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THE EFFECT ON CELL DIVISION OF INHIBITING AEROBIC GLYCOLYSIS.

R. J. O'CONNOR.

From the Westminster School of Medicine, London, S.W. 1.

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In the normal development of the chicken midbrain it has been shown that, as the number of dividing cells decreases, there is a corresponding decrease in the rate of aerobic glycolysis. In addition it was possible to demonstrate a linear relationship between the two (O'Connor, 1950*a*). On the other hand, the rate of oxygen consumption underwent no variation during the developmental period considered.

A dependance of cell division on aerobic glycolysis was thus suggested. If this is so, it would be expected that when aerobic glycolysis is inhibited there would be some associated effect on cell division. Using the isolated midbrain of the embryonic chick, aerobic glycolysis has been inhibited by sodium fluoride and sodium iodoacetate, and the effect observed on dividing cells and on oxygen consumption.

MATERIAL AND METHODS.

In all experiments isolated portions of the midbrain of the six-day chicken embryo were used. The tissue was isolated and the volume measured by the method, and in the medium, previously described (O'Connor, 1950*a*). Using this medium the rate of respiration was determined by the Cartesian diver micromanometer, using divers with central cups (O'Connor, 1948, 1949). The cups contained sodium hydroxide to absorb carbon dioxide so that the normal rate of respiration could be directly determined. These divers contained, in the neck, a solution of the reagent to be tested, which, after determining the normal rate of respiration, was transferred to the tissue in the bulb of the diver and the alteration in the respiratory rate determined for the period 1 to 2 hours after the addition of the reagent.

The effect of the reagents, sodium fluoride and sodium iodoacetate on aerobic glycolysis was determined in divers filled as described by O'Connor (1950*a*). As in the case of respiration, the reagent was added to the tissue from the neck

of the diver, and the alteration in the rate of aerobic glycolysis measured for the period 1 to 2 hours after the addition was made.

The estimation of the rate of aerobic glycolysis by this method requires the determination of the respiratory quotient, which was done separately (O'Connor, 1950*a*), and which, in the concentrations of fluoride and iodoacetate used, did not alter from the normal value of 1·0.

Mitotic damage was assessed by exposing fragments of the isolated midbrain to various concentrations of fluoride and iodoacetate in small test-tubes (O'Connor, 1950*b*). This method reproduces the conditions under which observations were made on the rate of respiration. It was considered necessary to make certain that the results would not be altered in the conditions under which aerobic glycolysis was measured, that is, in a bicarbonate-containing medium, and in an atmosphere of 5 per cent carbon dioxide/95 per cent oxygen. Results were, however, the same under both conditions. In both series of experiments the time of exposure of the tissue to the reagents was 90 min., to correspond to the period 1 to 2 hours after the addition of the reagent, for which alterations in the rate of respiration and aerobic glycolysis were measured.

EXPERIMENTAL RESULTS.

The six-day embryos used had an eye diameter of 3·5 to 4·0 mm. In such embryos the midbrain has the following normal values :

Respiration, 0·8 c.mm. O_2/c.mm. tissue/hr.
Aerobic glycolysis, 1·7 c.mm. CO_2/c.mm. tissue/hr.
Dividing cells, 25,000/c.mm. tissue.*
Respiratory quotient, 1·0.

The effect of sodium fluoride and sodium iodoacetate on glycolysis and oxygen consumption is expressed as percentage inhibition occurring, as already stated, in the period 1 to 2 hours after the addition of the reagent. It is possible in the case of iodoacetate that equilibrium has not been reached in this period, but this is of small importance in the present consideration, since the effect on cell division is measured for an equivalent time period.

The method of assessing mitotic damage did not permit of quantitative measurements but, in a series of experiments using serial dilutions by steps of two, the following concentrations were determined : Firstly, the concentration in which there was some detectable change in dividing cells when the tissue was examined by " squash " preparations. This consisted of an irregularity of the separated chromosomes in dividing cells. Secondly, the concentration was determined in which pycnotic fusion of chromosomes was regularly observed (see O'Connor, 1949, 1950*b*). Thus a range of concentrations was determined over which damage to chromosomes became visible and proceeded to their complete fusion. This range of concentration is indicated by the vertical lines A, B in Fig. 1 and 2. It should be pointed out that the " squash " preparations are inadequate to detect chromosome fragmentation that has been revealed in anaphase by more refined methods (see Loveless and Revell, 1949). This was seen, however, in some cases. Also, the value indicated by the line B in Fig. 1 and 2 is somewhat higher than values for the effective mitotic concentration referred to previously (O'Connor, 1950*b*).

* Possibilities of error in this measurement have been previously discussed (O'Connor, 1950*a*).

Results with sodium fluoride.

In the concentrations used there was no inhibition of respiration and no change in the respiratory quotient. The effect of sodium fluoride on aerobic

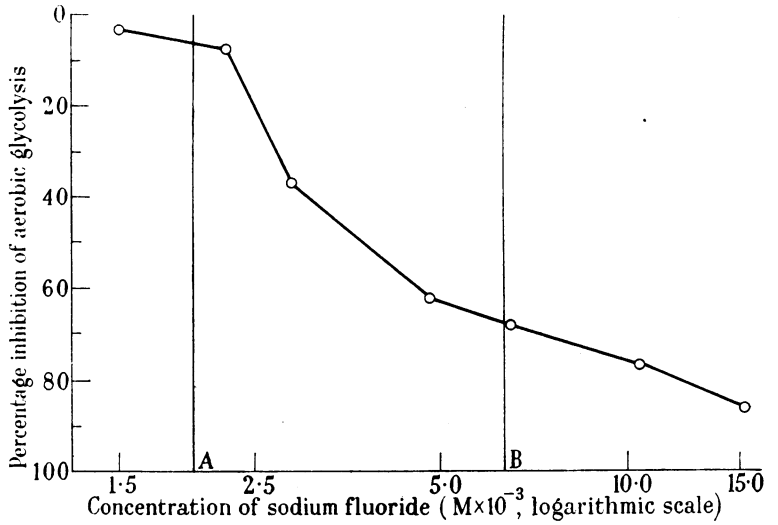

FIG. 1.—The effect of sodium fluoride on aerobic glycolysis and cell division. A, B, see text.

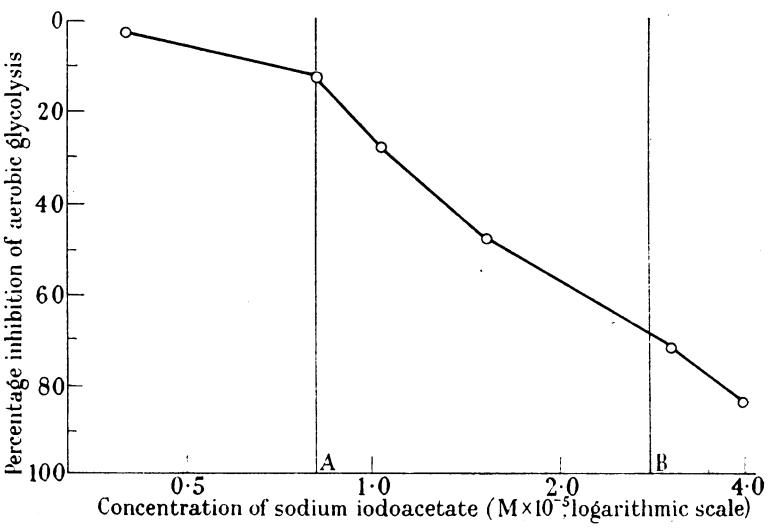

FIG. 2.—The effect of sodium iodoacetate on aerobic glycolysis and cell division. A, B, see text.

glycolysis is recorded in Fig. 1, the concentration of fluoride being recorded on a logarithmic scale. Each plotted point is derived from 4 to 8 separate measurements. As described above, the lines A, B indicate the range of concentration

over which visible damage to dividing cells begins and proceeds to complete pycnotic fusion of chromosomes, that is, concentrations of 2·0–6·3 M $\times 10^{-3}$. At these concentrations inhibition of aerobic glycolysis begins and increases to 70 per cent inhibition.

Results with sodium iodoacetate.

As with sodium fluoride respiration was not inhibited, and the respiratory quotient not altered, in concentrations investigated. The effect of sodium iodoacetate on aerobic glycolysis and dividing cells is recorded in Fig. 2, as with sodium fluoride. It will be seen that the range of mitotic damage, A–B, is between the concentrations 0·7–2·5 M $\times 10^{-5}$, in which concentrations inhibition of aerobic glycolysis begins and increases to 70 per cent inhibition.

DISCUSSION.

In normal adult tissue it has been shown that the action of fluoride on carbohydrate metabolism is due to its combination with magnesium, resulting in the inhibition of enolase (Warburg and Christian, 1942), while Rapkine (1938) has shown that iodoacetate affects carbohydrate metabolism due to its effect on the –SH groups of triosephosphate dehydrogenase. However, in the whole chicken embryo, Needham and Lehmann (1937) have produced evidence indicating that glycolysis proceeds by non-phosphorylating paths—a process referred to as " non-phosphorylating glucolysis."

One of the points of evidence for this path of carbohydrate breakdown is the difference in the inhibition of glycolysis produced by fluoride in muscle and in the chick embryo. At a concentration of M/200 the formation of lactic acid from glycogen in muscle is 90 per cent inhibited (Lohmann, 1931), while Needham and Lehmann show that the " non-phosphorylating glucolysis " in the chicken embryo is only 40 to 50 per cent inhibited by the same concentration. In the midbrain tissue of these experiments the inhibition at this concentration is approximately 60 per cent (Fig. 1), so that this observation cannot be used to identify the carbohydrate metabolism of the midbrain with that of muscle or with the process described by Needham and Lehmann in the whole embryo. In any case it is doubtful if the comparison would be valid, for the authors quoted measured glycolysis in anaerobic conditions. The process measured in these experiments is that occuring in the presence of oxygen.

Uncertainty as to the paths of glycolysis in the midbrain of the embryonic chicken must remain, and hence as to the mode of action of fluoride and iodoacetate on it. Nevertheless, the known properties of these two substances make it unlikely that they act in the same way. Thus the relationship of mitotic damage to inhibition of glycolysis produced by both substances is of significance. In both cases inhibition of glycolysis begins in concentrations where mitotic damage first becomes visible, and complete fusion of chromosomes occurs when the inhibition of glycolysis is 70 per cent. From this similarity it is concluded that the damage to mitosis is associated with common factor of the inhibition of glycolysis.

Such a conclusion requires consideration with regard to possibilities arising out of the work of Rapkine (1931) and reviewed by Brachet (1944). It is suggested that spindle formation and function is associated by a reversible denaturation

of protein controlled by oxidation and reduction of the contained -SH groups. It is further suggested the inhibitory action of iodoacetate on cell division is due to the interference with this mechanism due to the ability to combine with -SH groups demonstrated by Dickens (1933). Final conclusions are not justified by the present results, and such a possibility is not meant to be excluded. However, the similarity of the action of fluoride and iodoacetate discussed in the previous paragraph makes it necessary to favour the view that the effect of iodoacetate on cell division is associated with its effect on aerobic glycolysis.

Therefore, in the midbrain of the embryonic chicken the action of fluoride and iodoacetate leads to the conclusion that there is an association between cell division and aerobic glycolysis which is in accordance with the association previously demonstrated in normal development (O'Connor, 1950*a*).

If such findings are due to a dependance of cell division on aerobic glycolysis the conclusions of Bullough (1950) must be considered. Studying cell division in the skin of the mouse, Bullough concludes that cell metabolism alters both quantitatively and qualitatively during the mitotic cycle. The earlier stages, involving the addition of nucleic acid to the chromosomes, require the metabolism of carbohydrate with the use of oxygen and the energy requirements are high. Later stages, involving the separation of chromosomes into the daughter cells, require smaller amounts of energy, which, it is concluded, could be derived from carbohydrate breakdown without the use of oxygen, that is, by a process which is, presumably, glycolysis. The possibility must therefore be considered that any causal relationship between aerobic glycolysis and cell division may be confined to the later, rather than to the earlier stages of the mitotic cycle.

SUMMARY.

In the isolated midbrain of the six-day chicken embryo aerobic glycolysis was inhibited by sodium fluoride and sodium iodoacetate.

With both compounds, when aerobic glycolysis was 70 per cent inhibited, a pycnotic fusion of chromosomes in dividing cells was produced.

At concentrations producing these changes there was no inhibition of respiration.

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