration, and to suggest that an inquiry into the cause of this erythrocyte destruction might lead to a better understanding of host responses to tumor growth.

MATERIALS AND METHODS

In four patients with metastatic carcinoma of the breast and one patient with malignant melanoma, serial hemoglobin determinations, reticulocyte counts, and red cell survival studies were correlated with the progress of the disease as measured by symptoms and by serum and urinary calcium values. In one patient values for serum aldolase activity were correlated with a red cell survival curve.

1. The blood hemoglobin was measured by the Evelyn colorimeter.
2. The number of reticulocytes per 1,000 red cells was counted in a wet preparation with saline brilliant cresyl blue.
3. Red cell survival studies were done by the method of Ashby (1). Powdered anti-A or anti-M rabbit serum was added to a 1:200 dilution of venous blood in such proportions that a blank count of less than 10,000 unagglutinated cells/cu mm was obtained. The red cells in each of two tubes were spun and shaken twice, and the unagglutinated cells in 0.1 cu. mm. counted on both sides of a counting chamber. Packed red cells were given only after a Coombs cross-matching had been performed.

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Negative. In cases #1, 2, and 3, the red cells with a shortened survival had been found to survive normally in other patients.1
4. Coombs testing was done with a commercial Coombs serum,3 and with Coombs sera made by the technic of Emerson, Franklin, and Lowell (12), but with, as antigen, the serum of two of the patients reported here. The patient’s red cells had been frozen in glycerol-citrate so that they would not lose their antigenicity on storage (18).
5. Serum and urinary calcium values were determined by the method of Fiske and Logan (16).
6. Serum aldolase values were obtained by the use of Beck’s (2) modification of an assay method devised by Sibley and Lehninger (38). One aldolase unit here is expressed as equal to the chromagen concentration produced by 1 μmole of glyceraldehyde. A factor of 1.9 converts glyceraldehyde into glyceraldehyde phosphate. A factor of 22.4/2 converts 1 μmole of glyceraldehyde phosphate into 1 aldolase unit as used by Sibley and Lehninger (1 cu. mm. of hexosediphosphate split in 1 hour).

RESULTS OF CASE STUDIES

Chart 1 shows the results of three red cell survival studies in a 41-year-old woman with metastatic breast carcinoma who failed to respond to castration, androgens, or adrenalectomy. The hemoglobin, reticulocyte percentage, and erythrocyte survival were normal over a period of 8 months, despite the presence of diffuse metastases. However, an anemia with a hemolytic component did develop during the last 2½ months of life, when more rapid clinical deterioration accompanied by hypercalcuria and then hypercalcemia followed estrogen administration. During the period of shortened red cell survival, when her hemoglobin dropped from 13 to 6.5 gm., two direct Coombs tests were negative, and her serum did not agglutinate trypsinized cells. There was no evidence of blood loss, repeated stool examinations being guaiac-negative. One week before death a blood smear showed moderate variation in the size and shape of red cells, and eleven normoblasts/100 white blood cells. There was immaturity of the white cells with 10 per cent metamyelocytes.

1 The authors are indebted to Miss Eleanor Casna and Dr. M. Grove-Rasmussen in the Blood Bank of the Massachusetts General Hospital for setting up and Coombs cross-matching the blood used in this report.
2 Ortho Antihuman (Coombs) Serum.
Autopsy at an outside hospital was reported to show extensive metastases of a mucin-producing adenocarcinoma. Sternal, costal, and vertebral marrow was replaced by tumor. A slightly enlarged liver contained many metastatic nodules and a few foci of extramedullary hematopoiesis. A congested spleen weighed 300 gm. The ovaries and adrenal glands were absent.

Chart 2 shows the results of three erythrocyte survival studies on a 56-year-old spinster with metastatic breast carcinoma, who had responded to adrenalectomy at the beginning of the study. The hemoglobin values were normal or slightly decreased, and red cell survival was not shortened during a 15-month period, despite extensive metastases. The anemia and the precipitous early fall in the second erythrocyte survival was thought to be the result of gastrointestinal hemorrhage. Marked decrease in bone pain followed a course of 12 mc. of P32. Again clinical deterioration and an increase in urinary calcium excretion seemed to be precipitated by a trial of estrogens. This turn of events was accompanied by a progressive fall in hemoglobin from 12 to 5 gm., by a rise in reticulocytes to 55 per cent, and by a marked shortening of red cell survival. There was evidence of blood loss only at the time indicated, three stools during the final survival study being guaiac-negative. A direct Coombs test was thrice negative. A blood smear taken on the day of death showed normochromic red cells with marked variation in size and shape plus an occasional normoblast. No immature white cells were seen.

Autopsy revealed extensive metastatic disease to nodes, lungs, liver, and bones. No adrenal tissue could be found. The spleen was absent, but three small accessory spleens together weighed 10 gm. and contained many iron deposits. The sternal and vertebral marrow was quite cellular, containing greatly increased numbers of red cell precursors as well as many clumps of tumor cells. It is of interest that, while three of the other cases had splenomegaly, this patient’s spleen was removed during adrenalectomy for technical reasons. Nevertheless, the sequence of events was the same.

Chart 3 depicts one normal and one markedly shortened red cell survival curve in a 56-year-old man with metastatic carcinoma of the breast, who responded temporarily to first an estrogen and then an androgenic preparation. His hemoglobin had fallen from 11.5 to 7 gm. during the month before death, and both liver and spleen were found to be enlarged. Red cells trapped in an enlarging spleen may have contributed to the rapid fall in donor cells during the last survival study. Two stools were guaiac-negative. The day that his reticulocyte count rose to 21 per cent there appeared 50 normoblasts and 5 erythroblasts/100 WBC in the smear of peripheral blood. A direct Coombs test, which was positive in titer 1:8 at the beginning of these studies, became positive in titer 1:16 terminally. An autopsy was not permitted.

Chart 4 reveals the progressive shortening of erythrocyte survival in a 41-year-old woman with malignant melanoma. The first two survival studies revealed a shortened red cell life span, but the patient had received two courses of radiation treatment during each period. No x-ray treatment had been given during the last and markedly shortened erythrocyte survival, but there was striking tumor growth and clinical deterioration. Despite the rapid disappearance of donor cells, the hemoglobin concentration remained at 10 gm. per cent, and the reticulocytes rose to only 4.5 per cent. It is possible that the patient’s erythrocytes were not being destroyed as fast as the donor red cells. Had the donor cells become sensitized? The Coombs cross-matching had been negative, and no complete or incomplete isoantibodies were found in the serum with the use of a cell panel containing all the known red cell antigens. That this possibility still remains constitutes the major drawback to the Ashby technic for determining red cell survival. Concomitant tagging of the patient’s own erythrocytes with radioactive chromate would have been helpful in solving this problem. We are now combining both technics.

A direct Coombs antiglobulin test was repeatedly negative during the patient’s illness. In an attempt to demonstrate red cell coating by abnormal globulins, a Coombs serum was developed by injecting the patient’s own serum into rabbits. Table 1 shows a low titer agglutination of the patient’s red cells by this serum but not by commercial Coombs serum. That this is a quantitative problem. We are now combining both technics.

Chart 5 shows three of four red cell survival curves covering a period of 2 years, on a 65-year-old spinster with metastatic breast carcinoma. The first survival study, started in April, 1953, is not on the graph, but, like the first two depicted here, it was normal. There were diffuse bony metastases during this entire interval, but the patient felt well, except during the first few days of each course of androgen therapy. Immediately following a single injection of estradiol benzoate there was marked increase in bone pain, malaise, nausea, and weakness. The final red cell survival study, which was started a day after the estrogen injection, showed
CHART 1 (Case #1—M.C.).—Serial hemoglobin values, reticulocyte percentage, and red cell survival curves during a 10-month period in a female patient with metastatic breast carcinoma. The blood studies are correlated with symptoms, serum and urinary calcium values, and treatment.

CHART 2 (Case #2—E.G.).—Serial red cell survival studies in another female patient with metastatic carcinoma of the breast covering an 18-month period.
CHART 3 (Case #3—V.D.).—Two red cell survival studies in a male patient with metastatic carcinoma of the breast.

CHART 4 (Case #4—M.M.).—Serial red cell survival curves in a female patient with malignant melanoma during the last 8 months of life.
a more rapid rate of fall initially, and then leveled off to give a slightly shortened over-all life span. During this time there was no evidence of blood loss, stool examinations with each cell count being guaiac-negative. Both commercial Coombs serum and a rabbit antiserum, prepared by injecting rabbits with the patient's serum, did not agglutinate the patient's red cells; but a third Coombs serum, giving a higher titer on Rh-sensitized cells than both the others, caused agglutination in the first tube (titer 1:4). The terminal episode of deterioration was accompanied by a rapid fall in hemoglobin from 11.6 to 6.2 gm. per cent, and by the appearance of 31 normoblasts and 24 erythroblasts/100 white cells in the peripheral blood. A serum hemoglobin of 17.3 mg. per cent (normal less than 5 mg. per cent) and the appearance of methemalbumin in the serum indicated intravascular hemolysis. A fifth red cell survival study had been started the day before death.

Chart 6 provides a closer look at this patient's final red cell survival curve and introduces a graph of serum aldolase activity. The over-all erythrocyte survival might have been normal but for the precipitous fall in the percentage of donor cells during the first 10 days. This occurred immediately after a single injection of estradiol benzoate had caused an exacerbation of symptoms and a sharp rise in serum aldolase activity. The combination of increased hemolysis and rising serum aldolase occurred again before death.

Autopsy on this patient revealed metastatic

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<td><strong>RED CELL AGGLUTINATION BY TWO COOMBS SERA IN A PATIENT WITH MALIGNANT MELANOMA</strong></td>
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* Coombs serum A was developed by the technic of Emerson, Franklin, and Lowell (11), but with the patient's serum as antigen. Coombs serum B was the commercial Ortho Coombs serum.
cancer to skin, nodes, liver, pleura, pericardium, lungs, dura, adrenals, and bones. The adrenals and liver contained many nodules of tumor without complete replacement. The spleen weighed 260 gm., was congested, and contained hemosiderin deposits. Only tumor cells and fibrous tissue were present in the portions of vertebral and costal marrow examined. Femoral marrow contained marked erythroid hyperplasia around nests of tumor cells.

Testosterone 50 mgm q d Testosterone Propionate 100 mgm t.i.w. ÆEstradiol Benzoate 25 mgm. Meticorten 20 mgm. q.d.

CHART 6 (Case #5—G.G.)—A close-up of the last red cell survival study on Case #5, showing how the systemic symptoms that occurred after the estradiol injection and again terminally were accompanied by both a rise in serum aldolase and increased hemolysis.

DISCUSSION

No correlation has been found between the extent of malignancy and the degree of anemia or hemolysis, when only single determinations of hemoglobin, red cell mass, or erythrocyte survival are made (4, 34). Each survival study in the cases presented here, if taken alone, would bear out this contention, since hemoglobin and red cell survival were normal in the presence of extensive bony and parenchymal metastases. However, when these studies were repeated throughout the late phase of the disease, an anemia with a hemolytic component eventually appeared. Hyman and Harvey (21) found a further increase in hemolysis when they repeated Ashby counts on two patients dying of cancer. They wondered when in the course of this disease the hemolytic phenomenon might first appear. This period would seem to be when the patient begins to suffer the progressive systemic effects of his disease, when the tumor-host relationship has reached the critical point at which the host begins to deteriorate rapidly. Variations in types of neoplasia and treatment may alter the onset and duration of hemolysis. The one patient with malignant melanoma, for example, who was also receiving x-ray treatment, showed a pattern different from the others in that a shortened red cell survival was present during the entire last 9 months of life. Radiation-induced hemolysis in malignancy has been reported (34).

A progressive anemia with a hemolytic component coinciding with the onset of rapid clinical deterioration suggests that the factors responsible for this anemia may constitute part of the systemic effect of tumors. Because normal transfused cells exhibit a shortened survival, one can postulate that an extracorpuscular hemolytic factor is present and is at least partially responsible for the contracted life span of the patient’s erythrocytes. Hemolysis of this variety could be produced by an auto-immune mechanism, by a hemolysin coming from tumor or degenerating host tissue, or by lack of substances in plasma important for normal red cell survival.

Auto-immune mechanisms.—A positive Coombs test is not a usual finding in the anemia of malignant disease (21). However, Evans (14) has demonstrated auto-antibodies in certain cases of acquired hemolytic anemia by the use of a high-titered Coombs serum when the less potent commercial sera showed no agglutination. In one of his cases agglutination of red cells was obtained only with a Coombs serum made by injecting rabbits with the patient’s serum. In our five cases, one patient had a positive test with the commercial Coombs serum, and two others showed red cell agglutination in Coombs sera of greater potency. The significance of these results may be questioned, however, because the agglutinations are of low titer and because damage to erythrocytes other than by antigen-antibody reaction may cause coating with globulins and agglutination in Coombs serum. For example, Muirhead, Grover, and Bryan (29) found that in dogs phenylhydrazine produced a positive Coombs test not only when the drug was given intravenously, but also when mixed with whole blood in vitro. Jandl (24) discovered that red cells, suspended in plasma or
in the globulin fraction of plasma and exposed to dilute solutions of certain metallic ions, would agglutinate in a Coombs serum of high dilution.

**Tissue hemolysins.**—Tissue hemolysins may be the cause of accelerated red cell destruction in advanced carcinoma (32). The increased serum activity of certain enzymes such as aldolase (37), phosphohexose isomerase (7), and lactic acid dehydrogenase (19) in patients with malignancies suggests that tissue substances are accumulating in the blood stream. Warburg (40) believes that catabolizing host muscle tissue might be the cause of serum aldolase elevation, but recent evidence by Sibley (38) suggests that scattered areas of necrosis in rapidly growing tumors are a more likely source. Chart 6 shows a close correlation between a rise in serum aldolase and increased hemolysis, as suggested by the rapid fall in donor erythrocytes in the first instance and by a rapid drop in hemoglobin with a rise in reticulocyte count in the second. The amount of red cell aldolase released through hemolysis is insufficient to account for this rise, nor is aldolase itself hemolytic in vitro. Hemolysis may be the result of another or combination of other tissue substances acting directly on the red cells or indirectly by releasing potential plasma lysins. Leroy and Spurrrier (27) have shown that high concentrations of β-glucuronidase are hemolytic in vitro, having characteristics similar to tissue lysins. They found that, when red cells were sensitized immunologically, β-glucuronidase became hemolytic in physiological concentrations. Therefore, a number of factors of different pathogenesis may combine to cause hemolysis.

**Absence of vital substances.**—Cancer may cause death by utilizing substances vital to the host which are not sufficiently replenished in the diet (8). Accelerated red cell destruction may reflect the lack in the circulating plasma of one or more such substances which may be important for erythrocyte survival and perhaps may also be responsible for the rejuvenation of stored red cells when transfused back into active circulation (17).

In the many patients dying of cancer where massive hemorrhage, infection, or destruction of vital organs does not take place, the direct cause of death still remains unknown. Since host survival correlates poorly with both histology and extent of tumor, resistance factors must be important. The erythrocyte is a host cell. Knowledge of what causes progressive decrease in the viability of this cell at the time of rapid clinical deterioration might help us understand how the last barriers of host resistance are destroyed.

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**SUMMARY**

Serial red cell survival studies were performed on four patients with metastatic carcinoma of the breast and on one patient with malignant melanoma. The life span of the donor erythrocyte was initially normal in the patients with carcinoma of the breast, but an anemia with a hemolytic component appeared in each case within 1 month of death. In the patient with malignant melanoma, who was also receiving x-ray therapy, the red cell survival was shortened during the entire last 9 months of life. When these studies were correlated with the course of the disease as evaluated by symptoms, serum and urinary calcium values, and, in one case, serum aldolase activity, the degree of hemolysis seemed to parallel the systemic effects of malignancy. The possible etiologies of this hemolytic phenomenon were discussed.

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Hemolytic Anemia, a Host Response to Malignancy

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