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Metabolism of Leucocytes in Ringer-Phosphate and in Serum.

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Ponder and MacLeod[1, 2] described the oxidative rate of leucocytes obtained from peritoneal exudates in rabbits, but no studies of glycolysis were made, and Ringer-phosphate solution was the only medium employed. In this communication are presented the results of studies of aerobic and anaerobic glycolysis of exudate leucocytes, and a simple method by which their respiration in serum may be measured is described.

Bakker[3] stated that exudate leucocytes respired at a low rate ($QO_2 = 0.4$) but the rate of aerobic glycolysis was high ($Q_G^{O_2} = 6$). Fleischmann and Kubowitz[4] reported rates 10 times as great ($QO_2 = 4$) for the respiration of similar cells, and figures for aerobic and anaerobic glycolysis of 11 and 21 respectively. Both authors used Ringer-phosphate solution as a medium. Fujita[5] using rat blood leucocytes suspended in citrated rat plasma reported a high respiratory rate ($QO_2 = 9$) and a low rate of aerobic glycolysis ($Q_G^{O_2} = 2$).

The experiments of Bakker and of Fleischmann and Kubowitz have been repeated and the results are here reported. The leucocytes of peritoneal exudates induced in the rabbit were suspended in Ringer-phosphate solution. The technic for obtaining the cells has been described.[1, 2] The cell suspension used contained about 70,000 cells per mm^3, and 2 cc of the suspension were sufficient to allow good measurements of the metabolic rate over a period of 3 hours.

Respiration was measured in Ringer-phosphate at a pH of 7.3, in an atmosphere of pure O_2. For aerobic glycolysis, the cells were suspended in Ringer-glucose-bicarbonate, and the measurements made after equilibration with 95% O_2 and 5% CO_2. For anaerobic glycolysis the cells were suspended in the same medium equilibrated with 95% N_2 and 5% CO_2. The results express the average metabolic activity during the first hour in 10 experiments.

QO_2	$Q_G^{O_2}$	$Q_G^{N_2}$
4.6	17	25

1 Ponder, E., and MacLeod, J., *J. Gen. Physiol.*, 1936-37, **20**, 267.

2 Ponder, E., and MacLeod, J., *Am. J. Physiol.*, 1938, **123**, 420.

3 Bakker, G., *Klin. Woch.*, 1927, **6**, 252.

4 Fleischmann, W., and Kubowitz, F., *Biochem. Z.*, 1927, **181**, 395.

5 Fujita, A., *Klin. Woch.*, 1928, **7**, 897.

These figures are slightly higher than those of Fleischmann and Kubowitz, but are in the same range. We cannot confirm the low rate of respiration reported by Bakker. The cause of the abnormally high rate of aerobic glycolysis observed is obscure. It is possible that the effectiveness of respiration in preventing aerobic glycolysis has been damaged in these cells, either by the methods used in obtaining them, or by the use of Ringer-phosphate solution as the suspension medium.

The effect of serum on the respiration of exudate leucocytes has been investigated. Normal serum cannot be used in the presence of KOH because of the marked pH changes due to the loss of CO_2. It is possible to modify rabbit serum so that the bicarbonate content is reduced and the resulting pH change in the presence of KOH is relatively small. The method of preparation is as follows: 15 cc of serum are titrated with N/10 HCl until the pH is about 6.4. In the case of normal rabbit serum which has been exposed to the air for some time from 1.5 to 2 cc of N/10 HCl are required. The acidified serum is then thoroughly evacuated until the pH reaches 7.3. Measured quantities are then transferred to the Warburg vessel, the cells added, and respiration measured in the conventional manner in an atmosphere of O_2, KOH being used to absorb CO_2. In this way, special apparatus is not necessary.*

In 12 experiments the respiration of exudate leucocytes has been measured in neutralized serum. In all the respiration was greater than in Ringer-phosphate. This increase varied from 35 to 70% with a mean of 50%. The mean QO_2 in neutralized serum is 7 as compared to a QO_2 of 4.6 in Ringer-phosphate. Furthermore, maximum respiratory activity is maintained longer in serum than in Ringer-phosphate. The serum alone consumes sufficient O_2 to account for not more than 15% of the increase.

In several control experiments the change in pH of the neutralized serum in the manometer in the presence of KOH for a period of time equivalent to the usual duration of an experiment has been measured. The changes were from 0.2-0.3 toward the alkaline side (7.3-7.6). In the case of exudate leucocytes where aerobic glycolysis is of considerable magnitude this change of pH is offset by acid production The pH of the cell-serum system after 3 hours in the manometer in presence of KOH falls from 7.3 to approximately 6.9.

* Dr. C. O. Warren, Jr., has applied the neutralized serum technic to the respiration of bone marrow and compared the results obtained with those of experiments done in untreated serum in the Dixon-Keilin manometer; he found no appreciable difference in the QO_2 values in both systems though both showed a marked increase (50%) over that of Ringer-phosphate (private communication).

Summary. The rates of respiration and aerobic glycolysis of exudate leucocytes reported by Fleischmann and Kubowitz have been confirmed. A method of measuring the respiration of leucocytes in serum is described. The respiratory rate of leucocytes in serum is greater than the rate in Ringer-phosphate solution.†

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Effects of Female Sex Hormones in Young Opossums.

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In a recent communication[1] the writer described the effects of the male hormone, testosterone propionate, on the developing genital tracts in pouch young of the American opossum, *Didelphys virginiana.* This hormone has a dual action, stimulating the development of both male and female structures in animals of either genetic sex. However, male parts are uniformly better developed in male subjects, and conversely, female structures show greater growth in females. These results illustrate clearly the interaction of hormones and genotype in development. The present report deals with a similar experiment, in which the female hormones estradiol dipropionate and estrone were administered from the 4th to the 18th day of pouch life.

The group treated with estradiol dipropionate consisted of a litter of 12 young (8 males, 4 females) which received a total dose averaging 20-25 gamma daily. Externally no effects were observed until the ninth day, when several individuals showed a distention of the abdomen, and a pronounced enlargement of the phallus, of a different morphological character from that induced by male hormones. Later the abdominal condition had become worse, and 2 individuals appearing moribund, were sacrificed on the 12th day. From day to day others were preserved for the same cause, the last on the 18th day.

At first the symptoms were ascribed to toxicity of the solution; however, autopsy showed a greatly distended bladder, enormous sac-

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1 Burns, R. K., Jr., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, —