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# Distribution of Pteroylglutamates in Developing Chick Embryo (41258)

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*Abstract.* Distribution of poly- and monoglutamate forms of folate coenzymes was studied in the whole egg, the nonembryonic portion of the egg, and in the embryo at various stages of development. Total folate activity of the nonembryonic portion decreases significantly after 12 days of incubation, while the folate activity of the embryo gradually increases. In the nonembryonic portion of the egg folates are present in monoglutamate form. The embryo assumes monoglutamates and immediately synthetises polyglutamates. In fact, these compounds are the only folate forms present in embryo and they increase progressively during development.

In the course of our studies on the regulatory mechanism of biosynthesis and utilization of folate coenzymes, we have investigated folate metabolism in rapidly growing tissues. They represent a very useful experimental situation, since the intense biosynthetic processes occurring in growing tissue require a greater availability of folate coenzymes, essential as $C_1$ donors in the synthesis of purine and pyrimidine nucleotides and in the metabolism of some aminoacids (1, 2).

In the regenerating rat liver after partial hepatectomy, significant variations in folate derivatives were found, especially in the concentration of reduced forms. The enzyme activities involved in the synthesis of these compounds were also found to have significantly increased (3).

The developing chick embryo showed (4) a constant increase in folate-reduced forms during growth, particularly on Day 12 when biosynthetic processes are more active. A simultaneous increase in enzymatic activities involved in reduced form biosynthesis ($H_4$ folate dehydrogenase and 10-CHO synthetase) was also observed. Subsequent studies performed *in vitro* and *in vivo* (5, 6) by a number of laboratories have demonstrated that pteroylpolyglutamates, which represent almost all of the naturally occurring forms of folate, are the true coenzymes for folate-mediated reactions.

This paper presents the data concerning the distribution of folate polyglutamates in the chick embryo and in the nonembryonic portion of the egg at various stages of development.

**Materials and Methods.** White Leghorn fertilized eggs, obtained from a commercial source, were incubated at 39° with a relative humidity of 65% and forced air circulation. The eggs, placed on racks, were turned twice daily. After 6, 9, 12, 15, and 18 days of incubation the embryos, freed from extraembryonic membranes and yolk sac, and the nonembryonic portion of the egg (consisting of the egg content except the embryo), were pooled.

The nonembryonic portions of egg and the embryos were each rapidly minced in a Waring blender and dropped into adequate volumes of hot (95°) Na-ascorbate, pH 6, to a final concentration of 1%. The materials were placed in a 95° water bath for 5 min. After cooling the mixture was homogenized for 60 sec and centrifuged at 12,000*g* for 10 min at 4°.

An aliquot of supernatant corresponding to 1 g of tissue was applied to a 1.5 × 90 cm Sephadex G-15 column prepared according to Shin *et al.* (7) and eluted with 0.025 *M* K-phosphate buffer, pH 7, containing 0.2 *M* mercaptoethanol; 60 fractions of 3.4 ml were collected at a flow rate of 7 ml/hr.

The folic acid activity in the extracts and

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in the single fractions was determined by microbiological assay after conjugase treatment (8), in accordance with Waters and Mollin's procedure (9), with some modifications (10, 11).

The estimation of various folates was performed as previously described (12).

**Results.** The folate activity of the embryo (Table I) increases throughout development, and this is more evident if the activity is expressed per total embryo. In the nonembryonic portion of the egg (Table II) on the other hand, this folate activity decreases.

From the data of Table I and Fig. 1 regarding the distribution of polyglutamate forms of folate in developing chick embryo, a remarkable increase can be seen, especially of higher polyglutamates, while the monoglutamates are present in trace amount.

In the nonembryonic portion of the egg (Table II, Fig. 1), the higher polyglutamates are completely absent. Diglutamates are present in small amounts and tend to disappear after 18 days of incubation. Monoglutamates which represent the greater part of the activity begin to diminish significantly after 12 days of incubation.

**Discussion.** The data obtained in the present research demonstrate first of all that total folate activity of the whole egg does not change significantly during incubation and that at zero time it is represented only by monoglutamate forms. This is of vital importance since the embryo is dependent for its metabolic needs on the folic acid present in the egg and only monoglutamate forms can easily pass through the membranes. After absorption, the embryo promptly converts the vitamin from monoglutamate to polyglutamate forms, which are more easily retained by the tissues.

The gradual increase in the synthesis of these compounds during development, along with intensification of the folic acid-dependent biosynthetic processes, might be further proof in favor of the hypothesis (5, 6) that pteroylpolyglutamates are the true active forms of folic acid.

Consequently, the conversion of folic acid into coenzymes requires, besides reduction and the introduction of $C_1$ units at different oxidation levels, the addition of glutamyl residues to the $\gamma$-carboxyl group of the monoglutamate form.

This last process might be a further very important regulating step for all the meta-

TABLE I. DISTRIBUTION OF FOLATE FORMS IN THE CHICK EMBRYO AT DIFFERENT DAYS OF INCUBATION

| Incubation days | Total folate activity | | $PteGlu_5$ | | $PteGlu_4$ | |
|---|---|---|---|---|---|---|
| | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt |
| 6 | 181 ± 16 | 670 ± 54 | 117 ± 9.7 | 435 ± 37 | 15.7 ± 2.1 | 58 ± 7.2 |
| 9 | 1,017 ± 99 | 748 ± 63 | 692 ± 6.5 | 509 ± 49 | 87 ± 10.3 | 64 ± 8.4 |
| 12 | 3,285 ± 282 | 900 ± 79 | 2,402 ± 220 | 638 ± 60 | 274 ± 31 | 75 ± 8.1 |
| 15 | 12,672 ± 1007 | 1320 ± 113 | 9,120 ± 892 | 950 ± 81 | 1171 ± 97 | 122 ± 11.7 |
| 18 | 35,070 ± 3302 | 1817 ± 179 | 21,655 ± 1803 | 1122 ± 93 | 4921 ± 289 | 255 ± 20.5 |

*Note.* The values represent the means ± SEM of three pools of six embryos.

TABLE I—*Continued*

| Incubation days | $PteGlu_3$ | | $PteGlu_2$, PteGlu | |
|---|---|---|---|---|
| | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt |
| 6 | 39 ± 4.3 | 145 ± 16 | trace | |
| 9 | 181 ± 24.1 | 133 ± 14 | trace | |
| 12 | 609 ± 54 | 167 ± 13 | trace | |
| 15 | 2102 ± 187 | 219 ± 18 | trace | |
| 18 | 7566 ± 621 | 392 ± 29 | trace | |

TABLE II. DISTRIBUTION OF FOLATE FORMS IN THE NONEMBRYONIC PORTION OF EGG AT DIFFERENT DAYS OF INCUBATION

| Incubation days | Total folate activity | | $PteGlu_5$, $PteGlu_4$, $PteGlu_3$ | | $PteGlu_2$ | | PteGlu | |
|---|---|---|---|---|---|---|---|---|
| | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt | ng/total wt | ng/g wet wt |
| 0 | 55,650 ± 5300 | 1070 ± 93 | trace | | 5720 ± 460 | 110 ± 9.3 | 49,920 ± 3850 | 960 ± 85 |
| 6 | 54,475 ± 4700 | 1065 ± 97 | trace | | 5570 ± 410 | 109 ± 8.7 | 47,830 ± 4110 | 936 ± 91 |
| 9 | 50,190 ± 4237 | 1050 ± 77 | trace | | 4732 ± 362 | 99 ± 10.2 | 43,928 ± 4137 | 919 ± 77 |
| 12 | 44,840 ± 4067 | 950 ± 64 | trace | | 2785 ± 210 | 59 ± 6.5 | 40,639 ± 3871 | 861 ± 78 |
| 15 | 32,560 ± 3200 | 800 ± 60 | trace | | 2401 ± 230 | 59 ± 6.2 | 29,467 ± 3125 | 724 ± 69 |
| 18 | 10,760 ± 980 | 400 ± 39 | trace | | 861 ± 91 | 32 ± 4.3 | 9,738 ± 1017 | 362 ± 35 |

*Note.* The values represent the means ± SEM of three pools of six nonembryonic portions of egg.

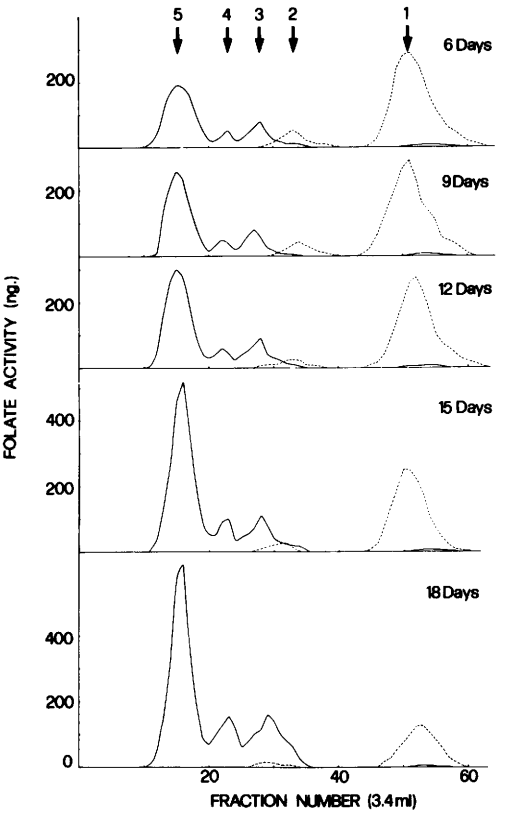


FIG. 1. Pattern of various folate forms in the embryonic (solid curve) and nonembryonic (dotted curve) portions of the egg at different days of incubation. Single compounds were separated on a Sephadex G-15 column and quantitatively evaluated by microbiological assays performed on the single fractions as specified under Materials and Methods. Number on the arrows show the number of glutamate moieties.

bolic processes in which folate coenzymes are involved.

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