RESPIRATION AND CELL DIVISION IN THE RED BLOOD CELLS OF THE CHICKEN EMBRYO.

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In the normal development of the midbrain of the chicken embryo it has been shown that there is a linear relationship between the number of dividing cells and the rate of aerobic glycolysis (O'Connor, 1950*a*). A dependence of cell division on aerobic glycolysis was thus suggested and further supported by investigations which demonstrated mitotic damage when aerobic glycolysis was inhibited by fluoride and iodoacetate. The concentrations of both substances producing this effect did not inhibit respiration (O'Connor, 1950*b*).

In order to investigate the possibility of similar relationships in the circulating blood cells of the chicken embryo the variations of cell division, aerobic glycolysis, respiration and respiratory quotient have been measured during normal development.

MATERIALS AND METHODS.

Preparation of blood for examination.

Chicken embryos of the third to ninth days of incubation were used, blood being removed from the heart or from a blood vessel by a capillary pipette. Except where a modification is described the blood was discharged into the following medium, anhydrous compounds being used: NaCl 0·9 g., KCl 0·02 g., $MgCl_2$ 0·02 g., $CaCl_2$ 0·02 g., glucose 0·1 g., water 100.0 ml., to which 10 ml. of M/15 phosphate buffer (Sørensen) was added to produce pH 7·4.

The cells were washed and concentrated to produce a suspension containing 10^5–10^6 cells per c.mm. No attempt was made to separate red cells from white, since the latter were too few to affect measurements made on all the cells. This investigation is therefore to be considered as one on the circulating red blood cells.

The measurement of cell volume.

Since the size of the circulating red cells decreases with development, the measurements of respiration and aerobic glycolysis were related to a unit volume of cells rather than to a unit number. The average cell volume was determined from the haematocrit, portions of the cell suspensions being centrifuged in capillary tubes of constant internal diameter. Since the cells were suspended in the medium described it was possible that some difference in the tonicity of cells and medium might affect the haematocrit reading. However, microscopic measurements revealed no significant differences between the average diameter

of cells suspended in the medium and of equivalent cells suspended in embryonic plasma directly after removal from the embryo.

The measurement of cell division.

A small drop of the suspension was placed on a microscope slide and mixed with the following stain: Lacmoid (resorcin blue), 2·2 g.; glacial acetic acid, 100·0 ml. This solution was diluted with an equal volume of water and filtered immediately before use. The mixture of suspension and stain was covered with a cover-slip, ringed with vaseline and examined forthwith. An even distribution of dividing cells was obtained and the percentage determined in 2000–3000 cells.

The measurement of the rate of respiration and respiratory quotient.

The Cartesian diver micromanometer, as previously described (O'Connor, 1950*a*), was used. About 10 c.mm. of the cell suspension, containing between 1 and 5 million red cells, was placed in the divers and rates of respiration determined per million cells. By means of the cell volume the rate of respiration was expressed as c.mm. oxygen per c.mm. red cells per hour. The measurements were not affected by variations in the amount of haemoglobin bound to the red cells because the observations were made in an atmosphere of oxygen and the haemoglobin remained saturated throughout.

The measurement of the rate of aerobic glycolysis.

This was determined as described previously (O'Connor, 1950*a*). The cell suspension was made in a bicarbonate-containing medium and the measurements made in Cartesian divers containing an atmosphere of 95 per cent oxygen/5 per cent carbon dioxide.

RESULTS.

Table I summarizes the results obtained from embryos of 3 to 9 days' incubation, each figure being obtained from 10–20 separate measurements. It will be seen that throughout the respiratory quotient does not differ significantly from 1·0 and there is no aerobic glycolysis. During development, however, there is a fall in the number of dividing cells and the rate of respiration.

The daily averages for cell division and respiration are plotted in Fig. 1. After the ninth day the rate of respiration continues to fall and these variations are recorded in the figure. In obtaining these daily averages the corresponding

TABLE I.—*Cell Volume, Respiration, Respiratory Quotient, Aerobic Glycolysis and Cell Division in the Circulating Red Cells of the Chicken Embryo.*

	Day.						
	3.	4.	5.	6.	7.	8.	9.
Respiration, c.mm. $O_2/10^6$ cells/hr.	0·47	0·26	0·19	0·14	0·11	0·056	0·044
Cell volume (u^3)	780	570	500	430	380	240	210
Respiration, c.mm. O_2/c.mm. cells/hr.	0·60	0·46	0·38	0·32	0·29	0·24	0·21
Dividing cells (per cent)	4·6	3·6	2·7	1·3	0·2	0	0
Respiratory quotient	1·04	0·98	1·02	0·96	0·98	1·04	1·06
Aerobic glycolysis	0	0	0	0	0	0	0

measurements were not necessarily made on the same blood suspension, and on the third and fourth days of incubation not only are the measurements changing rapidly but, in addition, there is considerable relative variation in the stage of development reached at any given time. For these reasons Fig. 1 includes observations in which the measurements have been made on the same blood sus-

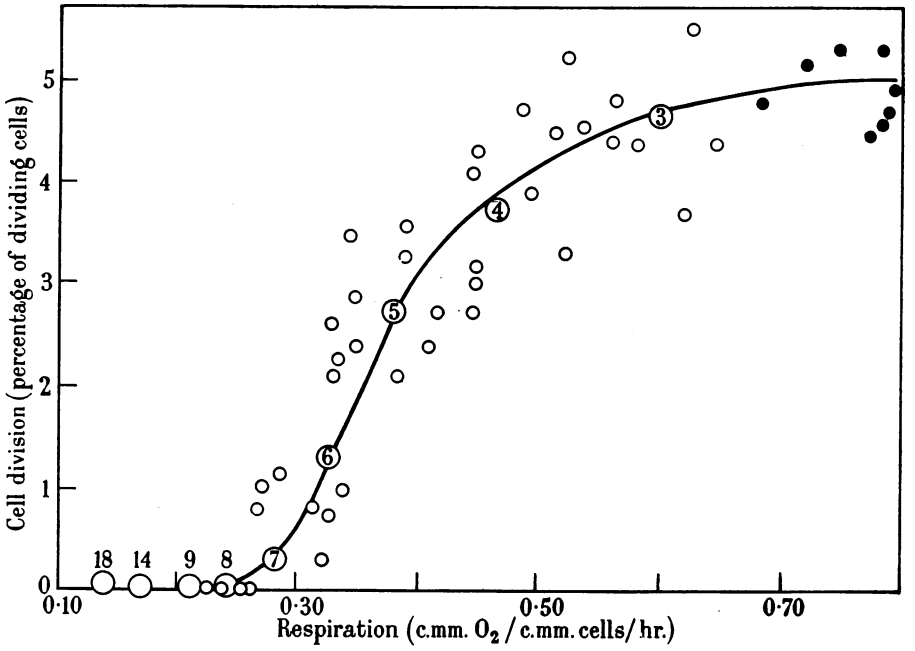

FIG. 1.—The relationship of respiration and cell division in the circulating red blood cells of the chicken embryo. Large circles—daily averages with day indicated. Small circles—corresponding values obtained from the same red cell suspension, the closed small circles being derived from embryos soon after the establishment of the intra-embryonic circulation.

pension ; these are therefore strictly comparable. These measurements include those made on the earliest stages technically possible, that is, on embryos of about 25 somites, soon after the establishment of the intra-embryonic circulation. For this purpose the blood from 5–6 embryos was pooled. These measurements are specifically indicated in Fig. 1.

DISCUSSION.

In considering a possible relationship between the number of dividing cells and the rate of respiration there is the question whether the circulating red cells are homogeneous as regards stage of maturation, and whether cell division is confined to a limited number of the total cells, which are at an earlier stage of development than the remainder. This is unlikely, for in Leishman-stained films no staining differences can be found between the cytoplasm of dividing cells and the immediately adjacent non-dividing cells. Further, the cytological investigations of Dawson (1936) show that throughout incubation the circulating

red cells of the chicken embryo are predominantly of one type, which increases in maturation as incubation proceeds.

Dawson gives figures for the percentage of dividing cells at each day of incubation. In general his figures are somewhat lower than those recorded in Table I, and, in one respect, require comment. On the third day Dawson's figures indicate a rise in the dividing cells from 1·49 per cent to 3·44 per cent, this rise continuing into the fourth day when a decrease, comparable to that recorded here, sets in. It has not been possible to confirm this rise in the percentage of dividing cells on the third day. Blood from three-day embryos at the earliest stage where there was an intra-embryonic circulation gave an average value of 4·8 per cent dividing cells, which is no lower than the average for all three-day embryos. It may be pointed out that Dawson's figures were obtained from the examination of dried smears, while the present results are derived from the wet preparations described. Dry smears were not used because they appeared to give an uneven distribution of dividing cells and because, even in the best preparations, the débris of dividing cells was often seen.

In Fig. 1 the plotted points group themselves around the line drawn, but its significance must be regarded as doubtful owing to the scatter of the individual results. Further, it has to be pointed out that the observations only begin at the third day of incubation when the red blood cells, in terms of haemoglobin formation, have undergone a considerable degree of differentiation and contain 30–40 per cent of the haemoglobin they will eventually acquire.* The observations, therefore, do not cover the earliest stages of red cell development. However, the results obtained on the earliest embryos examined suggest that, to cover the whole range of the variations in respiration and cell division a curved line would best fit the relationship between them.

Although the precise relationship of cell division to respiration cannot be defined there is a difference between these findings and those in the development of the midbrain of the embryonic chicken. There, dividing cells decreased as aerobic glycolysis decreased, while respiration remained constant. In the red cells there is an association between decreasing cell division and decreasing respiration while aerobic glycolysis does not occur. On the other hand, since in both tissues the respiratory quotient is unity, it is probable that the rate of respiration is a measure of the rate of the complete oxidation of carbohydrate, so that in both tissues a decreasing number of dividing cells is associated with a decreasing rate of total carbohydrate metabolism, the two tissues differing in their relationships to the respiratory and glycolytic paths. Further, both tissues can be said to differ from the epidermis of the mouse, where the findings of Bullough (1950) indicate that cell division is dependent on both paths of carbohydrate metabolism.

A possible explanation of these differences may lie in the connection between cell division and cell differentiation. In embryonic tissues, and in tissues capable of regeneration such as skin, the fate of dividing cells is to differentiate, that is, to undergo metabolic processes producing substances characteristic of the tissues concerned. Many observations (e.g., Doljanski, 1930*a*; *b*) show that there is an inverse relationship between cell division and cell differentiation, indicating a linkage between the two processes. Thus, in different tissues, cell division may

* This statement is based on quantitative estimations of haemoglobin content, subsequently to be dealt with in detail.

be linked to different anabolic processes, and this may be the basis for differences in the relationships to the catabolism of carbohydrate. In malignant tissue there is a breakdown of the linkage between cell division and cell differentiation, and it is possible that this breakdown is associated with an alteration of metabolic relationships of cell division, which might explain the differences in the response of normal and malignant or pre-malignant tissues to anti-mitotic agents. Lasnitzki (1948) has shown that urethane inhibits mitosis in cultures of normal cells, but stimulates the growth of C57 sarcoma and adenocarcinoma 63. More recently Green and Savigear (1951) have shown that the inhibitory effect of cortisone and shock on cell division in the skin of the mouse disappears after the application of a carcinogen.

SUMMARY.

In the circulating red blood cells of the chicken embryo the rate of respiration and the number of dividing cells decrease during normal development.

At all stages investigated the respiratory quotient is unity and aerobic glycolysis cannot be demonstrated.

The possibility is discussed that there is in the red blood cells of the chicken embryo a relationship between carbohydrate metabolism and cell division. Comparisons are made with the metabolic relationships of cell division in other tissues.

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