THE EFFECT OF SWELLING ON THE RESPIRATION OF ERYTHROCYTES*

By F. R. HUNTER AND W. G. BANFIELD

(From the Department of Zoology, Rhode Island State College, Kingston, Rhode Island)

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This investigation represents an additional attempt to test the hypothesis that a relationship exists between the respiratory activity of a cell and the maintenance of the selectively permeable properties of its membrane. If this hypothesis were true, it seemed reasonable to expect that a change in the tension of the plasma membrane might possibly bring about a compensatory change in the respiratory activity of the cell. All of the available evidence would indicate that this probably was not the case, but no such assumption can be made *a priori*. A number of investigators have studied the effect of a change in cell size on respiration. Inman (1921*a*) using the marine alga *Laminaria*, reported a decrease in carbon dioxide production in both hypotonic and hypertonic solutions, while Bodine (1933) using grasshopper eggs in hypertonic solutions and Johnson and Harvey (1938) using marine luminous bacteria in hypotonic solutions, reported a decrease in oxygen consumption. Ray (1927) reported a similar decrease in oxygen consumption of dog reticulocytes in both hyper- and hypotonic solutions. Tipton (1933) found no significant change in the rate of oxygen consumption of chicken erythrocytes using hypotonic and slightly hypertonic solutions of sodium chloride, while Hunter (1939) using the same type of cells in highly hypertonic sodium chloride solutions reported a marked decrease in oxygen consumption. Hypotonic solutions had no effect until a considerable number of cells were hemolyzed. In these solutions there was a marked decrease in oxygen consumption. Ponder (personal communication) found that hypotonic solutions had no effect on the rate of oxygen consumption of rabbit erythrocytes. All of these authors have reported either a decrease in respiration or no change resulting from anisotonic media.

The present investigation was undertaken in an attempt to determine

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what changes in respiration, if any, were associated with the actual swelling of a cell such as an erythrocyte. In the previous investigations, the cells were allowed to reach osmotic equilibrium in the anisotonic medium before respiratory measurements were made. The present data were obtained while the volume changes of the cells were taking place. Chicken erythrocytes obtained from defibrinated blood were the cells used. It was necessary to find some substance which would penetrate the cells slowly enough so that respiratory measurements could be made during the swelling process. Preliminary tests showed that erythritol penetrated chicken erythrocytes at 37°C. at such a rate that considerable hemolysis had occurred in about 45 minutes. Although this substance penetrated a little more rapidly than was desirable for these experiments, larger molecules could not be used because they failed to penetrate in a reasonable length of time. By lowering the temperature the rate of penetration could have been decreased, but this would also have decreased the rate of oxygen consumption. The experiments were made at a temperature of 37° ± 0.02°C., and all possible manipulations were made before the blood was added. This was done to minimize the amount of swelling which occurred before the respiratory measurements were begun.

The blood was centrifuged before using to remove as much of the serum as possible. Since small volumes of solutions had to be used, it was necessary to eliminate the osmotic effect of the serum. Although only a small amount would have been added, it would have reduced the volume of the experimental solution which could have been used. This in turn would have made the solution surrounding the cells more nearly isosmotic, which would have resulted in a smaller volume change of the cells. Oxygen consumption measurements were made using a Barcroft-Warburg microrespirometer as previously described (Hunter and Pahigian, 1940). Three vessels were used for a single experiment. The control consisted of one-half cc. of chicken erythrocytes suspended in 3 cc. of Ringer-Locke. One experimental vessel contained one-half cc. of cells in 3 cc. of 0.3 M erythritol, while the second experimental vessel contained one-half cc. of cells suspended in 3 cc. of a solution of 0.3 M erythritol in Ringer-Locke. In the first experimental solution, the cells swelled from their normal volume to their hemolytic volume. In the second experimental solution, the cells swelled from a shrunken condition (due to the loss of water into the hypertonic solution) back to their normal volume.

The results of a typical experiment are plotted in Fig. 1. The cubic millimeters of oxygen are plotted against the time in minutes. It can be seen that there is no marked change in the rate of oxygen consumption in

the experimental cells. At the beginning the shrunken cells consume oxygen at a slightly slower rate, as would be expected from previous experiments (Hunter, 1939). Although this previous work showed that the oxygen consumption was irreversibly decreased when the cells were shrunk, these cells were allowed to remain in the shrunken condition for at least 1 hour before they were swollen back to their normal volume. In the present experiments the cells began to swell back to their original volume almost

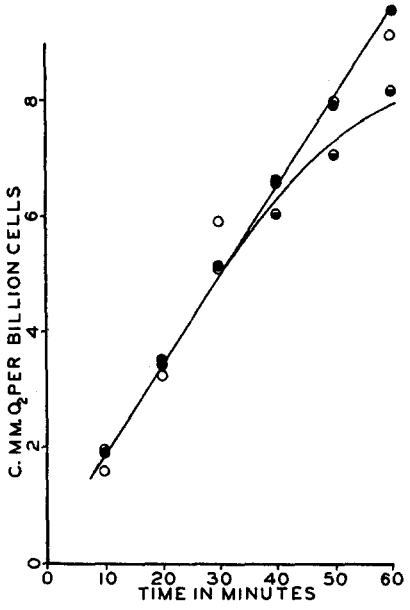

FIG. 1

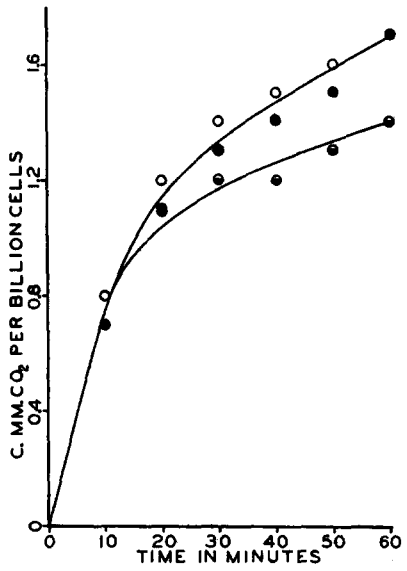

FIG. 2

FIG. 1. The effect of swelling on the oxygen consumption of chicken erythrocytes ●-Ringer-Locke; ○-erythritol in Ringer-Locke; ◒-erythritol.

FIG. 2. The effect of swelling on the anaerobic glycolysis of beef erythrocytes. ●-Ringer-Locke; ○-erythritol in Ringer-Locke; ◒-erythritol.

instantaneously. The fact that the oxygen consumption of the shrunken cells recovered to the normal rate when the cells began to return immediately to their normal volume is in accord with the observations of Inman (1921*b*). This author showed that the degree of recovery of respiration was inversely proportional to the length of time the cells had remained shrunk. Except for this initial lowering of the rate, it seems to be the same as that of the control cells. This would indicate that there was no change in the aerobic respiratory activity of the cells associated with swelling from a shrunken to a normal condition. The first portion of the

curve obtained from cells suspended in erythritol solution appears to be essentially the same as the control curve. As soon as an appreciable number of cells have hemolyzed, the rate falls off as would be expected on the basis of the data presented by Michaelis and Salomon (1930). Ramsey and Warren (1930) reported a large "burst" when various types of erythrocytes were hemolyzed. In view of these experiments, it might be expected that there would be an increase in the rate of oxygen consumption as the cells hemolyzed in the erythritol solution. A further investigation by these authors (1934) demonstrated that this burst did not always appear. They also pointed out that the sudden increase in oxygen consumption depended on the plasma. Since in the present investigation only cells were used, the burst would not be expected to appear.

Since we demonstrated that aerobic oxidations are not involved when the cell membrane is stretched (*cf.* Hunter, 1936, 1937), it seemed of interest to determine if the experimental treatment would affect anaerobic glycolysis. These measurements were made in the same manner as previously described (Hunter and Pahigian, 1940). Because the oxygen in the vessels had to be displaced by a nitrogen-carbon dioxide mixture, a longer equilibration period was necessary before the readings could be taken. Consequently, some other type of cell had to be used. It was found that erythritol penetrated beef erythrocytes at a much slower rate (*cf.* Jacobs, Glassman, and Parpart, 1935). Although non-nucleated erythrocytes consume very little oxygen (Michaelis and Salomon, 1930), it has been shown that they produce lactic acid anaerobically at a considerably faster rate (Kempner, 1939). In view of these two facts, beef erythrocytes were used for the glycolysis measurements. The blood was freshly drawn from the jugular vein into a sterile bottle and defibrinated. In most experiments it was used within a few hours of drawing, while in others it was kept in an ice box for 24 hours. The results were the same in either case. In these experiments the control solution was Ringer-Locke containing 200 mg. per cent glucose and 0.03 N $NaHCO_3$. One experimental solution contained 0.3 M erythritol, 0.03 N $NaHCO_3$, and 200 mg. per cent glucose, while the second experimental solution was the same as the above, except it was m e up in Ringer-Locke. The osmotic effect of the $NaHCO_3$ and glucose was negligible since the amounts added were small, and the changes in cell volume would still occur. The Warburg vessels used in these experiments were smaller (about 6 cc.) than those used in the preceding ones. Consequently, a smaller total volume of solution was used. In some experiments 0.3 cc. of centrifuged cells was added to 1.7 cc. of solution, while in others 0.6 cc. of cells was added to 1.4 cc. of solution. Half an hour was allowed for the displacement of the oxygen by the N_2-CO_2 gas mixture.

A typical pair of curves are plotted in Fig. 2. The cubic millimeters of carbon dioxide produced are plotted against the time in minutes. It should be remembered that 1 c. mm. of carbon dioxide is equivalent to 0.004 mg. of lactic acid. It is evident that the change in cell size has no marked effect on the anaerobic breakdown of sugars until the cells begin to hemolyze.

These results are what might have been postulated on the basis of previous experiments. Without experimental evidence, however, such predictions would not have carried much weight. It should be pointed out that a mammalian erythrocyte, because of its peculiar shape, does not undergo as great a change in surface area as would at first appear. That it does undergo a certain amount of stretching, however, is not denied (Ponder and Marsland, 1935). It is reasonable to assume that because of its initial shape an avian erythrocyte when it swells would have a greater change in surface area than a mammalian erythrocyte. The fact that a change in cell size, and hence a change in the surface area, is not accompanied by any compensatory change in either oxygen consumption or anaerobic glycolysis adds further evidence testing the hypothesis of a relationship between the cell membrane and its respiratory activity.

SUMMARY

1. Oxygen consumption measurements made while a chicken erythrocyte swells show no increase over the control value.

2. There is no change in the rate of anaerobic glycolysis in beef erythrocytes when they swell.

3. The above statements are true whether the cells swell from a shrunken condition back to the normal volume, or swell from the normal to the hemolytic volume.

4. These data add a further test of the hypothesis that a relationship exists between the cell membrane and its respiratory activity.

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