ENERGY METABOLISM OF CADAVER BONE MARROW OBTAINED BY COMPRESSION AND ASPIRATION

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In recent years, a great deal of attention has been paid to the problem of the transplantation of bone marrow. Transplantation of bone marrow tissue is used in the medical treatment of radiation injuries, hypoplastic and aplastic anemia, for the prophylaxis and therapy of complications connected with a depression in bone marrow hemopoeisis, for medical treatment of malignant diseases with radiant energy, or with cytostatic preparations [1, 2, 7, 10, 11, 14].

Nowadays special interest is being acquired by the transplantation of cadaver bone marrow, since the latter may be obtained in considerably greater quantities than from living donors of bone marrow. Cadaver bone marrow is prepared in various ways: by aspiration, compression, washing out of vertebrae and sternum with a preservative solution [3, 6, 12]. The advantages and disadvantages of each of these have been insufficiently studied. In experiments with animals, the functional activity of the bone marrow cells has been verified by its ability to exert a protective action during experimental radiation illness; for evaluation of the viability of human bone marrow (both donor and cadaver) other methods for determining its functional state are used. In particular, the ability of the bone marrow cells to synthesize DNA, the growth of bone marrow in tissue culture, etc., have been investigated. One of the methods for determining the functional composition of bone marrow cells may be the study of certain important biochemical indicators, beginning with the energy metabolism.

In this work we studied respiration, aerobic and anaerobic glycolysis of the cadaver bone marrow cell, and also determined the amount of adenosine triphosphate (ATP) and the content and rate of glycogen metabolism.

PROCEDURE

Cadaver bone marrow obtained by the method of aspiration and compression of vertebrae (henceforth denoted as aspiration and compression bone marrow), developed in the Laboratory of Tissue Preservation of the Leningrad Institute of Blood Transfusion, was studied [3]. Bone marrow was prepared from the cadavers of people 40-70 years of age, the cause of death of which was mainly cardiovascular diseases. The aspiration bone marrow was prepared mainly in the first 4-5 h after death; the compression bone marrow was prepared 10-12 h after death.

The bone marrow obtained by the method of aspiration usually was very diluted by peripheral blood; therefore, its cell nuclei (myelokaryocytes) were isolated by fractional centrifugation in a gelatin-citrate solution according to a procedure analogous to the method for the isolation of leukocytes from blood [5].

The compression bone marrow was a dense cellular suspension with a small amount of fat impurity, for the removal of which it was mixed with physiological saline in a 2 : 1 ratio and subjected to centrifugation for a short time. The liquid layer containing the fat was discarded. Then the myelokaryocytes obtained (both from aspiration bone marrow and also from compression bone marrow) were suspended in group IV blood serum and Kreb's ringer phosphate buffer at a pH of 7.4, which were taken in a 1 : 1 ratio; a cell count was made with 1 microliter of the obtained suspension. Versene in a final concentration of 0.1% was used to prevent coagulation of the bone marrow cells. The volume of each sample comprised 2.8 ml; the number of cells in the sample fluctuated from $0.4 \cdot 10^8$ to $1.5 \cdot 10^8$. The cellular suspension was incubated for 40 min at 37° in a Warburg apparatus. Respiration was determined manometrically, glycolysis was determined with parahydroxydiphenyl [8]. The amount of ATP was determined

TABLE 1. Respiration and Glycolysis of Cadaver Bone Marrow Myelokaryocytes

Method of obtaining myelokaryocytes	Absorption of O_2 (in $\mu l/10^9$ cells per h)	Lactic acid formation (in $\mu l/10^9$ cells per h)	
		Aerobic	Anaerobic
Aspiration	605±45(14)	9488±904(11)	18274±816(8)
Compression	426±35(15)	4980±460(13)	11472±1074(10)

Note. In both tables the numbers of experiments are indicated in parentheses. The cells were incubated in a mixture of group IV blood serum and Krebs ringer phosphate buffer at a pH of 7.4 (1:1) at 37° for 40 min.

TABLE 2. ATP Content, Amount and Rate of Respiration of Glycogen in Cadaver Bone Marrow Myelokaryocytes

Method of preparation of the myelokaryocytes	Amount of ATP (in $\mu g/10^9$ cells)	Glycogen	
		Amount (in $\mu g/10^9$ cells)	Specific radioactivity (in counts/min/mg)
Aspiration	643±58(11)	3481±±354(10)	4148±639(9)
Compression	341±34(15)	332±29(12)	5006±±482(11)

Note. Conditions of incubation were as in Table 1. C^{14}-glucose = 6 mg/2.8 ml of suspension with total radioactivity 500,000 counts/min.

in the hexokinase reaction, linked with the reaction of oxidation of glucose-6-phosphate and the simultaneous reduction of triphosphopyridine nucleotide and recorded spectrophotometrically according to the change in light absorption at 340 mμ. The glycogen was isolated after alkaline hydrolysis by precipitation with 60° alcohol [9]; a quantitative polysaccharide determination was carried out on glucose with thymol-sulfur reagent [13]. The rate of glycogen metabolism was determined according to the incorporation of C^{14} as a result of the incubation of cells with glucose uniformly labeled with C^{14}. The samples contained 6 mg of glucose-C^{14} with a total radioactivity of 500,000 counts/min.

RESULTS

In the examination of the data of Table 1, some general features of the metabolism of cadaver bone marrow may be noted independent of the method of its preparation. The moderate respiration, the high aerobic glycolysis, and the still higher glycolysis under anaerobic conditions indicates metabolic similarity of the bone marrow cells to the granulocytes of the blood, which is understandable upon consideration of the genetic relationship of their cellular elements. The Pasteur effect takes place both in the granulocytes and in the cadaver bone marrow cells: glycolysis is reduced by approximately half under aerobic conditions. The reverse Pasteur reaction (Crabtree effect) is also noted in these cells: in the presence of glucose, respiration is lower than without the addition of sugar.

At the same time, quantitative differences in the indices of metabolism between the aspiration and compression cadaver bone marrow are quite distinctly detected. Both respiration and glycolysis are higher in the bone marrow cells obtained by the aspiration method.

In the calculation of the coefficients Q_{O_2} (absorption of oxygen in microliters per mg of dry weight per hour) and Q_{CO_2} (glycolysis in equivalents of CO_2 in microliters per mg of dry weight per hour), the metabolic activity of aspiration and compression bone marrow in the presence of glucose proved to be the following: $Q_{O_2} = 4.4$, Q_{CO_2} (aerobic) = 17.4, Q_{CO_2} (anaerobic) = 33.5 for the aspiration and $Q_{O_2} = 3.1$, Q_{CO_2} (aerobic) = 9.2, Q_{CO_2} (anaerobic) = 21.2 for the compression bone marrow.

On the other hand, if these data are compared with the corresponding indices of donor leukocytes, for which $Q_{O_2} = 5.7$, Q_{CO_2} (aerobic) = 18.8, and Q_{CO_2} (anaerobic) = 31 [4], it may be noted that the myelokaryocytes of cadaver bone marrow obtained by the aspiration method are somewhat inferior to the leucocytes only in relation to the value of respiration, while that of glycolysis is completely comparable with glycolysis of the leukocytes. At the same time both respiration and aerobic and anaerobic glycolysis were considerably lower in the bone marrow cells obtained by compression.

In the evaluation of the functional state of cadaver bone marrow cells, like any other, data on the quantitative level and the rate of conversion of such compounds of importance in the energy and plastic metabolism as ATP and glycogen might be of vital significance. Table 2 presents the results of such an investigation of cadaver bone marrow.

In a comparison of the amounts of ATP and glycogen in bone marrow cells obtained by various methods, it may be noted that the ATP content in the cadaver bone marrow myelokaryocytes obtained by the compression method was about half that obtained by aspiration, while the amount of glycogen was approximately one tenth as great. Such a sharp depression in the ATP level and especially in the amount of glycogen in the myelokaryocytes in the latter case may be to a considerable degree explained by the fact that about 12 h elapse after the moment of death in the preparation of bone marrow by the compression method, due to technical difficulties. In such rare cases when the compression bone marrow is prepared 4-7 h after death, the amount of glycogen in the cells is 1075±60 micrograms/cells, i.e., it was approximately three times higher than in the later preparation. A similar dependence was noted also in regard to the change in the amount of ATP, which in the earlier preparation of compression bone marrow was close to the amount of ATP in the myelokaryocytes of bone marrow obtained by the aspiration method. It was of interest to note that in the aspiration bone marrow cells prepared 7-10 h after death on appreciable drop in the levels of ATP and glycogen in comparison with the bone marrow obtained in the first hours after death was also observed.

Thus, the poorer indices of metabolism and chemical mechanisms of the bone marrow cells obtained by the compression method indicate not so much the deficiencies of this method of preparation as much as the determining role of the time elapsed from the moment of death to the moment of the extraction of the bone marrow.

Experiments conducted with radioactive glucose-C^{14} indicated that it is intensively incorporated into the glycogen under in vitro conditions. Even after a short 40-min incubation, the specific radioactivity of the isolated polysaccharide was 4148 counts/min/mg and 5006 counts/min/mg for the compression bone marrow. These data indicate that complex, multicomponent enzyme systems participating in the synthesis and decomposition of glycogen retain their activity for several hours after death.

The data obtained permit us to conclude that bone marrow obtained by the aspiration method in the first 4-5 h after death is a functionally active tissue, since the basic indices of its energy metabolism are close to the analogous indices of donor leukocytes—the cells are very closely related. The compression bone marrow, judging by its biochemical indices, is inferior to the aspiration bone marrow, and this may be connected with the fact that it is prepared a longer time after death than is aspiration bone marrow. The indicated hypothesis is confirmed by the fact that compression bone marrow prepared 4-7 h after death has more favorable biochemical indices than 12 h after death, exhibiting a tendency to approximate those indices of aspiration bone marrow. Evidently, the time between the fourth and seventh hours after death is critical for the functional state and biochemical status of the bone marrow.

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