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Esterase Activity in Embryonic Development* (32957)

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Although the enzymes of the nonspecific esterase type (carboxylic acid ester hydrolases) are known to be widely distributed in animal tissues, relatively little is known about their activity in different stages of embryonic development. Reports by Rossi *et al.* (1), for example, suggest that esterase activity appears in the early stages of ontogenesis in the gastrointestinal mucosa of human embryos. In addition, Richardson *et al.* (2) observed that the activity in the chicken embryo duodenum increased about 10-fold between days 13 and 22 (second day after hatching).

Introduction of the electrophoretic "zymogram" method by Hunter and Markert in 1957 (3) provided a new, sensitive tool for the study of species- and organ-specific esterase patterns. Using this method for the study of developmental changes in mouse plasma and tissues, Hunter *et al.* (4) found that esterase patterns show distinct changes with a general tendency towards an age dependent increase of the number and intensity of esterase bands.

In this paper, polyacrylamide "disc" gel electrophoresis with and without inhibitors has been used to study esterases in chicken and rat tissues during embryonic development. The electrophoretic studies were supplemented with measurements of the total esterase activity in all tissue specimens used for electrophoretic experiments. The results of these studies show that the changes in esterase patterns were both species and organ specific. The most complex and extensive changes were observed in rat tissues. The wide range of these esterase specific activities during the course of embryonic development, however, suggest some limitations in the interpretation of impure zymogram preparations since protein concentrations may influence the electrophoretic mobility.

Materials and Methods. All tissues were removed immediately after sacrificing the an-

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TABLE I. Esterase Activity.[a]

	Duodenum		Pancreas		Liver	
	A	B	A	B	A	B
Chicken embryo	0.18	—	0.012		0.175	
Adult chicken	0.400	22	0.150	12	0.340	2
Rat embryo	0.062	—	0.083		0.020	
Adult rat	1.826	30	0.283	3.5	0.126	6

[a] Abbrev.: A = specific activity in μmoles/min per mg of protein; B = increase factor.

imal and frozen in a dry ice–alcohol slurry. After thawing, the tissue was briefly homogenized in an all glass homogenizer with a medium containing 0.05 M Tris–HCl buffer, pH 7.6, 0.35 M sucrose, 0.04 M magnesium acetate, 0.07 M potassium chloride and 0.006 M mercaptoethanol. The homogenate was then sonicated for 30 sec in a Branson power sonifer and centrifuged for 5 min at 1,100*g* and then 25 min at 35,000*g*. The supernatant was used for electrophoretic studies and for total esterase activity measurements. All procedures were carried out at 4°C.

The polyacrylamide disc gel electrophoresis technique was that of Davis (5) with the exception that a lower density of the small-pore gel (5.5–6.0% acrylamide, instead of 7.5%) was used. The sample was applied after mixing with 2.0 *M* sucrose solution in 3:2 ratio. α-Naphthyl butyrate coupled to fast blue RR was used for esterase staining. The staining was carried out according to the procedure of Allen *et al.* (6), with the exception that phosphate buffer of pH 7.5 was used. In some experiments the esterase inhibitors, eserine sulfate or diisopropylphosphofluoridate (DFP) were added to the incubation mixture.

Total esterase activity was measured with the substrate incubated at 25° in 20 m*M* pH 7.5 phosphate buffer. At the appropriate intervals, not exceeding 15 min, the absorbance was measured at 40 mμ in a Spectronic-20 spectrophotometer. Protein was measured by the method of Lowry *et al.* (7), using albumin as a standard.

Results. The changes of total esterase activity measured with *p*-nitrophenyl caproate in the developing chicken embryo are shown in Fig. 1. The values obtained for embryonic and adult tissues of chicken and rat are summarized in Table I. As might be anticipated, activity increases with embryonic age in all three tissues. The most striking difference between the two species under study was in liver esterase activity. In comparison with the other tissues, the liver esterase activity was most intense in the chicken embryo and least in the rat embryo. Significant differences were observed in the enzyme activity of adult compared with the embryonic tissues for both species, with a 22–30 × increase in the duodenum, 3–12 × in the pancreas, and a 2–6 × in the liver.

As shown in Fig. 1, the nature of the change was similar in the duodenum and in the pancreas. Activity was very low up to day 14 and then gradually increased 20–30-fold

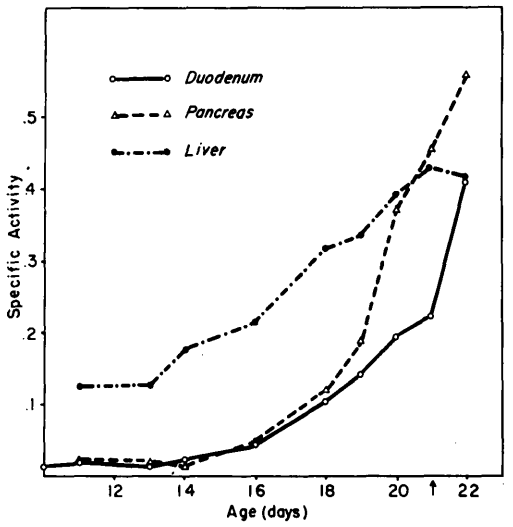

FIG. 1. Total esterase activity changes in the development of chicken embryo. The activity was measured with *p*-nitrophenyl caproate used in 0.1 m*M* concentration.

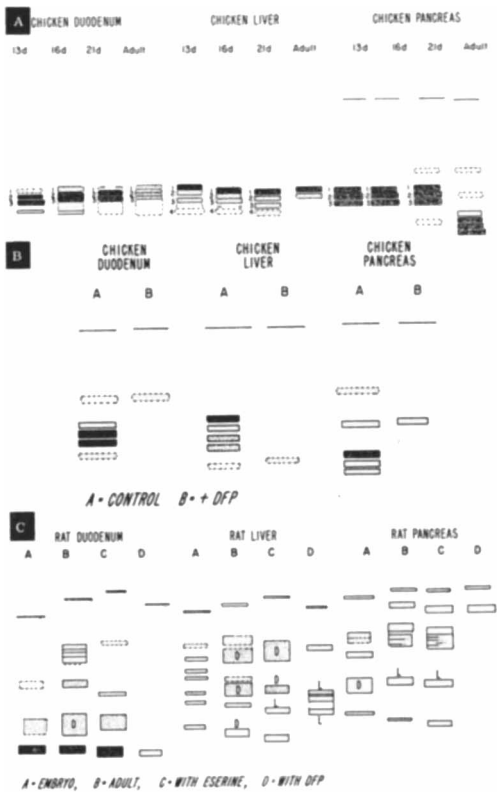

FIG. 2. Esterase pattern in polyacrylamide gel obtained for duodenum, liver, and pancreas of embryonic and adult chicken and rat; A. esterase in tissues of chicken embryo in various developmental stages; B. effect of diisopropylphosphofluoridate on esterases of chicken embryo tissues; C. esterase pattern of rat embryo and adult rat, effect of eserine and DFP; staining with α naphthol butyrate and blue RR salt. Electrophoresis in 5.5% gel for chicken and in 6.0% gel for rat tissues.

by the first day after hatching. In liver, the initial level (at day 11) was already appreciable and the rate of increase was more or less uniform with the progression of embryonic development.

Figure 2A presents the schematic diagram of the electrophoretic patterns of esterase activity observed in different stages of avian embryonic development at 13, 16, and 21 days as well as 3-day post hatching (adult). The embryonic esterase patterns on acrylamide gels were strikingly similar for these tissues. A typical pattern in 5.5% gel consisted of three major bands migrating close to the protein front and characteristic of the embryonic tissue from 13 to 21 days. In addition to these major bands in the duodenal and pancreatic tissue, the embryonic chicken liver contained an additional band of activity. The relative intensity of these three major activity bands differ for each of the tissues investigated. In the duodenum, for example, the two lower bands migrating closest to the protein front were most active, in contrast to the fact that the slowest migrating band in the embryonic liver was most intense, and that all three bands appear to have equal intensity in the embryonic pancreas.

The esterase activity bands persist throughout all developmental stages in all three tissues. The electrophoretic patterns of the adult resemble those of the embryonic duodenum and liver, but appear to change in the pancreas with the most complex enzyme profile observed with the adult tissue.

The esterase activity of different tissues may be further differentiated by the use of inhibitors. As shown in Fig. 2B, all major esterase bands in the adult tissues were inhibited by DFP, although none of these were appreciably sensitive to eserine (patterns identical to controls and not shown here). Since one of the slowly migrating bands in the duodenum, one of the most rapidly migrating bands in the liver, and a middle band in the pancreas were still active in the presence of DFP, it may be assumed that they represent organic phosphate resistant esterases. Since eserine is a specific inhibitor of acetylcholine esterase it may be assumed that these tissues are deficient in this enzyme.

The electrophoretic patterns obtained for the embryonic (14–19 day in utero) and adult rat were compared in the presence and absence of eserine and DFP, are shown in Fig. 2C. In contrast to the avian tissue, the rat tissue esterase patterns appear to be extremely complex with striking differences between the embryonic and adult tissues. For example, it is possible to demonstrate as many as 15 active bands migrating in four major groups in the duodenal embryonic tissue. Although the major groups of the duodenal patterns in embryonic tissue resembled the adult, the bands were much less active. The location and relative intensities of most

bands of the embryonic liver and pancreas were strikingly different from those of adult tissues. Although groups of bands may be observed to migrate near the anodal protein front in the embryonic pancreas, these proteins were absent or very weak in the adult tissue. The cathodal bands of the adult pancreas were most intensively stained.

The differential effect of esterase inhibitors was also evident in the rat patterns. Eserine inhibited only one band in the middle group, both in duodenum and liver, Fig. 2C (B vs C). None of the pancreatic esterases were sensitive to eserine. All the duodenal esterase bands except the most rapidly migrating was inhibited by DFP. In the liver pattern, four clearly resolved bands remained insensitive to DFP. All the pancreatic bands with the exception of the uppermost were inactivated.

Discussion. The results of these electrophoretic studies do not suggest any general trend in the developmental changes of the zymogram esterase patterns for either the avian or murine tissues. In the embryonic chicken duodenum and liver the changes appeared to be minimal. In contrast, extensive and characteristic developmental changes in the rat were observed for the individual tissues.

It is important to emphasize that any firm conclusions concerning the alterations of esterase zymogram patterns to be the result of embryological development must be viewed with some degree of caution. Thus the ideal conditions, permitting equal enzyme protein concentrations to be applied to the acrylamide electrophoretic gel, are frequently difficult to realize, particularly when it is known that the specific activity of the esterases are minimal in early embryonic tissue with the result that subtle changes in the concentration of some esterase bands may not be detected.

In spite of the limitations, however, it is possible to make some important observations concerning the esterase patterns during the course of development in the chicken and rat tissues. The fact that there were no changes in the esterase patterns of the chicken duodenum and liver, for example, suggest that in these tissues a complete complement of esterases is synthesized very early in development. In contrast, the fact that the chicken pancreas showed extensive change in the migration of the esterase bands when embryonic tissue was compared with that of the adult, would suggest developmental changes in this tissue that is not present in the duodenum and liver.

In the rat duodenum, liver, and pancreas striking changes were observed when embryonic tissue was compared with that of the adult. The disappearance of entire groups of esterase bands during the course of development suggests that these changes may indeed be significant in this species.

Because of the lack of specificity of esterase it is difficult to determine whether the observed electrophoretic bands correspond to entirely different proteins endowed with esterase activity, or whether they represent similar proteins forming a group of isozymes. Some observations seem to support the hypothesis that the three major bands of avian tissues actually represent esterase isozymes. First, the electrophoretic mobility of all three bands were similar. Second, all three bands were present in the tissues studied, showing only minor differences in the relative intensities. Third, all three bands were highly sensitive to DFP. On the basis of the latter observations, we would postulate the esterase isozymes belong to the β-type or aliester group.

Summary. Esterase patterns characteristic for duodenum, liver, and pancreas in different stages of avian embryo development were studied with polyacrylamide disc gel electrophoresis. Embryonic and adult rat tissues were studied for comparison. It was found that the electrophoretic patterns of chicken liver and duodenum were similar for all developmental stages studied. In contrast, definite changes in pancreatic tissues were observed during development. It is postulated that the three main esterase bands observed in chicken tissues represent isoenzyme forms of β-type nonspecific esterases. The developmental changes and the identity of some bands present in rat tissue zymograms are discussed.

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Thyroid Inhibition of Rats Bearing Transplantable, Hormone-Producing Pituitary Tumors* (32958)

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Previous reports from this laboratory (1) have described the significantly reduced uptake of radioactive iodine, and the histology of an understimulated thyroid gland, present in rats implanted with hormone-secreting pituitary tumors. Thyroid inhibition also has been described in rats implanted with a malignant nonendocrine tumor (2,3) and in humans with a variety of cancers (4).

The tumor strain employed here was the MtTW5 which secretes prolactin and growth hormone but not TSH or ACTH. The hormonal properties of this tumor have been previously described (5,6).

It has been suggested that the thyroid inhibition of rats implanted with tumors (2) and of humans with cancers or chronic debilitating illnesses (7) is operated through nonspecific mechanisms such as a decrease in the thyroxine binding proteins, diminution which is just a participant in the generalized decrease of protein synthesis that goes on in those diseases. These possibilities were explored.

Since thyroid suppression in the rats implanted with the pituitary tumors are not the result of the gland becoming unresponsive to TSH (1), our attention was directed to the pituitary content of TSH and to some of the aspects of intrathyroid iodine metabolism that are affected by TSH.

Materials and Methods. 1) *Correlation of tumor size and thyroid inhibition of iodine uptake.* Thirty-two adult Wistar-Furth female rats were placed on regular Purina rat chow and tap water *ad libitum*. They were implanted s.c. in the right subscapular area with the MtTW5 tumor and were distributed in four cages containing eight rats each. On days 5, 8, 12, 15, 20, 29, 36, 49 postimplantation, one rat from each cage was randomly selected, injected with 2 μC of carrier-free sodium ^{131}I per 100 gm of body weight, and 18 hours later anesthetized with ether, and bled by cardiac puncture. Their thyroids and anterior pituitary glands were removed and weighed. One ml of serum and the thyroids suspended in 1 ml of saline were counted in a well scintillation counter.

2) *Levels of free thyroxine* (T_4) in plasma were determined according to the equilibrium dialysis technique described by Ingbar *et al.* (8). The serum extracted from one single rat was insufficient to carry out these studies on an individual basis, hence the serum from 2–4 rats was pooled.

Control and tumor animals were matched by age and sex, but not by weight, because by virtue of the very same tumoral effects, the tumor-bearing rats were heavier.

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