CCIX. THE EFFECT OF RISE IN TEMPERATURE ON THE CARBOHYDRATE CATABOLISM OF CEREBRAL CORTEX.

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THE effect of rise in temperature on the respiration and glycolysis of animal tissues has been studied by Kubowitz [1929] and by Nakashima [1929]. Kubowitz using frog retina showed that aerobic glycolysis suddenly becomes apparent above 35°. The Pasteur effect is abolished above this temperature since the Meyerhof quotient descends to zero. Nakashima employed fish retina, which, like amphibian and unlike mammalian retina, shows little or no aerobic lactic acid production at normal temperatures. Nakashima found that in fish retina rise in temperature from 30 to 37·5° produces a large aerobic glycolysis, but here in contrast to frog's retina respiration is increased. The anaerobic glycolysis is also increased, but in spite of this Nakashima concluded that there is inhibition of the Pasteur effect embodying a fall in Meyerhof quotient at this high temperature. In the present communication the effects of abnormally high temperatures on the metabolism of cerebral cortex are described.

METHODS.

Slices of rabbit's brain cortex were employed. The respiration and glycolysis were measured by the two-vessel method of Warburg [1924]. Bicarbonate Ringer containing 0·2 % glucose was employed throughout.

The ordinary symbols denoting the rates of respiration and glycolysis are employed [*v.* Warburg, 1925; Negelein, 1925]. The symbols $Q_{C6}^{O_2}$ and $Q_{C6}^{N_2}$ as described by Dixon [1936] are also employed to represent the rates of sugar destruction in oxygen and in nitrogen respectively. These quotients are calculated from the respiratory and glycolytic rates as described by Dixon.

EXPERIMENTAL RESULTS AND DISCUSSION.

The results of a number of experiments at 37, 42 and 45° are shown in Table I.

Table I.

Temp. ... 37°					42°					45°				
Q_{O_2}	$Q_M^{O_2}$	$Q_{C6}^{O_2}$	$Q_M^{N_2}$	$Q_{C6}^{N_2}$	Q_{O_2}	$Q_M^{O_2}$	$Q_{C6}^{O_2}$	$Q_M^{N_2}$	$Q_{C6}^{N_2}$	Q_{O_2}	$Q_M^{O_2}$	$Q_{C6}^{O_2}$	$Q_M^{N_2}$*	$Q_{C6}^{N_2}$
−6·8	1·6	−1·9	—	—	−8·0	2·3	−2·3	—	—	—	—	—	—	—
−8·7	3·4	−3·1	—	—	−8·2	4·1	−3·4	—	—	—	—	—	—	—
−8·1	3·0	−2·9	19·5	− 9·8	−9·4	4·4	−3·8	21·0	−10·5	—	—	—	—	—
—	—	—	21·0	−10·5	—	—	—	26·0	−13·0	−17·0	11·4	−8·5	39	−19·5
—	—	—	{19·0	− 9·5}	—	—	—	—	—	−13·0	8·0	−6·2	{35	−17·5
			{19·0	− 9·5}									{32	−16·0
−9·0	2·6	−2·8	—	—	—	—	—	—	—	−16·8	9·3	−7·4	—	—
—	—	—	—	—	—	—	—	—	—	{−16·8	12·8	−9·0}	—	—
										{−16·7	8·0	−7·4}		

* At 45° the anaerobic glycolysis was measured over the first period of 20 min., since it falls rapidly. The other values were obtained over periods of 40 min. or 1 hour.

At 42° there is only a small rise in the rate of aerobic and anaerobic carbohydrate catabolism above those at 37°. None of the metabolic reactions as measured by the various quotients appears much increased by this rise in temperature.

At 45°, however, the picture is entirely different. There is an enormous increase in the rates of respiration and anaerobic and aerobic glycolysis above the values at the lower temperatures. The rate of carbohydrate catabolism (Q_{C_6}) is accordingly also much increased at 45° above that at 37 and 42°.

It is clear that both the anaerobic and the aerobic rate of carbohydrate catabolism suffer an enormous increase on raising the temperature to 45°. The respiration is also increased. These effects are like those described in fish retina by Nakashima [1929]. However, it does not appear that the Meyerhof quotient is markedly reduced at 45°, values between 1·6 and 2·1 being obtained from the above figures. In normal brain the Meyerhof quotient is about 2. It would thus appear that the aerobic glycolysis is due to the fact that the respiration is no longer sufficient to suppress the increased glycolysis. Specific inhibition of the Pasteur effect has probably not occurred. The Pasteur effect is certainly working at 45° since oxygen still reduces the rate of carbohydrate catabolism at this temperature. It is possible, had Nakashima been able to calculate the Meyerhof quotient from the values of glycolysis and respiration obtaining initially, that in fish retina also the Meyerhof quotient would have proved not to be subnormal at high temperatures.

In measuring the anaerobic glycolysis at 45° it is necessary to obtain readings in the first 20 min. (i.e. after 20 min. equilibration time). The anaerobic glycolysis at 45° falls very rapidly. This fall is not experienced at 37 or 42°. This will be clear from Table II, which also shows that aerobic glycolysis and respiration at 45°, although changing with time, remain much more constant than does the anaerobic glycolysis.

Table II.

Successive 20 min. periods from commencement of readings	1st	2nd	3rd	4th	5th	6th	7th
Exp. 1.							
$Q_M^{N_2}$ at 37°	21	19	22	—	—	—	—
$Q_M^{N_2}$ at 42°	26	28	25	—	—	—	—
$Q_M^{N_2}$ at 45°	39	26	10	—	—	—	—
Exp. 2.							
$Q_M^{N_2}$ at 37°	19	17	16	17	19	17	—
$Q_M^{N_2}$ at 45°	35	22	17	11	6	6	4
$Q_M^{N_2}$ at 45°	32	18	11	7	—	—	4
$Q_M^{O_2}$ at 45°	8	8	13	10	12	6	5
Q_{O_2} at 45°	-13	-13	-15	-9	-9	-6	-5
Exp. 3.							
$Q_M^{N_2}$ at 45°	33	22	13	6	4	4	3
$Q_M^{N_2}$ at 45°	36	24	11	9	5	5	4
$Q_M^{O_2}$ at 45°	6	6	9	11	10	9	6
Q_{O_2} at 45°	-15	-12	-15	-11	-10	-9	-5
Exp. 4.							
$Q_M^{O_2}$ at 45°	9		15		13		—
Q_{O_2} at 45°	-17		-16		-13		—

The reversibility of this effect of high temperature in increasing the rate of carbohydrate catabolism has been studied. Two parallel experiments with tissue from the same brain were carried out at 45°. The manometers containing the slices were then transferred to a bath at 37°. Finally the manometers were reintroduced to the bath at 45°. The results are shown in Table III. In this table figures in the same vertical column represent the metabolic rates of the same tissue at the different temperatures.

Table III.

Temp. ° C.	Exp. A Q_{O_2}	Exp. A $Q_M^{O_2}$	Exp. A $Q_{C6}^{O_2}$	Exp. B Q_{O_2}	Exp. B $Q_M^{O_2}$	Exp. B $Q_{C6}^{O_2}$
45	-16·8	12·2	-8·9	-16·7	8·6	-7·1
37	-8·8	2·6	-2·8	-7·7	3·0	-2·8
45	-7·5	12·2	-7·4	-7·4	11·5	-6·9

It appears that the effect on the aerobic glycolysis and carbohydrate catabolism is largely reversible. Respiration, aerobic glycolysis and carbohydrate catabolism ($Q_{C_6}^{O_2}$) all fall alike on transferring the tissue from the bath at 45° to that at 37°. The high glycolysis and carbohydrate catabolism are regained on reintroduction into the bath at 45°. It is not certain whether the effect on the respiration is reversible since the high rate cannot be recovered on reintroduction into the bath at 45°. The rate of respiration in any case falls at 45°. It is certain that the effect on the catabolism of carbohydrate is reversible. The low normal rate of destruction of carbohydrate is recovered at the lower temperature.

The main result of these experiments on the effect of temperature on the metabolism of brain is that a rise in temperature from 37 to 42° only produces a very slight increase in metabolic rate, whilst on raising the temperature to 45° the metabolism increases enormously. At 42° the respiration is scarcely raised above the normal value at 37°, nor is there any marked increase in aerobic glycolysis. At 45°, however, the respiration may be increased by 100 % whilst marked aerobic glycolysis becomes evident, this being sometimes as much as six times as great as the normal value at 37°. Table IV shows the aerobic rates of carbohydrate (hexose) catabolism ($Q_{C_6}^{O_2}$) calculated from the rates of respiration and glycolysis at these three temperatures.

Table IV. $Q_{C_6}^{O_2}$ *in brain at various temperatures.*

37°	42°	45°
-2·9	-3·8	—
-3·0	-3·4	—
-1·9	-2·3	—
-2·8	—	-7·45
—	—	-8·9
—	—	-7·0

This table emphasises the sudden rise in the rate of aerobic carbohydrate catabolism seen between 42 and 45°. I have not yet obtained many figures for the rates of anaerobic glycolysis at these various temperatures, but from those described above it would seem that a similar, though not so marked, rise in the rate of carbohydrate catabolism occurs between 42 and 45°. Here the question is complicated by the fact that at 45° the rate of anaerobic glycolysis falls rapidly with time.

The relation between temperature and rate of chemical reaction is given by the equation of Arrhenius. This may be expressed in the form

$$\log \frac{k_1}{k_2} = K\ (1/T_1 - 1/T_2),$$

where k_1 and k_2 are the rates of reaction at the absolute temperatures T_1 and T_2 respectively, and K is a constant. From this it is seen that the logarithm of the velocity of the reaction is proportional to the reciprocal of the absolute temperature. There should in fact be a linear relation between the logarithm of the velocity and the reciprocal of the absolute temperature in the reactions of living cells.

The results so far obtained on the rates of carbohydrate catabolism at different temperatures are represented graphically in Fig. 1. The logarithm of the velocity of carbohydrate destruction ($\log_{10} - Q_{C_6}$) is plotted against the reciprocal of the absolute temperature. It is clear from Fig. 1 that the results do not subscribe to the Arrhenius law. The velocity of the reaction increases much more rapidly between 42 and 45° than one would expect from the rates at 37 and 45°. Crozier [1924; see also Barcroft, 1932], has emphasised the fact that many biochemical processes apparently "evade" the Arrhenius equation. In general the velocity at high temperatures is not so high as would be anticipated from those at lower temperatures if the Arrhenius law were followed. A good example of this is shown by the effect of temperature on the rate of reduction of methylene blue by various substances in the presence of *Bact. coli*, as shown by Cook [1930].

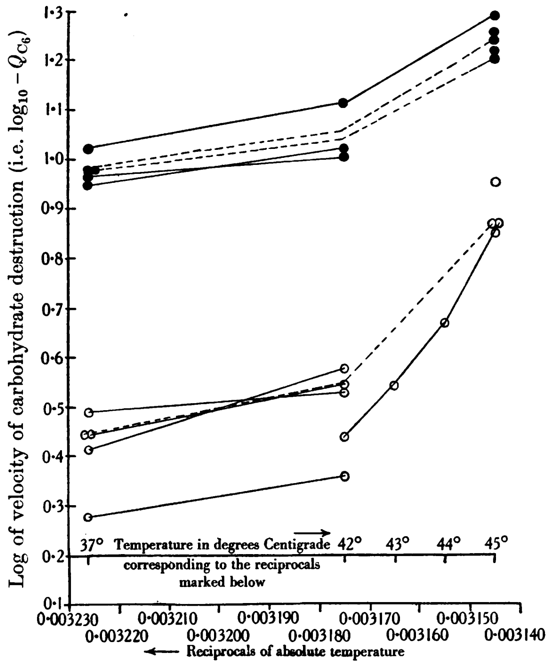

Fig. 1. The points connected by lines indicate observations obtained from the same brain. These lines are dotted where observations at the intermediate temperature (42°) were not obtained. • Signifies experiments conducted anaerobically. o Signifies experiments conducted aerobically.

Barcroft [1934] suggests that some governing mechanism is at work by which the cell resists the changes produced by a varying environment. In the case of cerebral cortex it would appear that this governing mechanism may break down above 42° and hence the abrupt rise in the rate of carbohydrate catabolism above this temperature.

These results are only of a preliminary nature and it is hoped later to extend the curves over a more complete range of temperature. One is, of course, only justified in regarding respiration and glycolysis as one reaction if both these processes have an initial common path, which is the reaction limiting them both. Otherwise we could not regard the total aerobic rate of carbohydrate destruction Q_{C_6} as measuring the rate of one reaction. But, at any rate, the same type of non-linear relation is obtained when the above treatment is applied to the respiration and glycolysis individually. The procedure followed above allows one to obtain readily a conception of the combined effect of respiration and glycolysis on the total rate of carbohydrate catabolism.

It is interesting to compare these effects of rise in temperature on the metabolism of rabbit's brain with the result of certain experiments of Marsh [1930]. Marsh noted that somewhere between 108 and 110° F. rabbits lose consciousness. 109° F. incidentally corresponds to 43° C. which is inside the temperature range (42–45°) where the metabolism of brain commences to become abnormal. It would seem that up to this level there is some regulating mechanism at work. When this breaks down carbohydrate catabolism runs riot in the brain cells and consciousness is simultaneously lost. It would thus appear that there may be a connection between the physiological process of consciousness and the rate of carbohydrate catabolism in the brain. Barcroft [1935] has pointed out the small size of the range of temperature over which the brain can function normally. It seems probable that the upper limit of this range is determined by this rapid rise in metabolic rate, though it also is possible that both the extent of this range and the metabolic rate are decided by some structural condition of the cell.

I have made one experiment so far in an attempt to ascertain more precisely the temperature at which this marked change in metabolism occurs. It consisted in measuring the metabolism of brain at intervals of 1° rise of temperature successively at the various temperatures between 42 and 45°. The results are seen in the following table (Table V):

Table V.

Temp. ° C.	Q_{O_2}	$Q_M^{O_2}$
42	− 8·4	2·7
43	−10·8	3·4
44	−11·3	5·5
45	−11·0	11·3

(Bicarbonate concentration used in this experiment was 0·04 *M*; usually 0·025 *M* is used. This concentration does not however change the metabolism at 42° from its normal value.)

It will be seen that the chief rise in respiration occurs between 42 and 43°. Rabbits lose consciousness at about 43° (Marsh). Above this temperature little further rise in respiration occurs, though the aerobic glycolysis is markedly increased. This experiment suffers from the fact that a time effect may be in part responsible for the change of metabolism. We do know, however, that time of incubation has little effect on the metabolic quotients at 42°. At 45° however respiration does fall considerably with time. It would further appear that the main rise in glycolysis occurs when the respiration has reached its maximum. This could be well interpreted on the view that respiration and glycolysis have an initial common path. When the oxidative removal of some intermediary in the glycolytic chain has reached its maximum, then further increase in the rate of the initial reaction merely increases the rate of accumulation of lactic acid.

Further work is required on the effect of temperature on glycolysis and respiration in brain. It is clear, however, that a marked change in the type of metabolism occurs when we surpass the temperature limit which is compatible with the life of the whole animal.

Summary.

1. The rates of respiration and of aerobic glycolysis in brain cortex at 42° are only slightly higher than these rates at 37°. At 45° however the respiration is increased by 100 % above that at 37° and the aerobic glycolysis is increased many fold.

2. A similar abrupt rise in rate above 42° is also observed in the anaerobic glycolysis though this falls extremely rapidly at 45°.

3. The aerobic glycolysis at 45° is due to the disproportionately great increase in glycolysis in relation to the increase in respiration. There is no specific inhibition of the Pasteur effect, since the Meyerhof quotient is normal.

4. The results obtained show that the rates of carbohydrate catabolism in brain at various temperatures do not follow the Arrhenius law. The rate of catabolism rises very abruptly above 42°.

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