THE DEVELOPMENT OF CYTOCHROME OXIDASE IN THE
CHICK EMBRYO*

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Studies on the development of enzyme systems with time in animal embryos are relatively rare. Especially is this true of the chick embryo, of which the only complete study of enzyme development appears in the work of Levy and Palmer (1) on dipeptidase accumulation. According to these authors, dipeptidase activity in the chick increases with time and this increase is correlated with the accumulation of total nitrogen which presumably comes from the yolk. The same kind of correlation between nitrogen increase and enzyme activity has been demonstrated in oat seedlings for catalase and dehydrogenase activity by Albaum, Donnelly, and Korkes (2).

In the chick, cytochrome c does not appear until about the 4th day, according to Yaoi (3), and Potter and DuBois (4) were unable to detect it until about the 6th day. The present work was undertaken to ascertain when the enzyme, cytochrome oxidase, which usually operates with cytochrome c as part of the same system, appears and how its concentration changes during the course of early development.

Material and Methods

Chick eggs were incubated at 37-38° for lengths of time varying from 2 to 12 days. The embryos were removed from the yolk and dissected away from the extraembryonic membranes. Extracts of the embryos were prepared in the following manner. One to eight embryos was used, depending on the age. These were ground in a small quantity of sand and taken up in 2 cc. of Mn/15 phosphate buffer (Na2HPO4, KH2PO4) of pH 7.4. After light centrifugation, the supernatant fluid was decanted and used immediately for enzyme assay.

Cytochrome oxidase activity was determined by measuring the oxygen consumption of the extracts in the presence of p-phenylenediamine (0.433 gm. per 100 cc.) and cytochrome c in a Warburg respirometer at 26°. The usual procedure was to use 0.2 cc. of extract, 1.0 cc. of p-phenylenediamine, 0.4 cc. of cytochrome c, and 0.4 cc. of Mn/15 phosphate buffer.

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Control runs were carried out in the absence of cytochrome c and p-phenylenediamine. The cytochrome c was prepared from beef heart by the method of Keilin and Hartree (5). The presence of cytochrome oxidase was tested for, in addition, by treating with 0.001 M sodium azide which, according to Keilin (6), specifically inhibits the oxidase. Measurements of total nitrogen were made on extracts by means of a micro-Kjehldahl technique in the usual way.

EXPERIMENTAL

The total oxygen consumption in the presence of p-phenylenediamine and cytochrome c, together with the total nitrogen content of the extracts on a per embryo basis, is shown in Fig. 1. It is apparent that, as in the work of Levy and Palmer (1) on dipeptidase activity, there is a correlation between nitrogen accumulation and cytochrome oxidase activity. Both curves show a break at about 4 days and a sharp increase in nitrogen content and enzyme activity at about 8 days. Levy and Palmer find a break at about 43 days and another at about 103 days for dipeptidase.

The earlier break in the curve for cytochrome oxidase is of especial interest. Its significance becomes much clearer when the oxidase data are replotted in terms of per cent stimulation over the oxygen uptake of the control, as in Fig. 2. Plotting the data in this manner shows that...
there is no stimulation in uptake until the 4th day. This can only mean that the enzyme does not appear until that time. That this interpretation is correct is indicated by the effect of sodium azide, shown in Table I. Sodium azide does not begin to inhibit oxygen uptake until the 4th day. The absence of enzyme until the 4th day fits in with the observation reported by Yaoi (3) that cytochrome is not present until this same time.

The data plotted in Fig. 2 also show the sharp break in enzyme activity at the 8th day. At this time, the nitrogen content and enzyme activity increase sharply. This may be related to the change in the rate and character of the metabolism of the embryo which occurs at about that time. All of the pertinent data are presented by Needham (7). Up until about the 9th day, carbohydrate is the chief source of energy and the metabolic rate is low. On about the 7th day, fat begins to be utilized and continues to be burned at an increasingly rapid rate up until the time of hatching.

SUMMARY

1. The change in cytochrome oxidase activity in the chick embryo has been measured from 2 to 12 days of incubation.

2. Cytochrome oxidase increase is correlated with increase in total nitrogen.

3. Cytochrome oxidase, as measured by oxygen uptake in the presence of cytochrome c and p-phenylenediamine does not appear until the 4th day of incubation. Neither can the oxygen uptake of extracts with or without p-phenylenediamine and cytochrome c be inhibited with sodium azide until the 4th day of incubation.

4. There is a sharp increase in enzyme activity at the 8th day of incubation. This increase is briefly discussed in terms of the change in the rate and character of the metabolism known to take place at about that time.
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