JCI The Journal of Clinical Investigation

THE METABOLISM OF HUMAN ERYTHROBLASTS

Walter Kempner

J Clin Invest. 1936;15(6):679-683. https://doi.org/10.1172/JCI100820.

Research Article

THE METABOLISM OF HUMAN ERYTHROBLASTS

By WALTER KEMPNER

(From the Department of Medicine, Duke University School of Medicine, Durham)

(Received for publication June 29, 1936)

The metabolism of human nucleated red blood cells has not previously been determined because of difficulties in procuring material. In most anemias the number of nucleated erythrocytes in the blood is so small in relation to the number of non-nucleated erythrocytes that the metabolism of the former is obscured by that of the latter. Moreover, it is impossible without damaging their very sensitive metabolic reactions to separate in sufficient amount the nucleated from the non-nucleated red blood cells and from the leukocytes.

The opportunity recently presented itself through the kindness of the Department of Pediatrics of examining at intervals of from three to five months the blood of a patient with erythroblastic anemia who had an extremely high proportion of nucleated red blood cells, 130,000 to 364,000 erythroblasts to 1.8 to 3.5 million non-nucleated erythrocytes in 1 c.mm. of blood. The patient was an eight year old boy of Greek parentage. One sister died at 2 years with symptoms similar to the patient's. Parents and a 3 year old brother are in good health. Since 1932 the patient has been given blood transfusions for his anemia regularly every few months. Splenectomy in 1933 was not followed by any evident improvement. At the time this study was begun there were 245,000 erythroblasts and 3.25 million non-nucleated red blood cells in 1 c.mm., that is, a percentage of 7 per cent of erythroblasts in his total red cell count. The percentage of reticulocytes was below 1 per cent. The leukocytes varied between 5,000 and 7,000 per c.mm.

METHOD

To determine the exact metabolism of animal cells in vitro it is imperative to avoid injury and changes in the physiological environment by physical and chemical factors. Allowing the blood to stand (anaerobiosis), cooling, centrifuging, washing with salt solutions, dilution, substitutions for the normal plasma constituents, changes in pH, in O_2 and CO_2 tension, etc., must be avoided. The error due to neglect of these precautions does not merely consist in the fact that the absolute metabolic values obtained are incorrect; respiration is also qualitatively changed, as for example, in its effect upon lactic acid fermentation or in the way it is influenced by HCN or CO. In dealing with sensitive cells, therefore, the metabolism must be measured in the unchanged blood, plasma, exudate, etc., though this makes the experiment more difficult, and as soon as possible after the cells are taken from the body.

The erythroblastic blood was taken in heparin (5 mgm. in 20 cc.) from the cubital vein, immediately saturated at 37.5° C. with a mixture of 5 per cent CO_2 and 20 per cent O_2, shaken gently with glass beads for five minutes and filtered through gauze. Then, avoiding cooling of the blood, 6 cc., 3 cc. and 3 cc. respectively were pipetted into three Warburg manometer vessels (plain rectangular vessels with side bulbs of 17 cc. capacity) (1). The side bulbs of the vessels contained 0.2 cc. of an M/40 solution of lactic acid, the exact concentration of which was determined manometrically. While shaking at 150 oscillations per minute, the vessels in the thermostat were again saturated at 37.5° with a mixture of 5 per cent CO_2 and 20 per cent O_2. Then the determination was begun in the aerobic manometer vessels 1 and 2. To obtain entirely anaerobic conditions vessel 3, containing 3 cc. of blood, was saturated for ten minutes with 2 liters of a mixture of 5 per cent CO_2 in CO (2), since in erythrocytes a saturation with 5 per cent CO_2 in nitrogen is not sufficient to produce complete anaerobiosis, because of the great amount of oxygen in combination with the hemoglobin. After the experiment the retention of lactic acid was determined (3) by tipping the 0.2 cc. lactic acid solution containing 112 c.mm. = 0.45 mgm. lactic acid, from the side bulb of the vessel into the blood. The retention of carbon dioxide was determined in a special vessel which contained 2 cc. of blood in the central space and 2 cc. of bicar-

bonate-Ringer solution in the outer space, by tipping the same amount of lactic acid into the bicarbonate-Ringer solution.

The metabolism, that is, O_2 consumption, CO_2 formation, aerobic and anaerobic lactic acid formation (glycolysis) was calculated according to the Warburg formulae (1). The dry weight was determined by Peschel's (4) method after centrifuging in special centrifuge tubes appropriate volumes of blood and drying at 100° C.

RESULTS

Table I shows the metabolism of the blood of a normal six year old child and of the blood of the patient with erythroblastic anemia. Calculated per milligram of dry weight of cells, the respiration of the erythroblastic blood is 19 times as great, the anaerobic glycolysis 7 times as great and the rate of aerobic glycolysis about 4 times as great as in normal blood. The ratio of aerobic glycolysis to respiration is 2.3 in erythroblastic blood and 10 in normal blood. This means that not only are the absolute metabolic values of respiration and glycolysis considerably greater, but through the greater increase in respiration the ratio of splitting metabolism to respiration metabolism in the erythroblasts has shifted decidedly in favor of the respiration metabolism.

TABLE I

Metabolism of erythroblastic blood and of normal blood

	I Q_{O_2} c.mm. oxygen consumed in 1 hour	II $Q_M^{O_2}$ c.mm. lactic acid formed under aerobic conditions in 1 hour *	III Q_M^{CO} c.mm. lactic acid formed under anaerobic conditions in 1 hour *	IV Inhibition of lactic acid formation by oxygen	V Aerobic lactic acid formation: respiration $\frac{II}{I}$
				per cent	
By 1 mgm. blood cells of the patient with erythroblastic anemia	0.94	2.17	4.08	47	2.3
By 1 mgm. blood cells of a normal 6 year old child	0.05	0.5	0.58	13.8	10

* 1 c.mm. = 0.004 mgm. lactic acid.

From the ratio of the metabolism figures of erythroblastic blood to those of normal blood, with the same number of leukocytes and thrombocytes one can obtain an approximate estimate of the metabolism of the erythroblasts themselves as distinguished from the metabolism of normal red blood cells (Table II). It must be remembered, however, that blood containing numerous young non-nucleated erythrocytes has a greater respiration rate than normal blood, as Warburg (5) demonstrated in the blood of young rabbits and Morawitz et al. (6, 7) in the blood of anemic patients with marked blood regeneration.

TABLE II

Calculation of the metabolism figures for 1 mgm. erythroblasts

	I Q_{O_2} c.mm. oxygen consumed in 1 hour	II $Q_M^{O_2}$ c.mm. lactic acid formed under aerobic conditions in 1 hour *	III Q_M^{CO} c.mm. lactic acid formed under anaerobic conditions in 1 hour *	IV Inhibition of lactic acid formation by oxygen	V Aerobic lactic acid formation: respiration $\frac{II}{I}$
				per cent	
1 mgm. blood cells of patient with erythroblastic anemia (7 per cent nucleated red cells and 93 per cent normal blood cells)	0.940	2.17	4.08	47	2.3
minus					
0.93 mgm. normal blood cells (93 per cent of the cells of erythroblastic blood)	0.047	0.47	0.54	13.8	10
0.07 mgm. erythroblasts (7 per cent of the cells of erythroblastic blood)	0.893	1.70	3.54		
1 mgm. erythroblasts	12.8	24.3	50.6	52	1.9

* 1 c.mm. = 0.004 mgm. lactic acid.

Damblé (8) repeated the experiments of Morawitz with the newer Warburg methods, determining quantitatively the oxygen consumption of anemic blood in different forms of anemia and comparing it with the number of reticulocytes. In pernicious anemia and in secondary anemias with a non-reactive bone marrow he found the respiration of the erythrocytes less than in normal blood, whereas in secondary anemias with reactive bone marrow and high reticulocyte count the respiration was markedly increased. The highest value, an oxygen consumption for erythrocytes two and a half times that of normal blood, he found in a case of posthemorrhagic anemia in a young patient in the stage of blood regeneration, with a reticulocyte count of 10.2 per cent (erythrocytes 2.5 million, hemoglobin 51 per cent).

With a decreasing content of reticulocytes the respiration returned to normal.

The anaerobic glycolysis of the erythrocytes of anemic patients was quantitatively determined for the first time by W. Burger (2). He found the anaerobic lactic acid formation increased in about 45 per cent of his anemic patients compared with that of the erythrocytes of normal people. In one patient with pernicious anemia he found the anaerobic blood glycolysis increased four times. Unfortunately, counts for reticulocytes and for nucleated red blood cells are not given so that no relationship can be established between the number of nucleated red blood cells and reticulocytes and the glycolytic activity of the blood.

Since our patient, in contrast to most of the cases of erythroblastic anemia described in the literature (9, 10), showed no increase of reticulocytes in the blood, the reticulocyte count at the time of the experiment being below 1 per cent, we have no reason to use the metabolism figures of anemic blood for the metabolism of his non-nucleated blood corpuscles, but the calculations must be based on the metabolism figures of normal non-nucleated blood cells. The respiration of erythroblasts, however, is of such a different order of magnitude compared to the respiration of non-nucleated red blood cells, including those of regenerative anemic blood, that even if one used for basic figures twice the highest respiration rate found in anemias, with a large number of reticulocytes, the respiration value of the erythroblasts would not be appreciably decreased.

Table II shows the calculation of the metabolism figures for 0.07 mgm. of erythroblasts. In 1 mgm. of erythroblasts an oxygen consumption of 12.8 c.mm. per hour results, aerobic glycolysis of 24.3 c.mm. (= 0.098 mgm. lactic acid) and anaerobic glycolysis of 50.6 c.mm. (= 0.203 mgm. lactic acid). The ratio of respiration to aerobic glycolysis, which is 1 : 10 in normal blood, is here 1 : 1.9. If one applies the highest respiration rates found by Damblé in severe anemias with 10 per cent of the reticulocytes, instead of the respiration rates of normal non-nucleated blood cells and, as stated before, there is no reason to do this in the case of our patient since he has a normal reticulocyte count, one would find a QO_2 of 11.5 for the respiration of erythroblasts instead of a QO_2 of 12.8. If one applies five times the respiration rate of normal blood cells which is twice the highest figure found in anemic blood, the respiration rate of erythroblasts would only be changed from 12.8 to 10.1.

The metabolism rates of human erythroblasts as compared with those of human leukocytes (4, 11) and of the nucleated red blood corpuscles of geese and alligators, which we examined by the same method at 37.5° C. in their own plasma are given in Table III. Similar values for the metabolism of blood cells of geese in bicarbonate-Ringer solution and for the oxygen consumption of alligator blood cells were found by Negelein (12) and Tipton (13). The respiration of human erythroblasts is seen to be 45 times greater than the respiration of the nucleated red blood corpuscles of alligators; about 24 times greater than the respiration of red blood cells of normal geese and about 12 times greater than the respiration of the erythroblasts of anemic geese. The metabolism of leukemic lymphocytes is considerably less than that of the erythroblastic cells. The ratio of aerobic glycolysis to respiration is in human erythroblasts 1.92, in human exudate leukocytes 0.74, in human leukemic leukocytes 0. The anaerobic glycolysis of human erythroblasts is 26 to 100 times greater than that of the nucleated red blood cells of geese and alligators and nearly

TABLE III

Metabolism of nucleated blood cells

Cell species	I Q_{O_2} c.mm. oxygen consumed in 1 hour	II $Q_M^{O_2}$ c.mm. lactic acid formed under aerobic conditions in 1 hour *	III Q_M^{CO} c.mm. lactic acid formed under anaerobic conditions in 1 hour *	IV Inhibition of lactic acid formation by oxygen	V Aerobic lactic acid formation: respiration $\frac{II}{I}$
				per cent	
Human erythroblasts...	12.8	24.3	50.6	52	1.92
Human exudate leukocytes (11).....	22.8	16.8	57.8	71	0.74
Human leukemic lymphocytes (4).....	5.8	0	11.1	100	0
Red blood cells of normal geese.....	0.54	0	0.51	100	0
Red blood cells of anemic geese.....	1.06	0.16	1.9	92	0.15
Red blood cells of alligators.....	0.28	0.11	0.65	83	0.39

* 1 c.mm. = 0.004 mgm. lactic acid.

as great as the anaerobic glycolysis of leukocytes in inflammatory exudates.

In Table IV is given a comparison of the metabolism of erythroblasts with the metabolism of animal tissue cells. Human erythroblasts are among the cells with the highest anaerobic glycolysis, being of about the same order of magnitude as the glycolysis of embryonic tissue.

TABLE IV

Metabolism figures for human and animal tissue cells

Cell species	I Q_{O_2} c.mm. oxygen consumed in 1 hour	II $Q_M^{N_2}$ or CO c.mm. lactic acid formed under anaerobic conditions in 1 hour *
Human erythroblasts	12.8	50.6
Smooth muscle of human stomach (14)	1.3	2.3
Adrenals of guinea pig (15)	6	3
Human liver (embryo 4th month) (16)	6.3	9.7
Human kidney (cortex) (16) (17)	10	10.8
Human malignant tumor (1)	5	28
Spermatozoa of steer (18)	9	28
Rat embryo (0.9 to 3.1 mgm.) (19)	12	25
Embryonic chicken heart (4 days old) (20)	30	52
Rat retina (21) (22)	31	88

* 1 c.mm. = 0.004 mgm. lactic acid.

SUMMARY

The metabolism of human nucleated red blood cells has been measured manometrically in the blood of a patient with erythroblastic anemia. The erythroblasts show very high oxidative and fermentative metabolism. The respiration is approximately 200 times greater than that of normal non-nucleated human red blood cells (12.8:0.05), 100 times greater than the respiration of blood cells of anemic patients with a great number of reticulocytes (12.8:0.12), and about 20 times greater than that of the nucleated red blood cells of geese (12.8:0.54). In erythroblasts, as in human inflammatory exudates, in leukocytes and in non-nucleated red blood cells, respiration is not sufficient to cause the lactic acid formed to disappear, but the ratio of respiration metabolism to glycolytic metabolism as compared to that of non-nucleated red blood cells, is shifted decidedly in favor of the respiration metabolism (1:1.9 as against 1:10).

The anaerobic lactic acid formation of erythroblasts is about 90 to 100 times greater than that of normal human blood cells and of normal erythrocytes of geese (50.6:0.58 and 0.51), and 25 times greater than that of erythrocytes of geese with marked anemia. The anaerobic lactic acid formation is of the same order of magnitude as that found in youngest embryonic tissue.

BIBLIOGRAPHY

1. Warburg, O., Über den Stoffwechsel der Tumoren. J. Springer, Berlin, 1926.
2. Burger, W., Über Glykolyse der roten Blutkörperchen. Arch. f. exper. Path. u. Pharmakol., 1930, **150**, 298.
3. Warburg, O., Kubowitz, F., and Christian, W., Über die Wirkung von Phenylhydrazin und Phenylhydroxylamin auf den Stoffwechsel der roten Blutzellen. Biochem. Ztschr., 1931, **242**, 170.
4. Peschel, E., Stoffwechsel leukämischer Lymphocyten. Klin. Wchnschr., 1930, **9**, 1061.
5. Warburg, O., Zur Biologie der roten Blutzellen. Ztschr. f. physiol. Chem., 1909, **59**, 112.
6. Masing, E., and Morawitz, P., Höhenklima und Blutbildung. Deutsches Arch. f. klin. Med., 1910, **98**, 301.
7. Morawitz, P., and Itami, S., Klinische Untersuchungen über Blutregeneration. Deutsches Arch. f. klin. Med., 1910, **100**, 191.
8. Damblé, K., Über die Atmung der roten Blutkörperchen bei Anämie. Ztschr. f. d. ges. exper. Med., 1933, **86**, 595.
9. Baty, J. M., Blackfan, K. D., and Diamond, L. K., Blood studies in infants and in children. I. Erythroblastic anemia. A clinical and pathologic study. Am. J. Dis. Child., 1932, **43**, 666.
10. Cooley, T. B., and Lee, P., Erythroblastic anemia; additional comments. Am. J. Dis. Child., 1932, **43**, 705.
11. Kempner, W., and Peschel, E., Stoffwechsel der Entzündung. Ztschr. f. klin. Med., 1930, **114**, 439.
12. Negelein, E., Versuche über Glykolyse. Biochem. Ztschr., 1925, **158**, 121.
13. Tipton, S. R., Factors affecting the respiration of vertebrate red blood cells. J. Cell. and Comp. Physiol., 1933, **3**, 313.
14. Rosenthal, O., and Lasnitzki, A., Über den Stoffwechsel stationärer und wachsender Gewebe. Biochem. Ztschr., 1928, **196**, 340.
15. Fujita, A., Über den Stoffwechsel der Körperzellen. Biochem. Ztschr., 1928, **197**, 175.
16. Krebs, H. A., Grösse der Atmung und Gärung in lebenden Zellen. Handbuch d. Biochem., 1933, Erg. **IB.**, 866.

17. György, P., Keller, W., and Brehme, Th., Nierenstoffwechsel und Nierenentwicklung. Biochem. Ztschr., 1928, **200**, 356.
18. Redenz, E., Über den Spaltungsstoffwechsel der Säugetierspermatozoen im Zusammenhang mit der Beweglichkeit. Biochem. Ztschr., 1933, **257**, 234.
19. Negelein, E., Über die glykolytische Wirkung des embryonalen Gewebes. Biochem. Ztschr., 1925, **165**, 122.
20. Warburg, O., and Kubowitz, F., Stoffwechsel wachsender Zellen (Fibroblasten, Herz, Chorion). Biochem. Ztschr., 1927, **189**, 242.
21. Warburg, O., Posener, K., and Negelein, E., Über den Stoffwechsel der Carcinomzelle. Biochem. Ztschr., 1924, **152**, 309.
22. Sellei, C., Weinstein, P., and Jány, J., Weitere Studien über den Stoffwechsel der Zelle. Biochem. Ztschr., 1932, **247**, 146.