QUANTITATIVE ASPECTS OF RESPIRATION AND GLYCOLYSIS IN THE WHITE CELLS OF HUMAN BLOOD

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In our previous investigations we have studied in detail the processes of energy exchange in the white cells of human blood [2, 3]. It was found that the energy exchange of the granulocytes is distinguished by three characteristic features: aerobic glycolysis, a reverse Pasteur reaction (inhibition of respiration by glycolysis) and complete resynthesis of adenosinetriphosphate (ATP) in both aerobic and anaerobic conditions. All these three metabolic features have previously been found in cancer cells [1]. In this way it was found that there was a qualitative resemblance between leucocytes and malignant tumors in certain important processes of cell metabolism.

In both his early and recent work on cancer, Warburg regards the respiration of cancer cells as "incomplete" in both the qualitative and quantitative sense, and attaches particular importance to the relationship between glycolysis and respiration. According to Warburg, for neoplastic tissues the ratio $Q^{O_2}_{CO_2}/Q_{O_2} = 3\text{-}4$, whereas for normal tissues (including growing tissues in which there is aerobic glycolysis) it does not exceed 1 [4].

Since the leucocytes showed, as pointed out above, such a profound similarity with malignant tumors in a number of fundamental processes of energy exchange, it appeared interesting to make an accurate quantitative analysis of the signs of qualitative resemblance of leucocytes and malignant cells which have been observed. Under these circumstances particular attention would have to be paid to the metabolic characteristics which are regarded by Warburg as specific for neoplastic tissues. For this purpose we determined the absolute values of respiration and glycolysis in the leucocytes in optimal conditions for these two processes, and calculated the ratio between the activities of respiration and glycolysis in each type of white blood cell.

EXPERIMENTAL METHOD

Human white blood cells, practically free from red cells and platelets, were suspended in blood serum at 37°C in the vessels of a Warburg apparatus. Respiration was recorded manometrically – glycolysis photometrically with p-hydroxydiphenyl. In order to detect the respiratory and glycolytic activity of the cells in optimal conditions, in our present investigation in contrast to the previous ones, we used suspensions with smaller concentrations of cells – 0.036 ml of leucocytes per 1 ml of experimental suspension (alongside the previously used concentrations). In this way maximum relative velocities of respiration and glycolysis were attained. Special experiments showed that the relative velocities of these two processes increase with increasing dilution of the leucocyte suspensions, reaching a stable maximum value of activity at cell concentrations of 0.04-0.05 ml per 1 ml of experimental suspension.

EXPERIMENTAL RESULTS

In the Table are given the results of the study of the rates of respiration and glycolysis in the different groups of human white blood cells in normal and some pathological conditions. The respiratory and glycolytic activities

TABLE

Respiration, Glycolysis and Their Ratio in Various Forms of Human Leucocytes

Leucocytes	0.03 ml of cells per 1 ml of experimental suspension					0.1 ml of cells per 1 ml of experimental suspension				
	$Q^{glucose}_{O_2}$	$Q^{air}_{CO_2}$	$Q^{N_2}_{CO_2}$	$Q^{air}_{CO_2} / Q_{O_2}$	$Q^{N_2}_{CO_2} / Q_{O_2}$	$Q^{glucose}_{O_2}$	$Q^{air}_{CO_2}$	$Q^{N_2}_{CO_2}$	$Q^{air}_{CO_2} / Q_{O_2}$	$Q^{N_2}_{CO_2} / Q_{O_2}$
Donors'	5,7±0,3	18,8±1,0	31±1,1	3,3	5,5	2,6±0,1	6,0±0,5	8,8±0,6	1,8	3,4
Chronic myeloid leukemia	5,0±0,3	13,2±0,7	23,3±0,8	2,6	4,7	3,1±0,2	5,6±0,2	8,9±0,8	1,8	2,9
Chronic lymphatic leukemia	7,9±0,5	0	27,2±1,0	—	3,4	6,6±0,3	0	11,1±0,8	—	1,7

are expressed as Warburg coefficients: Q_{O_2}, $Q^{O_2}_{CO_2}$, $Q^{N_2}_{CO_2}$ (in mm^3 gas per 1 mg dry weight of cells per hr).

It follows, first, from the Table that the metabolic indices depend to a large extent on the concentration of cells per unit volume of experimental suspension. In suspensions with a concentration of 0.03 ml of cells per 1 ml of experimental suspension, the respiration and glycolysis are as a rule significantly higher than in a concentration of cells of 0.1 ml per 1 ml of experimental suspension. The respiration of lymphocytes constitutes an exception to this for it changes only a little with changes in the cell concentration (Q_{O_2} equal to 7.9 and 6.6).

The figures given in the Table of respiration and glycolysis of leucocytes in optimal conditions (low concentration of cells, serum at 37°C) clearly show one of the most important laws of metabolism of the white blood cells – comparatively low respiration and very high glycolysis. This holds good even for aerobic cells – lymphocytes – in which, despite the absence of lactic acid formation in aerobic conditions, the anaerobic glycolysis is very high.

Normal donors' leucocytes absorb on the average 5.7 mm^3 O_2 calculated per 1 mg dry weight of cells per hour. At the same time they excreted under anaerobic conditions a quantity of lactic acid equivalent to 31 mm^3 CO_2 per 1 mg dry weight per hour. In aerobic conditions 18.8 mm^3 CO_2 was given off. Corresponding figures obtained for white blood cells of patients with chronic myeloid leukemia showed indices of respiration and glycolysis which were close to those of normal leucocytes: $Q_{O_2} = 5$; $Q^{air}_{CO_2} = 13.2$; $Q^{N_2}_{CO_2} = 23.3$. Calculation of the ratio $Q^{N_2}_{CO_2} / Q_{O_2}$ and $Q^{air}_{CO_2} / Q_{O_2}$ for normal leucocytes gives values of 5.5 and 3.3; for leucocytes of patients with chronic myeloid leukemia these figures were 4.7 and 2.6 respectively, and for lymphocytes, in which aerobic glycolysis does not occur and for which only the first coefficient may be calculated, the ratio $Q^{N_2}_{CO_2} / Q_{O_2}$ is 3.4.

The figures obtained acquire considerable importance when they are compared with the corresponding indices for cancer tissues. According to Warburg and his co-workers [4], epithelial carcinomas in man are characterized by the following values for respiration and glycolysis: $Q^{N_2}_{CO_2} = 41$; $Q^{O_2}_{CO_2} = 28$; $Q_{O_2} = 10.2$ (with corrections for "contamination" with connective tissue cells). For human sarcoma the same authors quote the following figures: $Q_{CO_2}^{N_2} = 35$; $Q_{CO_2}^{O_2} = 19.5$; $Q_{O_2} = 6.1$ (also calculated per 100% of malignant cells). As will be seen, the metabolic indices are of the same order as those which we obtained for human leucocytes.

In his very first papers on metabolism of malignant tumors, Warburg attached particular importance to the ratio $Q_{CO_2}^{O_2}/Q_{O_2}$, considering that a value of 3-4 for this coefficient was specific for a neoplasm. For the results given in the Table, it follows that this requirement is satisfied not only by malignant tumors but also by the white blood cells of a healthy person, in which the ratio $Q_{CO_2}^{air}/Q_{O_2} = 3.3$. In later papers on cancer [5], Warburg stresses the importance of the maximum values of glycolysis of tumors, when comparing the respiration of malignant cells with their anaerobic glycolysis. If the ratio of anaerobic glycolysis to respiration is calculated for Warburg's experiments with human carcinomas and sarcomas, the corresponding figures obtained are 5 and 5.8. As may be seen from the Table, the ratio $Q_{CO_2}^{N_2}/Q_{O_2}$ for human leucocytes is 5.5. Consequently there is no difference between malignant tumors and leucocytes in this ratio also. For ascitic carcinoma cells of mice, which Warburg regards as a classic object for biochemical tumor research, he obtained the following metabolic characteristics: $Q_{O_2} = 5-10$; $Q_{CO_2}^{O_2} = 25-35$; $Q_{CO_2}^{N_2} = 50-70$. The coefficient $Q_{CO_2}^{N_2}/Q_{O_2}$ in this case is approximately 8, with possible variations on either side. The last figures, however, are not fully indicative, since they relate to mouse cells and not to human cells, and cannot be used in calculations to make comparisons with human leucocytes.

Simple comparison of the findings in the Table on the respiration and glycolysis of human leucocytes with those obtained by Warburg for human malignant tumors indicates the absence of any quantitative difference between the absolute levels of respiratory and glycolytic activity of these two categories of cells, and also the absence of any difference between the ratios of the values $Q_{CO_2}^{N_2}/Q_{O_2}$ and $Q_{CO_2}^{O_2}/Q_{O_2}$.

The facts presented speak unanimously in support of the view that neither the old concept of Warburg, which rests on aerobic glycolysis, nor the new variant, based on the recognition of the specificity of low respiration and high anaerobic glycolysis for malignant tumors, can be regarded as valid in the light of these new findings.

SUMMARY

The study of the velocity of respiration and glycolysis of various forms of human leukocytes deomonstrated that these indicators of metabolism of white blood cells may be quantitatively compared with these indicators found in case of human carcinoma and sarcoma (formerly established by O. Warburg and his collaborators. Coefficients $Q_{CO_2}^{N_2}/Q_{O_2}$ and $Q_{CO_2}^{O_2}/Q_{O_2}$ were also found to be very similar in cancer cells and leukocytes. In case of leukocytes of normal blood Q_{O_2} (5.7); $Q_{CO_2}^{O_2} = 18.8$; $Q_{CO_2}^{N_2} = 31$; the ratio $Q_{CO_2}^{N_2}/Q_{O_2} = 5.5$; the ratio $Q_{CO_2}^{O_2}/Q_{O_2} =$ = 3.3.

As to the leukocytes of patients with chronic myeloid leukemia these values equalled: 5, 13, 2; 23, 3; 4.7 and 2.6, respectively. In case of lymphocytes the values of respiration and glycolysis were: $Q_{O_2} = 7.9$; $Q_{CO_2}^{O_2} = 0$; $Q_{CO_2}^{N_2} = 27.2$. The ratio $Q_{CO_2}^{N_2}/Q_{O_2} = 3.4$.

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