INTRODUCTION

Although there is considerable evidence that carbohydrate metabolism plays an important part in the process of cell division (see Bullough, 1952), conclusions have differed regarding the relative importance of glycolysis and catabolism involving respiration. In the adult mouse epidermis Bullough & Johnson (1951) found that cell division was dependent upon the respiratory oxidation of pyruvate by the tricarboxylic acid cycle. On the other hand, Pomerat & Willmer (1939) showed that, in tissue culture, agents that inhibited respiration had little immediate effect on cell division, while Laser (1933) found that growth of cultured fibroblasts could continue when respiration was greatly diminished by low oxygen tensions. Further, Warburg (1930) concluded that cell growth is associated with glycolysis.

A similar difference in the relationship of mitotic activity to the respiratory and glycolytic forms of carbohydrate metabolism was indicated by changes occurring in the midbrain and the red-blood cells of the chicken embryo during embryonic development. In the midbrain (O'Conor, 1950a) there was a corresponding decrease in the number of dividing cells in a unit volume of tissue and in the rate of aerobic glycolysis as measured by the production of acid by midbrain tissue isolated in a glucose containing medium (acid formation did not occur when glucose was omitted from the medium; O'Conor, 1949). Under these conditions the rate of respiration remained constant. Since the respiratory quotient was unity, this constant rate of respiration was taken to indicate a constant rate of glucose utilization by respiratory metabolism. Further investigations (O'Conor, 1950b) showed that, when the rate of aerobic glycolysis of the isolated midbrain was decreased by fluoride or iodoacetate, cell division was inhibited by concentrations which did not diminish the rate of respiration nor alter the respiratory quotient. Thus it was concluded that, in the midbrain, cell division was dependent on aerobic glycolysis, as measured by the acid production of the isolated tissue in a glucose-containing medium.

When the red-blood cells were investigated in a similar medium (O'Conor,
1951) it was found that acid formation did not occur but the rate of respiration decreased during development in a manner corresponding to the decrease in the proportion of dividing cells. The respiratory quotient of the red-blood cells was unity, so that these findings suggested that there was a corresponding decrease in mitotic activity and the catabolism of glucose by processes involving respiration. This suggestion was supported by subsequent investigations which showed that the inhibition of respiration by fluoride and by iodoacetate resulted in inhibition of cell division (O'Connor, 1952). Mitotic activity in the red-blood cells and in the midbrain thus differs in its relationship to carbohydrate catabolism, since in the former it is associated with the respiratory utilization of glucose, and in the latter with aerobic glycolysis, as measured by the acid production of the isolated tissue in a glucose-containing medium.

In order better to assess the significance of this difference, comparisons have been made of the changes undergone by mitotic activity and carbohydrate metabolism in the developing liver of the chicken embryo, the metabolism of which differs in certain relevant respects from that of the midbrain and red-blood cells. As shown previously (O'Connor, 1953a), in isolated liver tissue, the respiratory quotient is less than unity, indicating that oxygen is utilized in the metabolism of substrates other than carbohydrate. Further, the carbohydrate metabolism of the liver is complicated by the deposition of considerable amounts of glycogen during the course of its development.

**MATERIAL AND METHODS**

All observations were made on the liver of chicken embryos incubated at 38°C. The eggs were taken from the batches used previously (O'Connor, 1953a), and the observations were scattered at random among the batches. As previously, the embryos were grouped in arbitrarily chosen stages of development. Each stage represents an increase in the eye diameter of 0.7 mm. and is referred to below by its median eye diameter (M.E.D.). The mean time of incubation for each of these stages has already been recorded (O'Connor, 1953a), and, since the eggs used in these experiments are from the same batches, these times are applicable to the present observations and are repeated in Table 1.

*The estimation of glucose utilization by isolated liver tissue*

In the accepted scheme of carbohydrate catabolism in animal tissues the substrate, whether glucose or glycogen, is first transformed into pyruvic acid. In the presence of oxygen, part or all of the pyruvic acid is oxidized by way of the tricarboxylic acid cycle with the consumption of oxygen. On the other hand, even in the presence of oxygen, part of the pyruvic acid may be reduced to lactic acid. These two processes, respiratory catabolism and aerobic glycolysis, have been separately estimated from observations on the gaseous exchange of isolated liver tissue.

(a) *Acid production by isolated liver tissue.* In order to determine the rate of
aerobic glycolysis of the isolated liver, the rate of total acid production was measured by a Cartesian diver micromanometer. Although the technique used has already been recorded (O’Connor, 1950a), it possesses certain limitations requiring present consideration and is, therefore, recapitulated in part.

Livers were removed from the embryos and placed in the following medium: NaCl 0·9 g., KCl 0·02 g., MgCl₂ 0·02 g., CaCl₂ 0·02 g., glucose 0·20 g., water 100 ml., to which was added 10 ml. M/15 phosphate buffer (Sørensen) to produce pH 7·4; this is the medium previously used (O’Connor, 1953a) to determine the rate of respiration and the respiratory quotient. As in those determinations, fragments of liver were cut from the anterior surface of the right lobe of the liver and the volume measured; this volume varied from 0·3 to 0·8 c.mm. The fragments were then washed twice in the following medium: NaCl 0·9 g., KCl 0·02 g., MgCl₂ 0·02 g., CaCl₂ 0·02 g., glucose 0·20 g., NaHCO₃ 0·20 g., water 100 c.c. The liver fragments were then introduced into divers containing this medium. The gaseous phase of the divers was 95 per cent. O₂: 5 per cent. CO₂ and the arrangement of fluids within the divers, as well as their dimensions, were as described previously (O’Connor, 1950a). In such divers it should be noted that there is no alkali to absorb carbon dioxide so that the manometric reading is affected not only by carbon dioxide released from the bicarbonate in the medium as the result of acid formation, but also by the respiration of the liver tissue and by the carbon dioxide formed as a consequence. In order to take account of the last two factors the assumption was made that they occurred at the same rate under the conditions of the present observations as they did under the conditions in which respiration and respiratory quotient were investigated previously (O’Connor, 1953a), when the medium contained phosphate and not bicarbonate (see above) and the gaseous phase of the divers was oxygen. Using these results, a figure was calculated which represented the amount of carbon dioxide presumed to result from acid formation, and this will be referred to as ‘presumed acid formation’. In arriving at this figure the factor was calculated, both for oxygen and carbon dioxide, which related manometric change to the change in gaseous content of the diver. The formula given by Boell, Needham, & Rogers (1939) was used. Comparable observations and calculations were made when the isolated liver was suspended in the bicarbonate-containing medium from which glucose was omitted. All observations were made over a period of 2 hours, during which time the rate of change in the manometric reading did not vary significantly in any one particular observation.

(b) Respiratory utilization of glucose by isolated liver tissue. Since, as mentioned above, the oxygen consumed by isolated liver tissue is used in the catabolism of substrates other than carbohydrate, the rate of respiration cannot be used directly as a measure of the respiratory catabolism of glucose as it was in the case of the midbrain and red-blood cells. It was considered, however, that this rate could be estimated from the decrease in the rate of respiration which resulted when glucose was omitted from the medium in which respiration was
measured. Rates of respiration of the isolated liver in the presence and absence of glucose have been recorded previously (O'Connor, 1953a), and these were used in the present investigations to calculate the rate of the respiratory utilization of glucose at different stages of development.

The estimation of mitotic activity of the liver cells

This was carried out by determining, from histological sections, the percentage of hepatic cells in mitosis. Cells other than hepatic cells were not considered. Sections cut at a thickness of 6μ from livers fixed in Bouin's fluid were stained with iron haematoxylin. No counter-stain was used.

RESULTS

Glucose catabolism of isolated liver tissue

(a) Rate of 'presumed acid formation'. The mean value and the standard error of the mean is recorded for each developmental stage in Table 1, and results obtained in the presence and absence of glucose are given. In the latter case the values obtained were so regularly in the vicinity of zero that the results for more than one stage of development were treated together when the mean values and standard errors were determined.

(b) Rate of respiratory catabolism. The differences in the mean values for the rate of respiration in the presence of glucose and its absence, by which respiratory utilization of glucose is measured, are recorded for each of the developmental stages in Table 1. The standard error given for each difference is the standard error of the difference of the two means concerned and has been calculated from data already recorded (O'Connor, 1953a).
Mitotic activity in liver cells

To determine the percentage of hepatic cells in mitosis 2,000-3,000 cells were examined in each individual case. The mean value for a number of embryos in each developmental stage and the standard error of the mean are recorded in Table 1. Since the nuclei of dividing and resting cells differ in size an error is presumably introduced (Abercrombie, 1946). This error, however, would not differ greatly at different stages of development and would not, therefore, diminish the significance of the decrease during development of the percentage of cells seen in mitosis and particularly between the stages of M.E.D. 3·2 mm. and M.E.D. 4·6 mm.

DISCUSSION

Aerobic glycolysis by isolated liver tissue

The rates of ‘presumed acid formation’ were calculated in order to measure rates of aerobic glycolysis, that is the production of lactic acid from glucose. However, before the results are used for this purpose certain considerations are necessary. It will be recalled that the ‘presumed acid production’ was calculated on the assumption that the rate of respiration and the respiratory quotient were the same under the conditions of the present observations as in the conditions under which they were previously determined (O’Connor, 1953a); Dixon (1951) has pointed out that this assumption may not be justifiable. However, the gaseous exchange concerned, namely the uptake of oxygen and the resulting evolution of carbon dioxide, would affect manometric readings in opposite directions and so decrease any error introduced. Also, none of the results recorded in Table 1 for ‘presumed acid formation’ are significantly below zero. Since negative results can be considered ‘absurd’, their absence is in favour of the calculation being based on correct assumptions. Therefore, even if the possibility of error is not completely eliminated, it is considered that ‘presumed acid formation’ sufficiently represents true acid formation by isolated liver tissue to conclude that during development this decreases to reach a rate of nearly zero at stages of M.E.D. 5·3 mm. and later (see Table 1). In a previous publication (O’Connor, 1953a) the evidence was considered which excludes the possibility that this decrease is due to the addition, during development, of metabolically inert substances to the hepatic cells.

Although uric acid may be formed by isolated liver tissue (O’Connor, 1953a), the amount of carbon dioxide it releases from bicarbonate is unimportant because when glucose is omitted from the medium, acid formation nearly disappears (Table 1). Thus the acid formed is nearly, if not completely, derived from glucose, and can therefore be presumed to be lactic acid. For these reasons it is considered that ‘presumed acid formation’ is sufficiently a measure of lactic acid production from glucose to permit its use as a measure of aerobic glycolysis.
The relationship of cell division to carbohydrate metabolism

Using ‘presumed acid formation’ as a measure of aerobic glycolysis the rate of this process at different developmental stages has been compared, in Text-fig. 1, with corresponding rates of respiratory glucose utilization and with mitotic activity. Line $AB$ is added to the figure to indicate the first appearance of glycogen in embryos of the stage of M.E.D. 4·6 mm. These embryos have a mean incubation time of 6·9 days (O’Connor, 1953a). The figure shows that with the appearance of glycogen there is an alteration in the relationship between mitotic activity and the glucose metabolism of isolated liver tissue. Before glycogen appears there is a corresponding decrease in the proportion of dividing cells, the rate of ‘presumed acid formation’, and the rate of the respiratory utilization of glucose (Text-fig. 1). After the appearance of glycogen these metabolic processes almost disappear, although dividing cells persist and constitute about 0·6 per cent. of hepatic cells. Furthermore, both before and after the appearance of glycogen the relationship between mitotic activity and glucose metabolism by the isolated tissue differs from that found in the midbrain and in the circulating red-blood cells, for in the former decreasing mitotic activity is associated with decreasing acid production from glucose (O’Connor, 1950a), while
in the latter the association is with decreasing respiratory metabolism (O'Connor, 1951). These differences are summarized in Table 2, and it is possible that they represent a significant difference in the metabolic relationships of mitotic activity in the four tissues. On the other hand, the similarity of the mitotic mechanism in these and other tissues suggests the alternative possibility that

**Table 2**

*Changes in the rate of carbohydrate metabolism associated with the decrease in mitotic activity which occurs during the development of tissues in the chicken embryo*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Rate of carbohydrate metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Respiratory</td>
</tr>
<tr>
<td>Midbrain (O'Connor, 1950a)</td>
<td>Remains constant</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Red-blood cells (O'Connor, 1951)</td>
<td>Decreases as mitotic activity</td>
</tr>
<tr>
<td></td>
<td>decreases</td>
</tr>
<tr>
<td>Liver before glycogen appears (see text)</td>
<td>Decreases as mitotic activity</td>
</tr>
<tr>
<td>Liver after glycogen appears (see text)</td>
<td>decreases</td>
</tr>
<tr>
<td></td>
<td>Very low</td>
</tr>
</tbody>
</table>

The rates of carbohydrate metabolism are those of the isolated tissue in a glucose-containing medium. In the references quoted and in the text reasons are given for regarding acid production as a measure of aerobic glycolysis.

the differences recorded in Table 2 are nevertheless an expression of a common dependence of cell division on carbohydrate catabolism, as has been suggested by Bullough (1952) and which, in the case of the midbrain and the red-blood cells, has been indicated by the correspondence between the effect on cell division and the inhibition of carbohydrate catabolism produced by fluoride and iodoacetate (O'Connor, 1950b, 1952).

In the case of the midbrain, red-blood cells, and the liver before the appearance of glycogen, the findings of Table 2 could be accounted for if mitotic activity were dependent on the total amount of pyruvate disposed of by conversion to lactic acid in aerobic glycolysis together with its oxidation by the tricarboxylic acid cycle. Thus in the case of these three tissues it is possible that the reactions by which pyruvate is formed from glucose play an essential part in cell division. Such reactions could occur in the liver after the appearance of glycogen, in spite of the findings in Table 2, if pyruvate or compounds intermediate in its formation were incorporated into larger molecules. Such reactions leading to glycogen formation are well known in the adult liver, and it is likely that they occur in the embryonic liver after the appearance of glycogen, for Dalton (1937) has concluded that at this stage the liver of the chicken embryo becomes capable of adult functions. Thus the findings in all four tissues recorded in Table 2 could be accounted for on the assumption that mitotic activity was
dependent on the reactions leading to formation of pyruvate from glucose. Although there is not sufficient evidence to establish such a relationship, it is possible to consider its implications and compare them with results obtained by other investigators. Such is the purpose of the following discussion.

There is considerable evidence that in the metabolism of glucose there is a pathway alternative to the glycolytic pathway of the Meyerhof scheme. In this hexose monophosphate oxidative route\(^1\) glucose is converted into ribose-5-phosphate by reactions which involve the consumption of oxygen (Dickens & Glock, 1951). In the further metabolism of ribose-5-phosphate, a triose is formed which is incorporated into glucose-6-phosphate. Evidence for such reactions has been found in a number of tissues which include, in the adult, the liver (Glock, 1952), the red-blood cells (Dische, 1951), and brain tissue (Sable, 1952). Pyruvate could be formed as a result of such reactions since both the triose and the glucose-6-phosphate could be acted upon by enzymes of the glycolytic system. Thus the possibility that mitotic activity depends upon reactions leading to pyruvate formation gives rise to the further possibility that the actual reactions concerned may be those associated with the formation of ribose-5-phosphate. Since ribose-5-phosphate is similar to the pentose component of ribonucleic acid, this possibility would be in accordance with the well-established association of cell proliferation and ribonucleic acid (Caspersson, 1950; Brachet, 1947). Further, the association of cell division with the alternative hexose monophosphate oxidative route of glucose metabolism might constitute a basis for an explanation for a special form of metabolism which, it has been claimed, is associated with embryonic development (cf. Moog, 1944).

However, pyruvate formation by the hexose monophosphate oxidative route with ribose-5-phosphate as an intermediate compound involves the consumption of oxygen. Therefore, to suggest that such a process plays a dominant part in cell division makes it necessary to consider objections that might arise from observations suggesting that cell division can occur independently of oxygen consumption. In the case of the observations made on the isolated midbrain tissue, and recorded in Table 2, the association of decreasing mitotic activity with a constant rate of oxygen consumption might be the basis of such an objection. However, modifications of enzyme activity occurring in the course of differentiation might be adequate to meet this objection, since it is possible that any decrease of the oxygen utilized in the formation of ribose-5-phosphate may be balanced by an increase in the proportion of pyruvate oxidized by the tricarboxylic acid cycle. After the appearance of glycogen in the liver of the chicken embryo it might be questioned whether the oxidative formation of ribose-5-phosphate could occur because of the low values obtained for oxygen consumption associated with the catabolism of glucose (see Table 1). However,

\(^1\) There has been discussion about the most appropriate name for this metabolic pathway (Dickens, 1953). The term 'hexose monophosphate oxidative route', suggested by Dickens, will be used.
since measurements were made on isolated liver tissue they would not exclude the presence of the hexose monophosphate oxidative route in the intact embryonic liver, particularly since, in the adult liver, the necessary enzymes have been demonstrated (Dickens & Glock, 1951), and it is to be expected that such enzymes would be present in the liver of the chicken embryo after glycogen appears because of the evidence that, at this period of development, the embryonic liver is capable of adult function (Dalton, 1937).

Apart from the results recorded in Table 2 the possibility of a dependence of mitotic activity on an oxygen-consuming process might appear inconsistent with observations that, in some cells, division can be completed in the absence of oxygen (e.g. Lettre, 1951). Although, in such circumstances, glycolytic reactions could continue and be adequate to meet the energy requirements of the cell, the reactions leading to ribose-5-phosphate formation would presumably cease. However, if cell division were dependent on ribose-5-phosphate formation, its failure in such conditions might not inhibit mitosis, at least for a time, since the failure of ribose-5-phosphate formation might be balanced by a decreased rate of destruction. Further, if ribose-5-phosphate is concerned in the synthesis of ribonucleic acid (Cohen, 1951) any failure of its formation might be met by utilization of precursors of nucleic acid in the cell (cf. Walker & Yates, 1952).

Even if it were established that cell division was dependent on the reactions leading to pyruvate formation, it is unlikely in ordinary circumstances that pyruvate formation, whether by the glycolytic or by the hexose monophosphate oxidative route, meets the energy requirements of the dividing cell because the greater part of the energy produced in carbohydrate catabolism comes from the oxidation of pyruvate by the tricarboxylic cycle (Burton & Krebs, 1953). Further, Bullough & Johnson (1951) have shown by experiment that, in the isolated adult mouse epidermis, cell division is dependent on the oxidation of pyruvate in the tricarboxylic acid cycle. In most cells this would constitute a dependence of mitotic activity on carbohydrate catabolism, since pyruvate is derived from carbohydrate and in cells with a respiratory quotient of unity carbohydrate may be its sole source (e.g. the midbrain tissue and red-blood cells of the chicken embryo; O'Connor, 1950a, 1951). However, in the case of the liver after the appearance of glycogen it may be necessary to make an exception, because the respiratory quotient of isolated liver tissue is 0.69, which suggests that the oxygen consumed is utilized in the formation of uric acid from protein (O'Connor, 1953a). In this case, therefore, it may be that the energy requirements of dividing cells are met by the catabolism of protein rather than of carbohydrate. It is relevant to note that after glycogen appears no difference could be detected, by histochemical methods, in the amount present in dividing and non-dividing cells (O'Connor, 1953b).

This possible exception apart, the suggestion that the process of cell division depends on reactions leading to pyruvate formation can be regarded as additional to the dependence of cell division on carbohydrate metabolism for its
energy requirements. It may be that one or other dependence is limiting to cell division at different stages of the mitotic cycle, for Bullough & Johnson (1951) concluded that the respiratory oxidation of pyruvate is necessary for the initiation of cell division but not for its completion (see also Bullough, 1950). It might therefore be suggested that a continuance of cell division, once it has begun, depends upon reactions leading to pyruvate formation and in particular upon those associated with ribose-5-phosphate formation. Such a suggestion would be in accordance with the observations of Jacobson & Webb (1952) that ribonucleoprotein is formed in the nucleus during mitosis and extruded into the cytoplasm at anaphase. As mentioned above, such a formation of ribonucleic acid might, as an alternative, be possible from precursors already in the cell, and thus account for the ability of some cells to complete cell division, once it has begun, 'in almost any circumstances short of death of the cell itself' (Bullough, 1952).

If this suggested double dependence of mitotic activity on carbohydrate catabolism exists, cell proliferation might be controlled by a balance between the reactions leading to the formation of pyruvate and those of the tricarboxylic acid cycle. If the latter were limited by anaerobiosis an altered balance might cause a modification of proliferative capacity and in this way account for the association found by Goldblatt & Cameron (1953) between intermittent anaerobiosis and the malignant conversion of cultured fibroblasts. Again, it can be suggested that such a disturbed balance might occur if the respiratory mechanism of cells was damaged and replaced by a process involving lactic acid formation—a change that Warburg (1930) associated with the development of malignancy and one that might follow deficiencies in the tricarboxylic acid cycle for which evidence has been found in malignant tissue (Potter & Busch, 1950). Further, it might be possible to relate such a disturbed balance to changes in the cell fractions that can be separated by ultracentrifugation. It has been found that the reactions by which pyruvate is formed, whether by the glycolytic or the hexose monophosphate oxidative route, are associated with the soluble fraction (Le Page & Schneider, 1948; Glock & McLean, 1952), while the reactions of the tricarboxylic acid cycle are associated with the mitochondria (Harman, 1950).

**SUMMARY**

1. From micromanometric measurements made on liver tissue of the chicken embryo, isolated in a glucose-containing medium, estimates have been made of the rate of glucose catabolism ending in acid formation and that utilizing oxygen.

2. The variations undergone by these metabolic processes during normal development have been compared with the variations in mitotic activity of the hepatic cells as determined by the proportion of dividing cells seen in histological sections.

3. The comparison revealed two different associations in the embryonic liver...
between carbohydrate metabolism and mitotic activity. Before the appearance of glycogen, mitotic activity, the estimated rate of acid production from glucose, and the rate of respiratory glucose utilization decreased in a corresponding manner. After the appearance of glycogen, mitotic activity persisted, but the estimated rates of both metabolic processes fell nearly to zero.

4. Both before and after the appearance of glycogen these relationships of mitotic activity to carbohydrate metabolism differed from those previously recorded for the midbrain and the red-blood cells, which in turn differ one from the other. The suggestion has been made that these differences are consistent with a common dependence of mitotic activity on metabolic reactions leading to pyruvate formation, special consideration being given to the reactions of the hexose monophosphate oxidative route (Dickens, 1953), concerned in the formation of ribose-5-phosphate.

5. This possible dependence is considered to be additional to a dependence of mitotic activity on the energy produced from carbohydrate by the reactions of the tricarboxylic cycle. The implications of such a double dependence of mitotic activity on carbohydrate metabolism have been discussed.

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