# RESPIRATION AND GLYCOLYSIS OF EXUDATE LEUKOCYTES FROM NORMAL RABBITS AND RABBITS WITH ALLOXAN DIABETES

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In an earlier investigation [3], the phagocytic activity of the leukocytes of an exudate was found to be weaker in animals with diabetes. To explain the mechanism of this phenomenon, the metabolism of the leukocytes was investigated during diabetes. The little information in the literature on this question is conflicting [4-6].

The object of the present investigation was to study respiration and glycolysis of the exudate leukocytes in normal conditions and in alloxan diabetes.

### EXPERIMENTAL METHOD

Experiments were carried out on 58 rabbits of both sexes weighing 1.7-2.5 kg. Diabetes was induced by intravenous injection of alloxan in a dose of 150 mg/kg body weight. The leukocytes were isolated from an exudate obtained 16 h after intraperitoneal injection of 300 ml of sterile physiological saline. In smears made from the exudate, 90% of the cells were polymorphonuclear leukocytes. The results of vital staining of the leukocytes with 1:10,000 acridine orange and subsequent luminescence microscopy, like the reaction of granule formation with the dye neutral red [2], showed that 95% of the cells were viable and functionally intact.

The incubation medium consisted of equal parts of fresh rabbit serum and phosphate buffer, pH 7.4. Glucose (200 mg%) and versene (0.1%) were added to the medium. The respiration of the leukocytes was determined manometrically in a Warburg's apparatus and lactic acid was estimated colorimetrically with p-hydroxydiphenyl.

# EXPERIMENTAL RESULTS

In a concentration of leukocytes of between  $50 \cdot 10^6$  and  $100 \cdot 10^6/\text{ml}$  no essential differences were found in the oxygen consumption and the lactate formation by the leukocytes of the diabetic and control animals (see table).

Bearing in mind data in the literature [1, 5] indicating that in leukocytes respiration and glycolysis are inversely proportional to the cell concentration (the crowding effect), respiration and glycolysis of the leukocytes were investigated in different cell concentrations. The respiration and glycolysis of the leukocytes from the healthy and diabetic rabbits were maximal when the cell concentration was minimal. With an increase in the leukocyte concentration, their respiration and glycolysis diminished in intensity in both the experimental and the control animals. In the highest cell concentration the oxygen consumption and lactate formation by the leukocytes from both the rabbits with diabetes and the normal rabbits were minimal (see table).

In relation to the oxygen consumption, the crowding effect was the same for the leukocytes of the diabetic and control animals. In relation to lactate formation this effect was weaker in the diabetic rabbits than in the controls.

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Table 1. Oxygen Consumption and Lactate Formation by Exudate Leukocytes of Rabbits with Diabetes and Control Rabbits for Different Cell Concentrations, M  $\pm$  m

	Control		Diabetes		
luekocyte count (×10 <sup>16</sup> /ml)	oxygen consum- ption (µg/10 <sup>10</sup> leukocytes/h)	lactate form. (mg/1010 leukocytes/h)	oxygen consum- ption (μg/1010 leukocytes/h)	(mg/1010	p
3,5 10 20 30 50—100	35 750±3 854 18 770±914,6 10 680±615 8 340±593,4 4 690±303	$\begin{array}{c} 160,5\pm19,3\\ 135,41\pm5,59\\ 100,2\pm7,8\\ 69,2\pm5,19\\ 40,7\pm3,33 \end{array}$		91,1±9,11 54,4±4,87 46,0±2,83	<0,001 >0,05

The discovery of this rule for the leukocytes of diabetic and healthy rabbits helped to disclose a difference between their glycolytic activity. In cell concentrations close to physiological (10<sup>7</sup> leukocytes/ml) a significant decrease in lactate formation by the leukocytes of the diabetic animals was found. Consequently, when assessing the results of investigation of the respiration and glycolysis of the leukocytes of diabetic and healthy rabbits, it is important to make allowance for the cell concentration in the suspensions investigated.

The lower glycolytic activity of the exudate leukocytes in diabetes may evidently have a significant influence on their phagocytic activity, for glycolysis is the source of energy of the leukocytes for phagocytosis.

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