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SPECIES SPECIFICITY OF AN ENZYME DETERMINED BY AN ERYTHROCYTE NUCLEUS IN AN INTERSPECIFIC HYBRID CELL

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SUMMARY

When a chick erythrocyte nucleus is introduced into the cytoplasm of a mutant mouse cell lacking inosinic acid pyrophosphorylase, synthesis of the enzyme is induced. The enzyme induced in this way has the characteristics of chick, not mouse, inosinic acid pyrophosphorylase.

INTRODUCTION

In a previous study (Harris & Cook, 1969) it was shown that when a chick erythrocyte nucleus was introduced into the cytoplasm of a mutant mouse cell lacking the enzyme inosinic acid pyrophosphorylase (IMP: pyrophosphate phosphoribosyl transferase, E.C. 2.4.2.8.), synthesis of this enzyme was induced. However, it was not certain whether the enzyme induced in this way was mouse or chick enzyme, or both. Since the precise nature of the genetic defect in the mutant mouse cell is not known, the possibility existed that the introduction of the chick nucleus might stimulate the synthesis of mouse enzyme. The present paper shows that this not so. The enzyme induced by the chick erythrocyte nucleus is chick enzyme.

MATERIALS AND METHODS

Erythrocytes were obtained from 12-day-old chick embryos as described by Bolund, Ringertz & Harris (1969). A_9 cells (Littlefield, 1964), which lack inosinic acid pyrophosphorylase, were again used. The erythrocyte nuclei were introduced into irradiated A_9 cells (6000 rd (60 J kg^{-1})) by the use of inactivated Sendai virus, as previously described (Harris & Cook, 1969). The assay used for the measurement of enzyme activity in cell homogenates was also described in this paper.

Chick and mouse inosinic acid pyrophosphorylase were separated by electrophoresis on Cellogel (Chemotron, via G. Modena 24, Milan, Italy). Before use, Cellogel strips (16 cm × 6 cm × 0·25 mm) were soaked in the electrophoresis buffer for at least 3 h, during which the buffer was changed twice; the strips were then blotted dry. The electrophoresis buffer (pH 8·9) contained 82·6 mM tris, 3·74 mM EDTA and 12·9 mM boric acid. Before the samples were applied, the strips were subjected to a current of 12 mA for 15 min in a Shandon Universal electrophoresis apparatus (Shandon Scientific Company, Ltd., 65 Pound Lane, London, N.W. 10).

Cell homogenates were dialysed overnight at 4 °C against the electrophoresis buffer. One μl of cell homogenate (containing 1–100 μg protein) was applied to the Cellogel. The samples were subjected to electrophoresis at a constant current (9 mA) for 2 h. The Cellogel was cut

into a number of strips, 4 mm wide, from the origin to the anode. The amount of enzyme in each strip was assayed by immersing the strip in the enzyme assay mixture (Harris & Cook, 1969), which was incubated at 37 °C for 2 h. Radioactive inosinic acid, the product of the reaction, was then separated from the substrate, [8-^{14}C]hypoxanthine, by the washing procedure previously described. Under the conditions of the assay, enzyme activity is proportional to the number of p-moles of inosinic acid produced.

RESULTS

Chick enzyme preparations were made from the erythrocytes of 12-day-old chick embryos (Bolund *et al.* 1969), and mouse enzyme preparations from L cells (Earle, 1943). (The A_9 cell used in these experiments was derived from the L cell.) These enzyme preparations served as markers. Enzyme extracts from the erythrocytes of 5-day-old chick embryos, from adult hen erythrocytes and from primary cultures of chick embryo fibroblasts had the same electrophoretic mobility as the enzyme

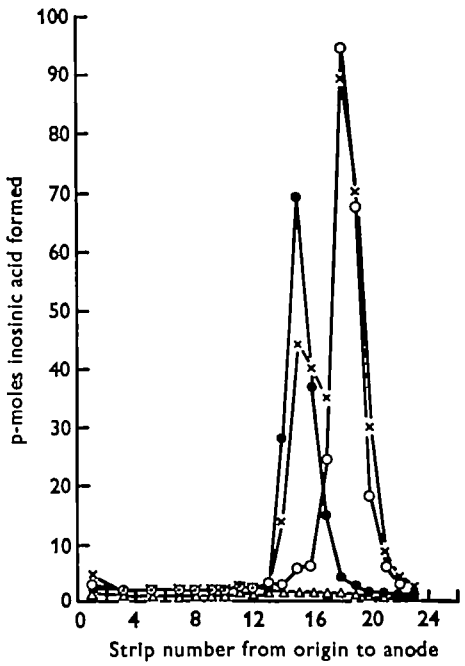

Fig. 1

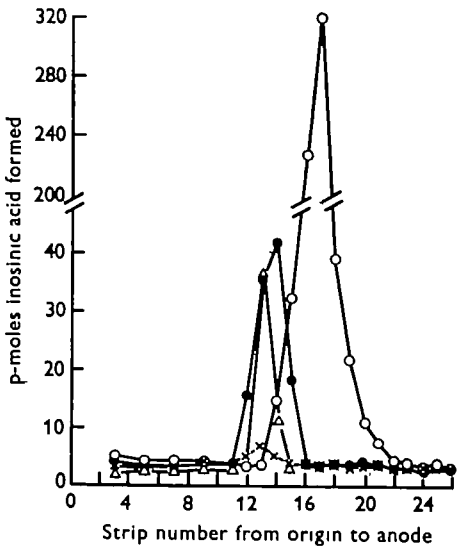

Fig. 2

Fig. 1. Electrophoretic mobilities of inosinic acid pyrophosphorylase from mouse and chick cells. ○, L-cell extract; △, A_9-cell extract; ●, extract from 12-day-old chick embryo erythrocytes; ×, mixture of extracts from L cells and 12-day-old chick embryo erythrocytes. The 3 bands of embryonic chick haemoglobin appeared in strips 8, 10 and 13.

Fig. 2. Electrophoretic mobility of the inosinic acid pyrophosphorylase induced in A_9-chick erythrocyte heterokaryons compared with that in L cells and 12-day-old chick embryo erythrocytes. ○, L-cell extract; ●, extract of 12-day-old chick embryo erythrocytes; ×, extract of A_9-chick erythrocyte heterokaryons 1 day after cell fusion; △, extract of A_9-chick erythrocyte heterokaryons 9 days after cell fusion. The 3 bands of embryonic chick haemoglobin appeared in strips 9, 10 and 12.

extract from 12-day-old chick embryos. Enzyme extracts from Ehrlich ascites tumour cells grown in Swiss Albino mice, from primary cultures of mouse embryo fibroblasts and from mouse erythrocytes had the same electrophoretic mobility as the enzyme extract from L cells.

A typical separation of the chick and mouse enzymes is illustrated in Fig. 1. A_9 cells and irradiated A_9 cells, both before and after treatment with the inactivated Sendai virus, contain no inosinic acid pyrophosphorylase activity. Embryonic haemoglobin resolves into 3 bands in this system and serves as a convenient visible marker. Chick inosinic acid pyrophosphorylase has a mobility slightly greater than the fastest haemoglobin band. Fig. 2 shows the mobility of the inosinic acid pyrophosphorylase induced in the A_9-chick erythrocyte heterokaryons compared with that of the mouse and chick markers. The enzyme appearing in the heterokaryons moves with the chick enzyme.

These results show that the inosinic acid pyrophosphorylase induced in A_9 cells by the reactivated chick erythrocyte nucleus is electrophoretically indistinguishable from chick inosinic acid pyrophosphorylase. The induced enzyme is clearly not mouse inosinic acid pyrophosphorylase. This finding, together with the kinetic experiments previously described (Harris & Cook, 1969) permit the conclusion that the chick erythrocyte nucleus reactivated within the cytoplasm of a mouse cell determines the synthesis of chick inosinic acid pyrophosphorylase.

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