8888 P

Effect of Low Oxygen Tension upon Respiration and Fermentation of Isolated Cells.

WALTER KEMPNER. (Introduced by W. A. Perlzweig.)

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

Warburg has devised the methods which make it possible to examine the respiration of isolated cells at a constantly maintained equilibrium between the cell and the gas medium and to measure quantitatively the influence of various oxygen tensions upon respiration in such a way as to eliminate diffusion as a limiting factor.[1] By these methods Warburg has determined the effect of low oxygen tensions upon the respiration of bacteria and isolated animal cells. He found the rate of oxidation of the nucleated red blood cells of geese, which he examined at 0°C., to be unchanged at oxygen pressures varying between 5 and 75 mm. Hg. (=0.7 and 10 vol. % O_2).[2] In *micrococcus candicans*, at 1°C. the rate of respiration even at an oxygen tension of 10^{-5} atm. (=0.0076 mm. Hg., =0.001 vol. % O_2) was the same as in air.[3] Also the respiration of non-nucleated red blood cells of rabbits poisoned by phenylhydrazin, examined at 38°C. showed no dependence upon oxygen tension.[4] Since these investigations it has been a generally accepted axiom of physiology that cell respiration is independent of variations of oxygen tension.[5]

The only exceptions seemed to be the nitrogen-fixing bacteria and pneumococcus which were both examined with the Warburg methods under conditions of optimal gas diffusion. The relative rate of respiration of azotobacter at 2 vol. % O_2 and in air was 1:2 (Meyerhof[6]) and that of pneumococcus at 2 vol. % O_2 and in air was even 1:4.5 (Schlayer[7]).

I have examined the effect of oxygen tension upon the rate of respiration of isolated animal cells and bacteria at temperatures between 1° and 42°C. Like Warburg, I did not find any difference in the rate of respiration at oxygen tensions between 1 and 20 vol. % O_2 when working at low temperatures, nor did I find any difference

1 Warburg, O., *Stoffwechsel der Tumoren*, Berlin, 1926.

2 Warburg, O., *Erg. d. Physiol.*, 1914, **14**, 253.

3 Warburg, O., and Kubowitz, F., *Biochem. Z.*, 1929, **214**, 5.

4 Warburg, O., Kubowitz, F., and Christian, W., *Biochem. Z.*, 1931, **242**, 170.

5 Meyerhof, O., *Chemische Vorgange in Muskel*, Berlin, 1930, p. 9.

6 Meyerhof, O., and Schulz, W., *Biochem. Z.*, 1932, **250**, 35.

7 Schlayer, C., *J. Bact.*, 1936, **31**, 181.

at temperatures between 25° and 42°C. in old bacterial cultures, in injured nucleated blood cells, and in non-nucleated human erythrocytes. Young undamaged body cells, however, examined in their physiological environment, and bacteria in suspension media, in which they were able to grow, showed at temperatures between 25° and 42°C. a great decrease in respiration at an oxygen tension as high as 5 vol. % as compared with air (20 vol. % O_2). Under these conditions I have found a marked effect on respiration of variations of oxygen tension in *Micrococcus candicans, Staphylococcus aureus, Pseudomonas pyocyanea, Escherichia coli, Monilia albicans,* human erythroblasts and leucemic leucocytes, red blood cells of fowls and alligators and young green plant cells (pine needles). The dependence of the respiration upon oxygen tension is greatest in the youngest cells and is influenced by physical and chemical changes in the cell medium such as pH, CO_2 concentration and bicarbonate content.

There was no increase in the fermentative metabolism corresponding to the decrease of respiration. Although the rate of respiration, *e. g.,* of geese erythrocytes, was decreased by 60% at an oxygen tension of 3.4 vol. % compared with the respiration in air, no acid formation occurred.

A typical experiment is described in the following:

Blood was taken under sterile conditions in heparin (5 mg. in 20 cc.) from the wing vein of a goose without contamination with tissue fluid. The blood was gently shaken with glass beads for 5 minutes and filtered through gauze. It was then saturated at 40°C. with a gas mixture of 2.5% CO—5% CO_2—18.75% O_2, 73.75% N_2, so that the hemoglobin oxygen was almost completely displaced by CO,[8] and pipetted into 4 Warburg manometer vessels of about 18 cc. capacity. Vessel 1 (4 cc. of blood) and vessel 2 (2 cc.) were saturated again with the same gas mixture while shaking in the thermostat. Vessel 3 (4 cc.) which contained 0.2 cc. 10% NaOH in the side bulb, in order to absorb the carbon dioxide, was saturated with 2.5% CO—18.75% O_2—78.75% N_2. Vessel 4 was saturated with 5% CO_2—95% CO in order to produce anaerobic conditions. The dry weight of cells in 4 cc. blood was 237 mg., the temperature was 40°C., the shaking speed 160-210 oscillations per minute. The readings were made every 5 minutes without stopping the manometers.

In vessels 1, 2, and 3 the metabolism was determined at 18.75% O_2 for a period of 30 minutes. Then the vessels were saturated in the thermostat with a gas mixture containing 3.4% O_2 and an equal proportion of CO to O_2 (0.45% CO—3.4% O_2—5% CO_2—91.15%

8 Warburg, O., *Biochem. Z.*, 1929, **214**, 4.

N_2). After another 30 minutes' observation the vessels were resaturated with the original gas mixtures of 18.75% O_2 concentration. CO_2 retention and metabolism figures were calculated according to Warburg.[4]

Table I shows that at 18.75% O_2 there is only a very small difference in the absolute respiration values between the blood cells examined at a normal pH and a physiological CO_2 tension and the cells examined at an alkaline pH in the absence of CO_2. Decrease of oxygen tension, however, causes in the physiological medium a great decrease in respiration which is completely reversible if the cells are not kept under low oxygen pressure too long; whereas in the alkaline carbon dioxide free medium the respiration rate remains nearly unchanged.

TABLE I.
Metabolism of 100 mg. Blood Cells of Anemic Goose in 1 Hour at Various Oxygen Tensions, 40° C.

Vol. % O_2	c.mm. oxygen consumed in alkaline, CO_2 free medium	c.mm. oxygen consumed in unchanged physiological medium with 5 vol. % CO_2	c.mm. lactic acid formed (1 c.mm. = 0.004 mg.) in physiological medium with 5 vol. % CO_2
18.75	—101	—106	+ 16
3.4	— 96	— 44	+ 0
0			+190

This proves that the decrease of respiration at low oxygen tensions is not due to insufficient diffusion of oxygen from the gas space or the suspension medium into the cells, since all the conditions which might influence the diffusion (cell volume, oxygen tension, shaking speed) are identical throughout.

The fact that cellular respiration in an alkaline, CO_2-free medium as well as at low temperatures is largely independent of oxygen tension, is probably the chief reason why previous workers did not find the marked effect of oxygen tension upon respiration. Even the dissociation of oxyhemoglobin at decreasing oxygen tension might have been overlooked if the dissociation had only been measured at low temperatures and in alkaline CO_2-free media. For each of these three factors affects the affinity of hemoglobin for oxygen to such an extent that one must go down to very low oxygen tensions before the dissociation of oxyhemoglobin begins.

In complete absence of oxygen a great amount of lactic acid is formed while at an oxygen concentration of 3.4 vol. % in spite of 60% decrease in respiration no lactic acid appears. No relationship was found between the decrease in respiration and the occurrence of glycolysis. The Pasteur reaction, the disappearance of the anaero-

bic splitting metabolism under aerobic conditions, was not dependent upon the rate of respiration but upon the concentration of oxygen.

8889 P

Effect of Crystalline Vitamin C (Ascorbic Acid) on Tolerance to Tuberculin.

M. MAXIM STEINBACH AND SIDNEY J. KLEIN.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

One of the characteristics of the reaction of tuberculous animals to a large dose of tuberculin is congestion and capillary hemorrhage in all tissues, particularly in those containing tubercles. This is attributed to altered cell permeability and capillary dilatation. Since vitamin C has been shown to decrease capillary fragility (Dalldorf[1]), and has been employed with encouraging therapeutic results in certain types of hemorrhage (Willstaedt[2]), we were led to study this substance in relation to tuberculin intoxication. It is of interest to note that about 10 years before the discovery of the anti-infectious and antitoxic action of vitamin C (Harde,[3] Jungeblut and Zwemer,[4] King and Menten,[5] Jungeblut[6]), Bieling[7] had already found that a dose of tuberculin which killed only one of 2 tuberculous guinea pigs maintained on a normal diet, sufficed to kill 2 scorbutic tuberculous guinea pigs.

The effect of crystalline vitamin C (l-ascorbic acid*) on tuberculin was investigated in tuberculous guinea pigs fed a normal diet which included an abundance of fresh lettuce as a natural source of vitamin C. Ascorbic acid mixed *in vitro* with skin test doses of tuberculin prior to intracutaneous inoculation in tuberculous guinea pigs failed to inactivate the tuberculin in tests on 12 animals. Nor did prolonged pretreatment of 7 tuberculous guinea pigs with ascorbic acid reduce the reactivity of the skin to small doses of

1 Dalldorf, G., *J. Am. Med. Assn.*, 1935, **104**, 1701.

2 Willstaedt, H., *Klin. Woch.*, 1935, **14**, 1705.

3 Harde, E., *Compt. rend. Acad. Sci.*, 1934, **199**, 618.

4 Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

5 King, C. G., and Menten, M. L., *J. Nutrit.*, 1935, **10**, 129.

6 Jungeblut, C. W., *J. Exp. Med.*, 1935, **62**, 517.

7 Bieling, R., *Zeit. f. Hyg.*, 1925, **104**, 518.

* We are indebted to Merck & Co. for a generous supply of crystalline vitamin C (Cebione; Cevitamic Acid Merck).