ON THE TISSUE SPECIFICITY AND BIOLOGICAL SIGNIFICANCE OF ALDOLASE C IN THE CHICKEN*

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Recent studies in this laboratory have revealed that aldolase exhibits an extensive heterogeneity in the tissues of the fowl; at least three distinct types of activity being present - aldolases A, B, and C - together with hybrids of these parental forms. In general, the isoenzyme patterns in this species are tissue specific, but differ markedly from those recently reported for the rabbit (Penhoet, Rajkimar, and Rutter, 1966). One particularly noteworthy point of contrast between these animals is the fact that aldolase C activity has been demonstrated in several of the major chicken tissues, whereas with the rabbit, it was found only in the brain. This finding would appear to necessitate

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a reappraisal of recent proposals as to the biological significance of aldolase C, based on the localization in the latter animal (Penhoet, Rajkimar, and Rutter, 1966).

Furthermore, an ontogenetic progression of aldolase multiple forms has been demonstrated in the present study, with aldolase C being the major early embryonic type. This establishes the developmental importance of aldolase C in this species and suggests an alternative, broader significance for this form of the enzyme.

Methods--Fresh tissues were excised and homogenized in 0.01 M Tris-HCl buffer, pH 7.5, which contained EDTA(0.001, M) and β-mercaptoethanol (0.01 M). The homogenates were then centrifuged at 100,000 g and 2° for 60 min. Zone electrophoresis of the supernatants was carried out on starch blocks at 4° and pH 7.0 by the method described by Fine and Costello (1963). After 15 hr at a current density of 3 mA/cm, the gels were sliced and stained for aldolase activity by a modification of the method of Dewey and Conklin (1960). Agar (1.5%) was dissolved in pyrophosphate buffer (0.05 M, pH 8.5), and 50 ml of this solution maintained at 55° in the dark while the following reactants were added: 1 ml of 0.3 M sodium arsenate, 2 ml of 0.2 M fructose-1,6-diphosphate, 1 ml of DPN^+ (30 mg/ml), 1 ml of thiazolyl blue (10 mg/ml), 0.25 ml of phenazine methosulphate (10 mg/ml), and 0.5 ml of triosephosphate dehydrogenase (10 mg/ml. This mixture was then poured over the starch gel and allowed to develop in the dark for 3 hr.

Results and Discussion--The recent realization that many enzymes exist in multiple forms has raised a number of questions of considerable biological import, and not the least of these is a specification of the physiological significance of this enzyme heterogeneity. In many cases, an examination of the tissue

distribution and catalytic properties of the multiple molecular forms has enabled a deeper understanding of their separate roles in cellular metabolism. With aldolase (E.C. 4. 1. 2. 7) for example, it has been recognized for some years that two distinctive forms of this enzyme exist in the tissues of vertebrates. Aldolase A typically derives from muscle and catalyzes the cleavage of fructose-1,6-diphosphate several times more efficiently than aldolase B, the liver enzyme. Aldolase A also shows a far greater specificity toward fructose-1,6-diphosphate rather than fructose-1-phosphate when compared to the liver enzyme. Based on these facts, a physiological significance of the two forms has been proposed, which relates the properties of the dominant form in liver (aldolase B) to the more diversified carbohydrate metabolism in this tissue, e.g., the pathway of utilization of fructose through the cleavage of fructose-1-phosphate and the emphasis on gluconeogenesis (Rutter, Blostein, Woodfin, and Weber, 1962).

More recently, it has become evident that aldolase possesses a more extensive heterogeneity than had been considered previously (Penhoet, Rajkimar, and Rutter, 1966; Foxwell, Cran, and Barron, 1966; Anstall, Lapp, and Trujillo, 1966; Herskovits, Masters, Wassarman, and Kaplan, Unpublished data). A third distinctive type of aldolase activity has been identified in mammalian brain (aldolase C), along with the possibility of hybridization between any two of these three parental forms (aldolase A, B, and C). On the basis of an apparently unique localization of rabbit aldolase C in nervous tissue, Rutter and coworkers (1966) have postulated that the functional significance of this form of the enzyme may be related to brain metabolism and, in particular, the subcellular distribution of the enzyme in this tissue.

Parallel studies in this laboratory on other species, however, while also revealing the extensive nature of the multiplicity of aldolase, had shown, in addition, that aldolase C, far from being restricted to brain, was a major contributor to the aldolase activity of many other tissues. Some of this evidence is presented in Fig. 1, where the distribution of multiple forms of aldolase is shown in a number of tissues from the chicken. As indicated, most of the isoenzyme patterns are derived from skeletal muscle at various stages of development, but patterns are also included from adult heart and brain. From the latter distribution, aldolase-1 is indicated as the parental C type in this species, and this identity has been confirmed immunologically.

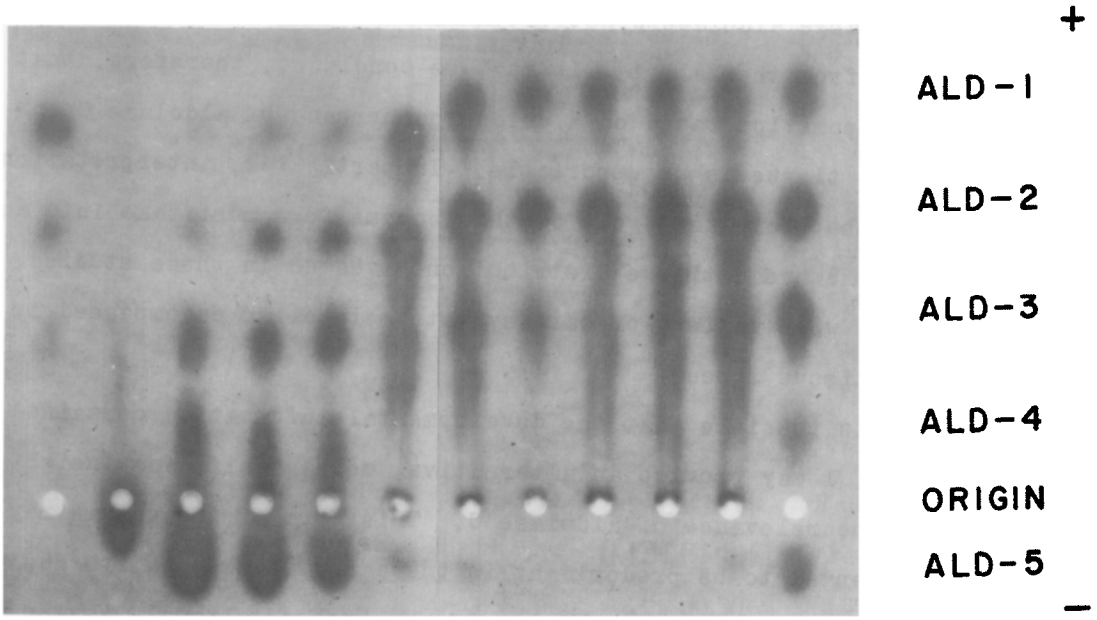

Fig. 1. Starch gel electrophoresis of chicken tissue homogenates stained to reveal the multiple forms of aldolase. The adult brain pattern is in the extreme left and adult heart on the extreme right. The remaining tissues are thigh muscle at sequential stages of development. From left to right, 7 days post hatching, 2 days post hatching, 20 days after fertilization, 16 days, 10 days, 8 days, 6 days, 5 days.

Similarly, the band position, aldolase-5, has been established as aldolase A, with the intermediate bands (aldolase-2, -3, and -4) representing hybrids of these two molecular forms. From Fig. 1 it may be noted that aldolase-1 and the A-C hybrids are present in appreciable quantities not only in brain, but also in heart, and embryonic and infant leg muscle. Since aldolase C is less efficient in its activity against fructose-1,6-diphosphate than the aldolase of adult skeletal muscle, these patterns would, if anything, underestimate the contribution of C-type aldolase.

Zymograms derived by a similar technique have demonstrated that considerable aldolase activity of the C type occurs in many other tissues of the adult fowl - kidney, spleen, lung, and many red muscles - indeed, the majority of the tissues of this species. Appreciable aldolase C activity has also been identified in leg muscle from the rabbit. It must be concluded, therefore, that any proposition based on a unique occurrence of aldolase C in nervous tissue is unsupportable. In particular, interpretations as to the biological role of this enzyme form must take into account the broad tissue distribution revealed in these studies and the wide variety of carbohydrate metabolism encompassed by these tissue types.

It is possible that the developmental information contained in Fig. 1 may provide an alternative, more significant insight into the relevance of aldolase C. This form of the enzyme may be observed to be predominant in the early chicken embryo and is replaced by A-type activity in skeletal muscle only in the late stages of ontogeny. This, then, establishes the basic developmental importance of aldolase C in this species, and, in addition, this form of the enzyme has been shown to play a major part in the ontogeny of other animals, as well.

It is of interest to note that developmental progressions of at least three isoenzyme systems have been reported for the fowl - lactate dehydrogenase (Cahn, Kaplan, Levine, and Zwilling, 1962), creatine kinase (Eppenberger, Eppenberger, Richterich, and Aebi, 1964), and aldolase (Fig. 1). In each of these cases, the early embryonic pattern of multiple forms approximates that in adult brain. This should serve to emphasize again that the isoenzyme pattern in brain may derive from more fundamental considerations than specific nervous tissue metabolism; and that a more rewarding line of investigation into the biological significance and epigenetic control of synthesis of aldolase C may lie in a study of the factors common to the occurrence of this form of the enzyme in the different tissue specified.

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