DEPENDENCE OF THE PHAGOCYTIC REACTION OF LEUKOCYTES ON THEIR CARBOHYDRATE METABOLISM

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N. V. Puchkov's work [1, 2] has shown that introduction of small concentrations of adrenalin into the animal's blood stream enhances the phagocytic activity of the blood. Since adrenalin is involved in the regulation of carbohydrate metabolism a study of the dependence of the phagocytic activity of the blood on the state of carbohydrate metabolism within the phagocytes was undertaken at this Department.

EXPERIMENTAL METHODS

A suspension of microbes prepared from a 1-2 day culture of Staphylococcus albus in dilution of half a billion per 1 ml was placed into a special test tube; the suspension was drawn up into a white-cell mixing pipette to the mark 1 and a similar amount of Ringer solution was added (in experiments with addition of glucose, insulin and other substances these were introduced in Ringer solution); blood taken from the finger was then added to the mixture. The test tube was then agitated carefully by rotary movement around its axis until a uniform mixture was obtained and the test tube was then placed in a thermostat for 30 minutes (phagocytic time). After 30 minutes a smear was made from the contents of the test tube and fixed in a mixture of alcohol and ether. The smears were stained by the Romanovskii method; the counts were always made along the edge of the smear on both sides. One hundred leukocytes – neutrophils – were counted. Among these, those that had behaved as phagocytes (positive) and those that had not (negative) were noted. The total number of positive leukocytes in relation to the total number counted (100) indicates the extent of phagocytosis.

It must always be borne in mind when investigating phagocytosis that the phagocytic reaction is not a constant entity and depends on many causal factors not always perceptible. In order to establish the degree of variability of this reaction under identical conditions a series of experiments was staged in which the phagocytic reaction of blood leukocytes was determined without any extraneous influences. Two test tubes were prepared simultaneously with the same blood (K_1 and K_2) for this purpose.

Twenty control experiments on 40 blood smears were carried out. The range of variation in the values was ± 8-10%. Therefore figures deviating from the control values by more than 10% could be taken as true deviation. In addition each experiment was accompanied by its own control.

EXPERIMENTAL RESULTS

Simultaneous addition of 1.6 international units insulin and 5% solution of glucose to the blood (25 experiments) leads to a sharp increase in the number of phagocytic leukocytes and also to enhanced phagocytic activity of the leukocytes which far exceeds the limits of chance variation (Fig. 1a). The average increase of the phagocytic index was 32%.

Experiments with separate addition to the blood of glucose (10 experiments) and of insulin (10 experiments) (Fig. 1, b and c) gave a directly converse result. When glucose alone was added the percentage of leukocytes showing phagocytic activity diminished by an average of 18-20%; insulin alone decreased phagocytosis to a still greater degree – by almost 25%.

Another method of affecting the carbohydrate metabolism of leukocytes was used in our experiments (14 experiments) and consisted of addition of monoiodoacetic acid in 1 : 10,000 dilution to the blood, i.e., interfering with glycolysis in the leukocytes (Fig. 2, b). It was found that abolition of glycolysis lowered phagocytosis by an average of 18-20% but did not pervent it completely.

Since lactic acid is a product of glycolysis it could be expected that its addition to phagocytes poisoned with monoiodoacetic acid would restore their normal activity. This proved to be in fact the case: when 40 mg% lactic acid was added to the blood simultaneously with the monoiodoacetic acid the phagocytic activity remained practically unchanged (Fig. 2a).

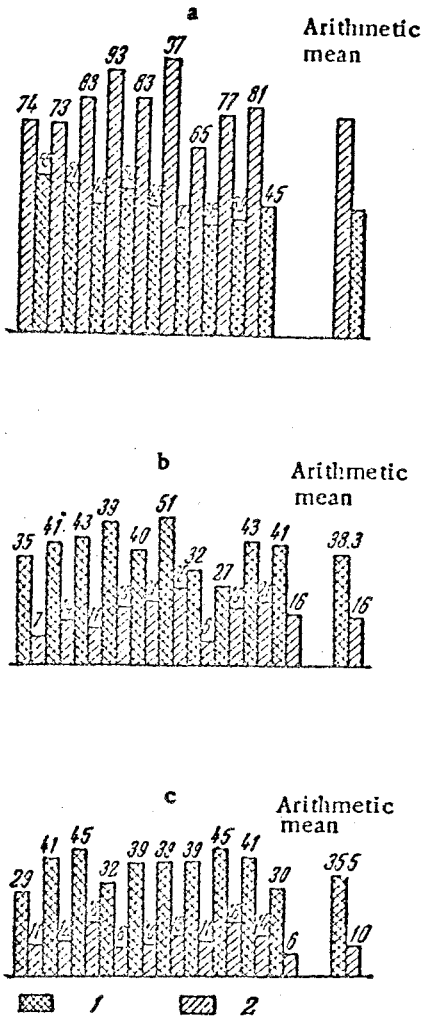

Fig. 1. Effect of insulin and glucose on phagocytosis. a) Addition of 1.6 international units insulin and 5% glucose solution; b) addition of 5% solution of glucose; c) addition of 1.6 international units insulin; 1) control; 2) experiment.

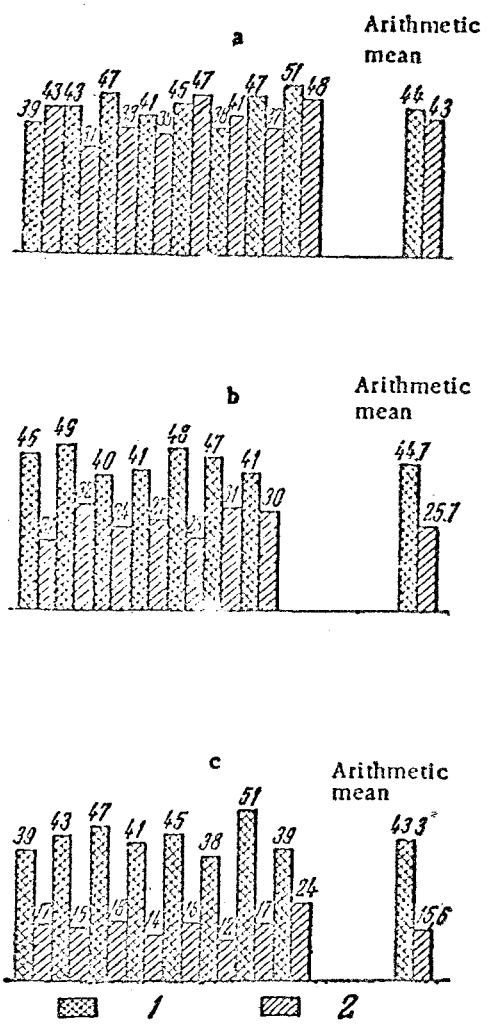

Fig. 2. Effect of monoiodoacetic acid and lactic acid on phagocytosis.
a) Addition of 40 mg% lactic acid and monoiodoacetic acid in 1::10,000 dilution; b) addition of monoiodoacetic acid in 1 :10,000 dilution; c) addition of 40 mg% lactic acid; 1) control; 2) experiment.

For the purposes of control, the effect of lactic acid alone was investigated, using the same concentration (9 experiments) (Fig. 2b). Under these conditions there was an extremely marked drop in phagocytic activity — by an average of 20%.

In order to exclude the possibility that the changes in phagocytic index obtained were determined by a shift in pH the latter was checked electrometrically. It was found that the addition to the blood of these substances in the concentrations used in our experiments left the pH practically unaltered with variations within the limits of 7.5 to 7.8.

The data presented indicate that phagocytic activity depends to a considerable extent on the carbohydrate metabolism of the phagocytes; in other words, carbohydrates are an important source of energy required for the performance of this function.

SUMMARY

Glucose and insulin added together to the media causes a considerable increase of phagocytic activity of leukocytes of the human blood. Glucose and insulin added separately decrease this activity. Elimination of glycolysis induced by the addition of monoiodoacetic acid, likewise diminishes phagocytosis; this effect is abolished by the addition of lactic acid. The phagocytic activity of leukocytes depends to a great extent on their carbohydrate metabolism.

LITERATURE CITED

[1] G. G. Golodets and N. V. Puchkov, Fiziol. Zhur. SSSR, No. 1, 135-150 (1948).

[2] N. V. Puchkov, Doklady Akad. Nauk SSSR, nov. ser., 61, No. 5, 945-948 (1948).