D-AMINO ACID OXIDASE IN EXTRA-EMBRYONIC MEMBRANES OF THE CHICK

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Summary. D-amino acid oxidase is present in the extra-embryonic membranes of the developing chick, and can be detected on the third day of incubation (37°) four days prior to its earliest appearance in the hepatic tissue and mesonephros. As the membranes grow, those which form the yolk sac continue to show enzymatic activity. After the sixth day the mesonephros and hepatic tissue both contain activity and on the thirteenth day of development the enzyme appears in the metanephros. Post-hatch, when the mesonephros disappears, the metanephros maintains a specific activity higher than the liver. Enzymatic activity persists in the yolk sac membranes until several days after hatch.

The extra-embryonic membranes of the amniote vertebrates (reptiles, birds and mammals) are responsible for nutrition, respiration and hydration of the developing embryo. There is also evidence that the primordial germ cells migrate from the yolk sac endoderm to their future site in the genital ridge (1, 2, 3) and this event governs the differentiation of the gonadal tissues. Accordingly, it is of interest to relate enzymatic activity in the yolk sac membranes with that in specific developing organs. During studies on D-amino acid oxidase in hepatic and renal tissues of embryonic chick, we discovered substantial enzymatic activity in the area opaca vasculosa of the extra-embryonic membrane system. The earliest activity was found in the 25 somite embryo corresponding

to stage 15 of Hamburger and Hamilton (4).

Materials and Methods. The embryos were white leghorns supplied by a local poultry farm and incubated at 37°. Organ samples were dissected out in 0.1M sodium pyrophosphate buffer (pH 8.3) and homogenates were prepared in the same buffer with a motor-driven teflon-glass homogenizer at 3°. Homogenates were centrifuged at 1000 x g and supernatant solutions were used for enzymatic assay according to a method described previously which is based upon the enzymatic dehydrogenation of D-allohydroxyproline to Δ^1-pyrroline-4-hydroxy-2-carboxylic acid (5). The reaction mixtures consisted of crystalline catalase (10 units), D-allohydroxyproline (10 µmoles), sodium pyrophosphate buffer (30 µmoles), flavin adenine dinucleotide (2 µg) and enzyme preparation to yield a final volume of 0.5 ml. The mixtures were shaken (100 oscillations per minute) at 38° for 60 to 120 minutes. Then 0.1 ml of 50 percent trichloroacetic acid was added, the precipitated protein centrifuged down and 0.3 ml of the clear supernatant was reacted with 3.7 ml of 1 N H_2SO_4 to dehydrate the Δ^1-pyrroline-4-hydroxy-2-carboxylic acid to pyrrole-2-carboxylic acid. This was decarboxylated by heating at 70° for 10 minutes and the pyrrole was reacted with 1 ml of 5 percent p-dimethylaminobenzaldehyde. After 10 minutes at 26°, the red color was measured in a spectrophotometer at 550 nanometers. Protein was determined with biuret reagent (6) or by the method of Lowry, et al., (7).

Results and Discussion. Table 1 shows that from the third day on (25 somite embryo), enzymatic activity is present in the membranes. Embryos excised from these membranes failed to show activity. On the fifth day, the extra-embryonic

TABLE 1. D-AMINO ACID OXIDASE IN EXTRA-EMBRYONIC MEMBRANES

Age of Embryo	Specific Enzymatic Activity[a]
3 day	5
5 day a.o.vasculosa[b]	20
5 day a.o. vitellina	2
6 day	12
8 day	10
16 day	40
18 day	25
5 day post-hatch	20

[a] mμmoles substrate oxidized per mg protein per hour.

[b] a.o. vasculosa: area opaca vasculosa
a.o. vitellina: area opaca vitellina

areas were subdivided into opaca vasculosa containing the circulatory network and opaca vitellina not yet vascularized (1). In older embryos, activity was found in the membranes which become enclosed in the abdomen as the yolk sac. It is surprising that significant enzymatic activity was still present in yolk sac membranes dissected from a chick seven days after hatch. The contents of this yolk sac were devoid of activity. Sodium benzoate competitively inhibits vertebrate D-amino acid oxidase (8), and it acted similarly on the enzyme from an eighteen-day yolk sac, 1 μmole producing 60 percent inhibition and 10 μmoles inhibiting 90 percent of the activity. Table 1 presents the results of assays on homo-

genates of membranes from embryos of different ages.

The earliest enzymatic activity in the mesonephros occurs on the seventh day of incubation (Hamburger-Hamilton stage 30) and by the eighth day total activity increases four-fold. The specific activity reaches a maximum on the twelfth day and remains constant until after the nineteenth day when

TABLE 2. D-AMINO ACID OXIDASE IN CHICK EMBRYO TISSUES

Age of Embryo	Source	Specific Enzymatic Activity[a]
8 day	liver	18
8 day	mesonephros	24
8 day	brain	0
9 day	liver	14
9 day	mesonephros	120
9 day	stomach	0
12 day	liver	20
12 day	mesonephros	300
12 day	metanephros	0
14 day	liver	15
14 day	mesonephros	300
14 day	metanephros	30
17 day	liver	15
17 day	mesonephros	180
17 day	metanephros	60
3 day post-hatch	liver	8
3 day post-hatch	metanephros	30

[a] mμmoles substrate oxidized per mg protein per hour.

the gradually decreasing tissue mass becomes insufficient for assay. The metanephros contains D-amino acid oxidase after the thirteenth day of development and shows an increase in activity to the sixteenth day. The hepatic tissue shows slight enzymatic activity on the seventh day which then increases. However, the renal tissues always have higher specific activity than the liver. Table 2 summarizes experiments done with renal and hepatic tissues.

Stereospecific oxidation of D-amino acids was first reported in the liver and kidneys of a number of vertebrates including sheep, cows, pigs, pigeons, tortoises, frogs and trout (8, 9). D-amino acid oxidase was subsequently found in numerous invertebrates (10, 5) as well as in microorganisms (11, 12). The occurrence of these enzymes in arthropods and molluscs implies that D-amino acid oxidase evolved at a time predating the geological origin of the vertebrates. D-amino acids are found in microbial cell walls (13), polypeptide antibiotics (14) and invertebrate species representing annelids, arthropods and molluscs (15). Clearly, several factors operate to conserve the genes responsible for the biosynthesis of the enzymes. The hypothesis that, in animals, these enzymes serve to destroy D-amino acids of microbial origin has been considered by Meister, et al., (16) and the recent discovery of D-amino acid oxidase in the granule fraction of human neutrophilic leukocytes (17) is consistent with this proposal. It is noteworthy that D-amino acid oxidase activity has been reported in brain tissue (18, 19, 20). The presence of the enzyme in extra-embryonic membranes suggests that it may play a unique role either metabolically or as a component of cellular structure.

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