Erythrocyte Glycolysis in Patients with Malignant Neoplasms and Other Chronic Diseases

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MANY LABORATORIES, including ours, have demonstrated the presence of occult hemolysis in certain patients with cancer.[1] In these patients, the routine hematologic tests are usually normal and hemolysis is shown only by actual study of the red cell life span by the Ashby, chromium^{51}, or other red blood cell labeling technic.

It has been known for many years, that most of the in vitro glycolytic activity of whole blood is due to erythrocytes.[2] Several studies have demonstrated that in vitro glucose utilization of reticulocytes is greater than that of mature red blood cells.[3, 4, 5] In various hemolytic conditions, there is an increase in glycolytic rate of whole blood which appears to be related to an increase in *young* cells, reticulated as well as nonreticulated.[5]

The present report deals with a study of erythrocyte glycolysis in patients with various malignant neoplasms and other chronic diseases, in whom there was no elevation of reticulocyte count.

MATERIALS AND METHODS

About 35 ml. of venous blood was drawn with minimal stasis from various patients and healthy volunteers. No attempt was made to collect the blood in a fasting state, in fact, most bloods were drawn about 30–45 minutes after breakfast. The blood was collected in a sterile syringe wet with a small amount of liquid heparin (5,000 u/ml.). If the red blood cell: white blood cell ratio (Rbc:Wbc ratio) was equal to or more than 500:1, that is, for example 5,000,000 Rbc/mm.^{3} to <10,000 Wbc/mm.^{3}, the sample was used without further alterations (*vide infra*). When the Rbc:Wbc ratio was less than 500:1, the sample was spun for 20 minutes at 400 g in a cold centrifuge (0 C.), the plasma aspirated and saved, and the buffy layer removed. The packed red cells were then resuspended in their own plasma, gently mixed, and recentrifuged. After the second centrifugation, separation, removal of remaining buffy coat and reconstitution with plasma, the blood samples usually had a Rbc:Wbc ratio above 500:1. If the minimum optimal ratio was not reached after this separation, the results were excluded from the study. The reticulocyte count, if initially below 1.5%, was little altered by this procedure.

Either the original or the reconstituted blood sample from one patient was then divided into two aliquots of about 5 and 11 ml. each. The smaller portion was used to determine in duplicate hemoglobin,[6] hematocrit,[7] red and white blood cell counts,[8] and reticulocyte counts.[5] The larger portion was placed into a 50 ml. Erlenmeyer flask, incubated in a water bath at 37.5 C. under an atmosphere of 5%CO_2 and 95%O_2, and agitated at a rate of 88 cycles per minute.

Blood sugar was measured in duplicate before incubation and at hourly intervals for three hours by the use of Dreywood's anthrone reagent. For the purposes of this study, the

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anthrone method as described by Roe[9] was modified using 9 ml. of 1.35*N* perchloric acid as the precipitant with 1 ml. of whole blood. In addition, after boiling 1 ml. of filtrate with 10 ml. anthrone reagent for 15 minutes in a metal water bath admitting minimal light, we cooled the solutions for 20 minutes in a tap-water bath, covered to keep out light. The solutions, prepared in Coleman tubes, were then read in a Coleman 14, using 620 mu filter immediately after removal from the cooling bath.

For the final analysis of data, glucose consumption rates were calculated from the linear slopes and glycolysis was expressed as mg.% carbohydrate consumed per hour with the red blood cell count corrected to 5 million red blood cells per cu. mm. (mg.% glucose consumed/hr. with 5 × 10^6 Rbc/cu. mm.), although comparable results were obtained when calculated on a hematocrit basis.* Only bloods showing an initial reticulocyte count of 1.5% or *less* were used for the purposes of this study.

Results and Discussion

Preliminary Studies. A number of preliminary studies were performed to validate our technic and confirm observations of others. It was found that:

1) Heparin has no effect on glycolysis, whereas glycolysis is depressed by oxalate[4, 10-12];

2) The initial level of blood sugar, if below 500 mg./ml., has no effect on the glycolysis of the red blood cells, whereas higher values inhibit glycolysis;[4, 13] with very low blood sugar levels, measurement of erythrocyte glycolysis is difficult, glycolysis, however, continues until all glucose is utilized;

3) Plasma alone has no significant glycolytic activity[12, 14];

4) The concentration of red blood cells per se has no effect on glycolysis except that dilution with saline rather than plasma reduces glycolysis;[4]

5) Gentle centrifugation in a cold centrifuge at 0 C. and 400 g for 20 minutes and the procedure for separation of the white blood cells per se have no effect on glycolysis, while vigorous centrifugation at 800 g in a non-refrigerated centrifuge markedly reduced glycolysis[10];

6) For purposes of this study the values obtained by the anthrone procedure, which in essence measures carbohydrates, are comparable with results obtained by the Somogyi method[15];

7) As can be seen in figure 1, when the Rbc:Wbc ratio is raised (i.e. altered

* *Example:* Patient D. M. #231 Dx P.A. Illustrating calculations as well as absence of effect of separating white blood cells when initial Rbc/Wbc ratio is >500.

	Original Blood	"Reconstituted" Blood
Red blood cell count	4, 17 × 10^6/cu. mm.	3, 95 × 10^6/cu. mm.
White blood cell count	7,650/cu. mm.	1,800/cu. mm.
Rbc/Wbc ratio	545	2,194
Reticulocyte count	1.4%	1.0%
Initial blood sugar	120 mg%	118 mg%
1 hr. specimen	105 mg%	103 mg%
2 hr. specimen	85 mg%	87 mg%
3 hr. specimen	72 mg%	74 mg%
Glucose consumed mg%/hr (uncorrected for red cell count)	16.0 mg%	14.7 mg%
Glucose consumed mg%/hr (Red blood cell count corrected to 5 × 10^6 Rbc/cu. mm.)	19.2 mg%	18.6 mg%

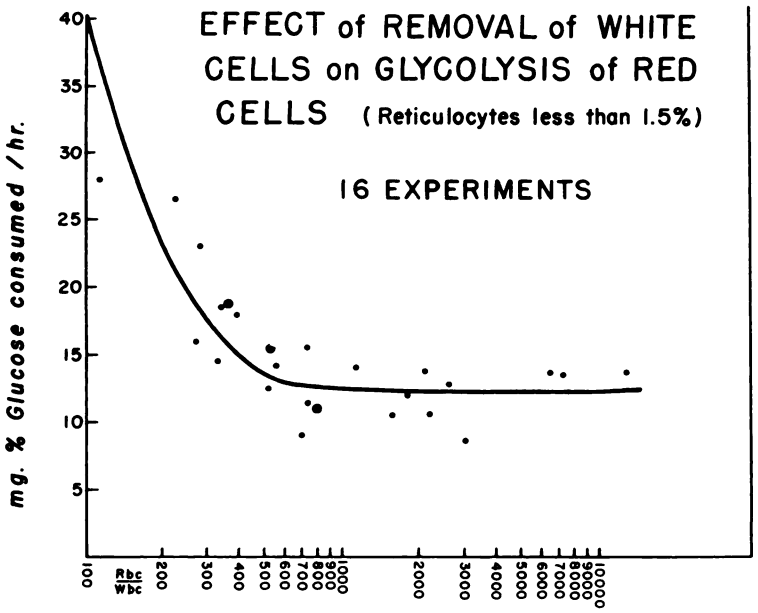

FIG. 1.—Schematic representation of the effect of removal of white blood cells on glycolysis of red cells. (Red blood cell counts corrected to 5 × 10^6 Rbc/cu.mm.)

from a very large number of white blood cells towards a small number of white cells), there is a marked decrease in glycolysis. In sixteen experiments, in which the Rbc:Wbc ratio was altered to various degrees, glycolysis was reduced significantly only if the initial Rbc:Wbc ratio was *below* 500:1. However, once the Rbc:Wbc ratio exceeds 500:1, little is gained by further separation as the small number of white blood cells contributes insignificantly to the total glycolytic rate[11-13];

8) No bacterial growth is demonstrable in cultures taken from the flasks at the end of a three hour run; and

9) There are no significant changes in pH after equilibration in 5% CO_2 and 95% O_2 compared to the specimen taken at the termination of the experiment. The most extreme pH range was from 7.35 to 7.61.

Control Subjects

Twenty-nine normal volunteers, each with a reticulocyte count below 1.5% and with an average red blood cell count of 4.9 million/cu. mm. and an average hematocrit of 44.2%, all had initial or adjusted Rbc:Wbc ratios *above* 500:1. In this group the mean glycolytic rate was 12.0 mg.% glucose consumed/hr. with 5 × 10^6 Rbc/cu. mm. with a standard deviation (S.D.) of 1.5 and a standard error (S.E.) of 0.29 (fig. 2).

Tumor Patients. Forty-three patients with biopsy proven malignant neoplasms were admissible to the study having a Rbc:Wbc ratio *above* 500:1 and a reticulocyte count *below* 1.5%. Their red blood cell counts averaged 4.1 million/cu. mm. and hematocrits 37.1%. The mean glycolytic rate of these patients was 14.5 mg.% glucose consumed/hr. with 5 × 10^6 Rbc/cu. mm. with a S.D. of 3.1 and a

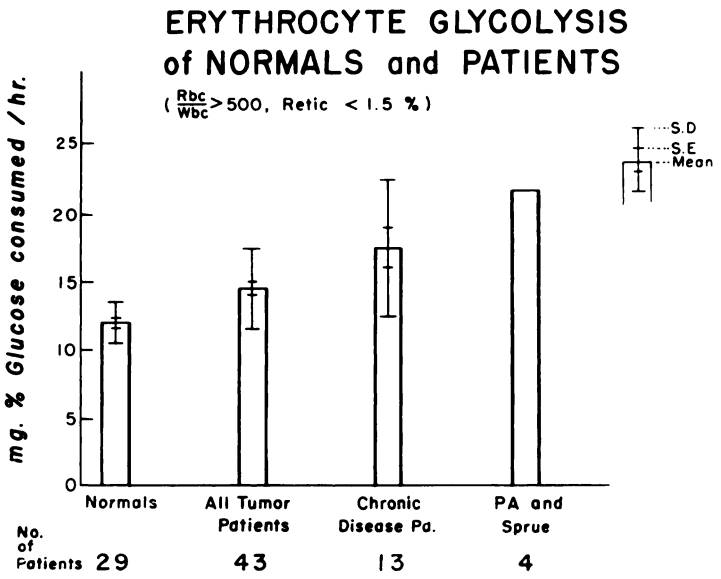

FIG. 2.—Erythrocyte glycolysis of normals and patients. (Red blood cell counts corrected to 5 × 10^6 Rbc/cu. mm).

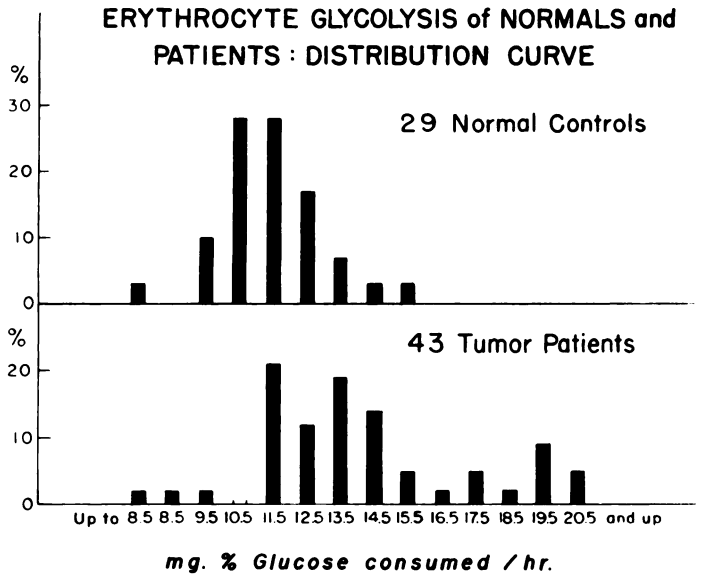

FIG. 3. Distribution curve of erythrocyte glycolysis of normals and patients. (Red blood cell counts corrected to 5 × 10^6 Rbc/cu. mm.)

S.E. of 0.47 (fig. 2). Figure 3 is a distribution curve of the red cell glycolysis values in the control subjects and in the tumor patients. About a third of the patients have a normal red cell glycolytic rate, while the others have moderately or markedly elevated red blood cell glycolytic rates. The patients having the highest erythrocyte glycolysis did not fall into any consistent category of diag-

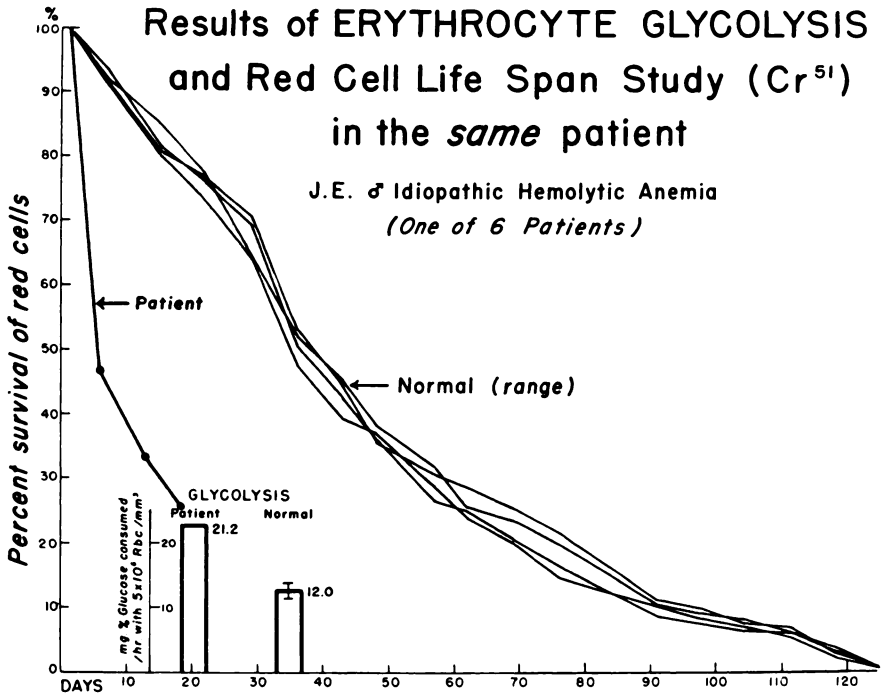

FIG. 4.—Results of erythrocyte glycolysis and red cell life span study (Cr^{51}) in the *same* patient.

nosis, were not necessarily more anemic nor the ones with the more wide-spread metastases. The difference between the mean red cell glycolytic rates of the controls and the tumor patients is statistically significant with a "t value" of 4.5; thus, "p" being smaller than 0.01.

Red cell life span determinations with chromium[51] tagged red cells[1] performed in twelve of the tumor patients showed a significantly shortened red cell survival in 8 patients with increased red cell glycolytic rates (fig. 4 and table 1, patients 1–8). In one patient (table 1, patient 12), the red cell lifespan and erythrocyte glycolysis were both normal. In the other three patients (table 1, patients 9–11), no correlation is demonstrable.

Patients with Chronic Diseases other than Tumors. Thirteen patients with various chronic diseases other than tumors and acceptable Rbc:Wbc ratios and low reticulocyte counts were studied. Among these were patients with iron deficiency anemia (3), pernicious anemia (3), sprue (1), duodenal ulcer (2) and one patient each with tuberculosis, severe heart failure, rheumatoid arthritis, and Laennec cirrhosis. The average red blood cell count was 4.0 million/cu. mm. with a hematocrit of 36.4%. The mean glycolysis was 17.4 mg% glucose consumed/hr. with 5×10^6 Rbc/cu. mm. with the S.D. 5.6 and the S.E. 1.54 (fig. 2). This is significantly different from the normal mean with a "t value" of 3.5 and "p" < 0.01. As can be seen in the separate bar-graph for the patients with pernicious anemia and sprue (fig. 2), the values obtained for erythrocyte glycolysis prior to therapy and with very low reticulocyte counts were markedly elevated.

In two patients with pernicious anemia and the one patient with sprue, sequen-

TABLE 1.—*Results of Erythrocyte Glycolysis and Red Cell Life Span Studies in Twelve Patients*

Patient	Mg% Glucose Cons./Hr. with 5×10^6 Rbc/cu. mm	Red Cell Life Span (As Per cent of Normal)
1*	21.2	30
2	19.6	43
3	19.6	45
4	17.8	71
5	15.4	71
6	14.5	71
7	13.6	62
8	13.0	67
9	13.0	91
10	12.2	62
11	11.6	77
12	11.5	100

* See also figure 4.

tial studies were performed after the administration of large doses of intramuscular vitamin B_{12}. Figure 5 summarizes the results obtained in the patient with sprue. In all three, there was an initially elevated red cell glycolytic rate even when the reticulocytes were normal. Following therapy, there was the expected reticulocytosis and a marked increase in red cell glycolysis. Of particular interest was the fact that *after* the reticulocytes returned to normal levels (below 1.5%), the glycolytic rate of the red cells remained elevated for a variable period of time.

The results of our studies of erythrocyte glycolysis in patients *with* reticulocytosis are in agreement with those reported by others[5, 10, 12, 13] showing, in general, an increase in the erythrocyte glycolysis as the percentage of reticulocytes increases (fig. 6).

Patients with various malignant tumors and others chronically ill may have an increased glycolytic rate of blood in absence of demonstrable reticulocytosis. This suggests that their red cell population may be younger than that of the normal subjects and that only some of the young cells stain with reticulocyte stain. This finding is in agreement with Hollingsworth[5] who feels that the glycolytic rate may actually give a rough index of the mean red cell age and thus, indirectly, of bone marrow activity. Of particular interest in this regard is the observation by Hollingsworth[5] of a patient with homozygous C-hemoglobin disease with only 2.5 to 3.4% reticulocytes but an erythrocyte glycolytic rate of 3 to 4 times normal. It is postulated, therefore, that the percentage of cells staining for reticulum in a population of red blood cells "may vary in different diseases depending upon the poorly understood mechanism of loss of reticulum by the cells, and the equally obscure mechanism of release of cells from the bone marrow[5]." The finding of increased erythrocyte glycolysis is compatible with the previously reported[1] increased marrow activity unassociated with reticulocytosis in cancer patients with a shortened red cell life span. It draws attention to the possibility that in these patients with shortened red cell life spans, there may be concurrent abnormal mechanisms of red cell maturation with premature release of red cells into the peripheral blood stream and early loss of reticulum substance.

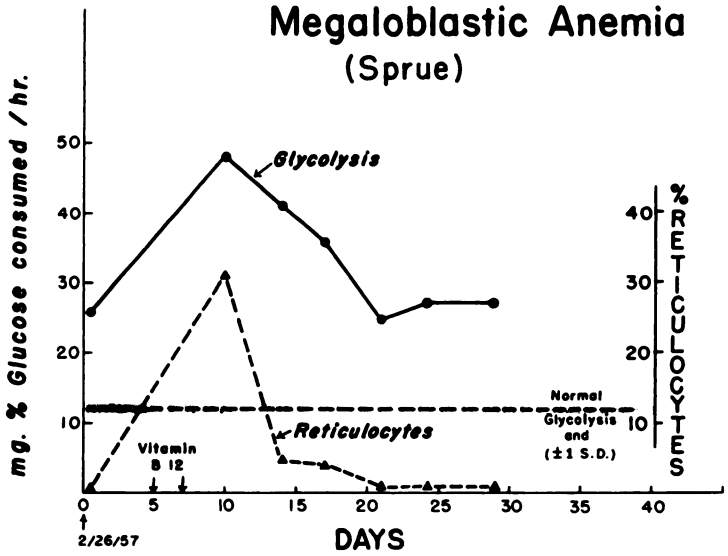

FIG. 5.—Changes in erythrocyte glycolysis and reticulocyte count in response to vitamin B_{12} therapy in a patient with sprue. (Red blood cell counts corrected to 5×10^6 Rbc/cu. mm.)

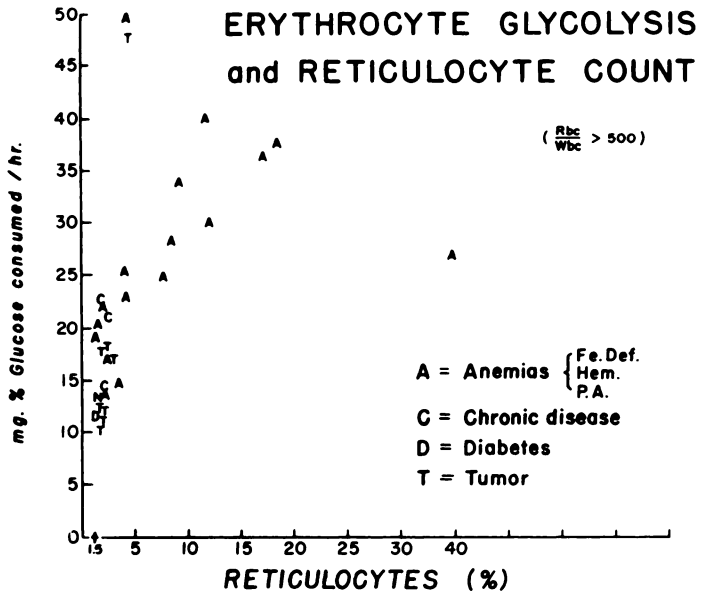

FIG. 6.—Erythrocyte glycolysis in patients with elevated reticulocyte counts. (Red blood cell counts corrected to 5×10^6 Rbc/cu. mm.)

These so-to-speak "precocious" cells may actually contribute to the abnormal rate of red cell destruction.

SUMMARY

The rate of erythrocyte glycolysis of blood from normal volunteers was 12.0 mg.% glucose consumed/hr with 5×10^6 Rbc/cu. mm. The mean glycolytic rate

of blood from tumor patients (14.5 mg.% glucose consumed/hr. with 5×10^6 Rbc/cu. mm.) and from patients with other chronic diseases (17.4 mg.% glucose consumed/hr. with 5×10^6 Rbc/cu. mm.) was found to be greater to a statistically significant degree than that of normals in the absence of reticulocytosis. This suggests that the red cell population of the patients with neoplastic and chronic diseases may be younger than that of normal subjects and that only *some* of the young cells have demonstrable reticulum.

Summario in Interlingua

Le mesura del glycolyse erythrocytic in sanguine ab voluntarios normal esseva 12,0 mg pro cento de glucosa consumite per hora con 5×10^6 erythrocytos per mm^3. Le mesura medie del glycolyse erythrocytic in sanguine ab patientes con tumores (14,5 mg pro centy de glucosa consumite per hora con 5×10^6 erythrocytos per mm^3) e ab patientes con altere morbos chronic (17,4 mg pro cento de glucosa consumite per hora con 5×10^6 erythrocytos per mm^3) se monstrava plus grande a statisticamente significative grados in comparation con le valores pro normales in le absentia de reticulocytosis. Isto pare indicar que le population de erythrocytos in patientes con morbos neoplastic e alteremente chronic es forsan plus juvene que in subjectos normal e que solmente *certes* del juvene cellulas ha un reticulo que es demonstrabile.

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