CCVIII. CARBOHYDRATE CATABOLISM IN CEREBRAL CORTEX.

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PASTEUR [1861; 1875] suggested that the presence of oxygen decreases the rate of sugar destruction by yeast, and also suppresses or diminishes the accumulation of anaerobic cleavage products. Accordingly the Pasteur effect may be defined as the effect of oxygen in reducing carbohydrate catabolism and in diminishing or suppressing the accumulation of the products of anaerobic metabolism. This definition involves two characteristics. It is evident that the first characteristic of oxygen in causing the Pasteur effect of necessity involves the second, since if there is decreased catabolism of carbohydrate less cleavage products must be formed. However, decrease in the formation of anaerobic cleavage products does not necessitate decreased carbohydrate catabolism. It is thus merely redundant to add the second characteristic in defining the Pasteur effect, although in the loose definition often given, it is often this characteristic which is mainly accentuated.

Accordingly it is necessary to demonstrate that the presence of oxygen causes a decrease in the rate of carbohydrate catabolism for it to be certain that the Pasteur effect is in operation. This has been shown by Meyerhof [1920] in the case of the destruction of glycogen by frog muscle and by Negelein [1925, 1] in the case of the red blood corpuscles of the goose and the rabbit acting on glucose. Negelein, however, measured the rate of carbohydrate destruction in the presence of cyanide instead of the anaerobic rate.¹ In no other tissue has the Pasteur effect been unequivocally demonstrated. Most work on the subject involves measurements of oxygen uptake as well as of glycolysis.² From the values of the respiration and the glycolysis under aerobic conditions and of the anaerobic glycolysis it may be inferred that the carbohydrate destruction is reduced in the presence of air, although this inference does not amount to a rigid proof.

The present communication shows how the rates of anaerobic and aerobic carbohydrate catabolism by brain cortex slices can be calculated indirectly from the rates of respiration and glycolysis and further demonstrates that this method of calculation is justified by actual measurements of the rates of sugar disappearance under these various conditions. It is thus shown definitely that the Pasteur effect is in operation in slices of cerebral cortex acting on glucose *in vitro*.

EXPERIMENTAL RESULTS AND DISCUSSION.

In considering this problem we adopt certain new symbols to express the rate of carbohydrate disappearance. The rate of disappearance of hexose is expressed by the symbol Q_{C_6} ; this represents the number of μ l. of hexose sugar

¹ Except in the case of two experiments which he performed on tumour tissue and in which the effect of oxygen on the rate of sugar destruction was to cause only a small percentage diminution.

² By the term glycolysis we mean either the formation of lactic acid in animal tissues or of ethyl alcohol and carbon dioxide in plants.

expressed as a gas at N.T.P., which disappears per mg. dry weight of tissue per hour. Since it is conventional to represent the rate of disappearance of a substance by a negative number, Q_{C_6} is always negative if sugar is being destroyed. The symbol $Q_{C_6}^{N_2}$ represents the rate of sugar disappearance under anaerobic conditions, whilst $Q_{C_6}^{O_2}$ is the aerobic rate of disappearance. When $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$ the Pasteur effect is in operation, since the rate of carbohydrate destruction is less in oxygen than in anaerobiosis.

In cerebral cortex it is possible to calculate Q_{C_6} from the rates of glycolysis and respiration.

represent respectively the glycolytic and respiratory processes occurring in brain supplied with glucose. The respiration of other intracellular substrates apparently does not occur in the presence of added glucose or lactate, since with these substances the respiration remains constant, whilst without substrate it falls rapidly [*v. Dixon, 1935*].

Further by equation (1)

$$Q_{C_6}^{N_2} = -\frac{Q_M^{N_2}}{2}^*,$$

and by equations (1) and (2)

$$Q_{C_6}^{O_2} = -\frac{Q_M^{O_2}}{2}^* + \frac{Q_{O_2}}{6}^*.$$

Q_{C_6} is naturally negative as it expresses a rate of disappearance; this explains the negative signs preceding Q_M in the above equations, since Q_M is positive when lactic acid is being formed. Q_{O_2} is always negative.

We will now consider an example of the calculation of $Q_{C_6}^{N_2}$ and $Q_{C_6}^{O_2}$. In an experiment on slices of rabbit's brain cortex, using the two-vessel method of Warburg [1924], I found the following metabolic rates (see Table I):

Table I.

Q_{O_2}	$Q_M^{O_2}$	$Q_M^{N_2}$
-8.3	2.8	20.7

From these figures $Q_{C_6}^{N_2} = -\frac{20.7}{2} = -10.35$,

while $Q_{C_6}^{O_2} = -\frac{2.8}{2} + \frac{-8.3}{6} = -2.78$.

Thus $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$. We therefore conclude that the Pasteur effect is operating. It might, however, be argued, although the sugar disappearance, as calculated above, is reduced by the presence of oxygen, that the absolute rate of disappearance is not really reduced, but that some intermediary other than lactic acid is accumulating. This was rendered unlikely by the results of some experiments kindly communicated to me by Dr E. G. Holmes.

Dr Holmes measured the aerobic rate of sugar destruction by brain cortex slices and obtained simultaneous measurements of the lactic acid production (by chemical method) and of the CO_2 of respiration (by the method of M. Dixon & Keilin). It is clear from his results that the sugar destroyed can be mainly accounted for by lactic acid production and respiration and that at any rate

* These symbols are defined by Warburg [1925] and Negelein [1925, 2].

there is no accumulation of an intermediary (other than lactic acid) in amount sufficient to account for the effect of oxygen in reducing glycolysis.

To justify the above conclusions it was, however, necessary to measure simultaneously the rates of sugar disappearance in oxygen and in nitrogen. Accordingly experiments were performed in which definite amounts of glucose were added to brain slices suspended in bicarbonate Ringer. The slices were then incubated both under aerobic and anaerobic conditions, and the sugar remaining in the Ringer was estimated by the Hagedorn-Jensen method.

In actual practice 0.2 ml. of 0.3–0.4 % glucose was added to the brain slices (3–10 mg. dry weight) suspended in Ringer. At the end of the experiment 1 ml. of 40 % trichloroacetic acid was added to the suspension. After standing 15 min. the suspension was filtered quantitatively into a 25 ml. graduated flask and the sugar content estimated. The slices remaining on the filter paper were dried and weighed. The original sugar present was estimated in the presence of identical concentrations of trichloroacetic (and Ringer's solution). In one set of experiments the original sugar was incubated and filtered in the same manner as the solutions containing the slices. This did not affect the final result. The initial concentration of sugar in the Ringer was 0.04–0.05 %, the total amount of sugar added being 0.5–0.8 mg. The experiments lasted for 1 hour. The results of these experiments are tabulated below (Table II):

Table II.

	mg. sugar loss per hour	Dry wt. of slices in mg.	Sugar loss in mg. per mg. dry wt. per hour	Q_{C_6}
Exp. 1.				
A slices incubated in O_2 containing 5 % CO_2	0.147	7.70	0.019	2.4
B slices incubated in O_2 containing 5 % CO_2	0.133	6.24	0.021	2.6
C slices incubated in N_2 containing 5 % CO_2	0.287	4.64	0.062	7.7
D slices incubated in N_2 containing 5 % CO_2	0.247	5.05	0.049	6.1
Exp. 2.				
A slices incubated in O_2 containing 5 % CO_2	0.161	5.81	0.028	3.5
B slices incubated in O_2 containing 5 % CO_2	0.209	8.04	0.026	3.2
C slices incubated in N_2 containing 5 % CO_2	0.384	5.51	0.070	8.7
D slices incubated in N_2 containing 5 % CO_2	0.350	5.37	0.065	8.1
Exp. 3.				
A slices incubated in O_2 containing 5 % CO_2	0.159	5.04	0.032	– 4.0
B slices incubated in O_2 containing 5 % CO_2 (0.1 M KCl added to Ringer)	0.318	3.54	0.090	– 11.2
C slices incubated in N_2 containing 5 % CO_2	0.292	3.48	0.084	– 10.5

(In all the experiments the vessels containing the slices were shaken in a bath at 37°.)

It is clear from the above results that $Q_{C_6}^{O_2}$ is always numerically less than $Q_{C_6}^{N_2}$. In other words the Pasteur effect is operating in cerebral cortex. Further, the addition of $M/10$ KCl inhibits the Pasteur effect and raises the aerobic catabolism of carbohydrate approximately to the normal anaerobic level [v. Ashford and Dixon, 1935]. We thus find that the indirect method of demonstrating the Pasteur effect is, in brain at any rate, substantiated by absolute measurements of the rate of sugar disappearance.

SUMMARY.

1. The data necessary for the demonstration of the Pasteur effect are defined.
2. Symbols representing the rates of carbohydrate destruction under aerobic and anaerobic conditions are introduced—the symbols are $Q_{C_6}^{O_2}$ and $Q_{C_6}^{N_2}$ respec-

tively. The calculation of these rates from the rates of respiration and glycolysis is described. If $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$ the Pasteur effect is in operation. This is shown to follow from the values of the respiration and glycolysis in cerebral cortex.

3. The absolute rates of glucose destruction by brain cortex slices under aerobic and anaerobic conditions have been measured. It is shown that $Q_{C_6}^{O_2}$ is numerically less than $Q_{C_6}^{N_2}$ when these quotients are measured directly as well as when they are calculated from the respiration and glycolysis.

4. The addition of potassium chloride (one of the so-called inhibitors of the Pasteur effect) raises the aerobic destruction of sugar to the anaerobic level.

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