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Invertase activity in the intestine of the developing chick

The microvilli of the epithelial cells of the small intestine are complex organelles that undergo structural differentiation during the formative stages of the early life of the organism[1], and also during the life of the individual cell as it glides up the villus from crypt to extrusion zone[2]. The microvilli bear alkaline phosphatases[3,4] and disaccharidases[5,6], apparently bound to their membranous coats. We have used these observations to investigate the question whether the enzymic components of these intestinal organelles are subject to independent controlling mechanisms, or whether an alteration in structural configuration implies a unidirectional shift in biochemical constitution.

In the duodenum of the chick embryo, the microvilli begin to assume their mature form at 18 days, and then become steadily longer and narrower through the second day after hatching[1]. These form changes closely parallel a 30-fold increase of alkaline phosphatase activity that goes on during the same time[7]. Yet invertase, which appears to be equally a component of the microvilli[6], shows a quite different developmental tendency.

Although invertase activity has been extensively studied in mammalian intestines, it has not been examined in the chick since MENDEL AND MITCHEL[8] reported its presence in 1907. The enzyme is active in the human from the third month of gestation[9], but in rats and mice it does not appear until two weeks or more after birth[10]. In the dog, activity is low at 5 days but increases with age[11], and in the pig it is absent at birth[12]. In adult life, invertase activity in rats is highest in the proximal and middle thirds of the intestine[13], but in the pig it is localized mainly in the distal part[14]. The dog's intestine has highest activity in the proximal and middle thirds, with a striking decrease toward the distal end[11].

In the present study, entire intestines of white leghorn chicks from 9 days of incubation to four weeks after hatching were removed after decapitation of the animal. The excised tissue was rinsed in cold 0.9% NaCl, dried lightly, weighed, and homogenized in 0.9% NaCl. The homogenate, containing 250 mg/ml, was centrifuged at 9000 rev./min for 15 min to remove cellular debris and nuclei. Enzymatic activity of the supernatant was measured in 0.1 M maleate buffer (pH 6.5) containing sucrose at a final concentration of 3%. These conditions were found to be optimal for chick invertase. The reaction mixture was incubated at 37° for 60 min, and the degree of hydrolysis was determined with the 3,5-dinitrosalicylic acid reagent of SUMNER[15], according to DAHLQVIST's method[16]. One unit of enzyme activity was equal to the formation of 0.5 mg of glucose under these conditions. Protein was measured according to LOWRY *et al.*[17]. In a subsequent study, the duodenal loop, jejunum, and ileum and caecal papillae from chicks of 17 days of incubation to 4 days after hatching (25 days) were examined for invertase activity by the same procedure. Only hatched chicks were used for the 21-day assays. The chicks had access to food after hatching.

At 9 days of incubation, invertase activity was barely detectable. There was then a gradual 7.5-fold increase to 19 days (Fig. 1). At 20 days, a significant decrease ($P<0.01$) to below the 17-day level occurred. However, the activity at 21 days increased to that of the pre-hatching level of 19 days. From 21 days to 4 weeks after

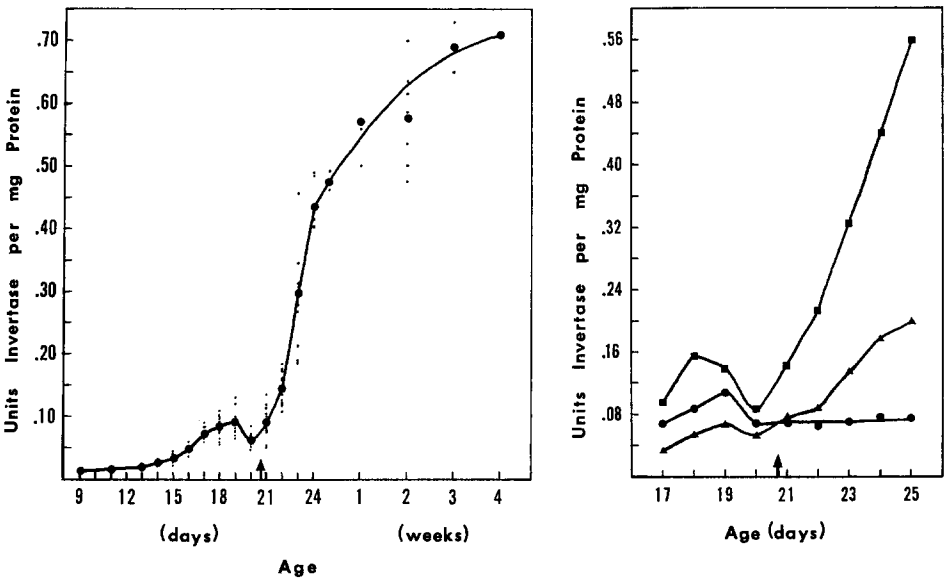

Fig. 1. The developmental pattern of invertase activity in the entire intestine of the chick. The small spots show individual assays, and the large spots are the averages of the individual assays for each day. The broad arrow denotes time of hatching.

Fig. 2. The developmental pattern of invertase activity in the three parts of the chick intestine. Averages of 6 to 10 determinations for each day are shown. ■—■, jejunal activity; ●—●, duodenal activity; ▲—▲, activity of the ileum and caecal papillae.

hatching, there was a very rapid increase (8-fold) in enzyme activity, with the greatest increase (5-fold) being between 21 and 25 days.

The duodenal invertase activity increased approximately 1.6-fold between 17 and 19 days of incubation (Fig. 2). However, at 20 days there was a statistically significant drop to the 17-day level, and this low activity was maintained to 25 days. In the embryonic jejunum the invertase activity increased between 17 and 18 days and then fell off to a low level at 20 days. Between 20 and 21 days there was a significant increase in enzyme activity, which then continued rising rapidly (Fig. 2). The invertase activity of the ileum and caecal papillae roughly paralleled that of the jejunum, although the rise from 20 to 25 days was not as great (Fig. 2).

The developmental pattern of invertase in the chick intestine thus appears to be different from that in any of the mammals that have been studied. In the chick, invertase activity is relatively high before hatching. In all regions the activity declines during the 19–20 day period when the chick is in the process of emerging from the shell, and then it rises sharply in all but the most proximal part. This early increase most probably reflects the varied character of the young chick's diet; by contrast, a mammal such as the rat, which is sustained at first entirely on milk, has abundant lactase but no invertase in its intestine in infancy[10,18]. In the hatched chick, the failure of doudenal invertase to rise even to its pre-hatching level suggests that the duodenum plays little part in the digestion and absorption of sucrose.

An especially interesting aspect of the developmental pattern of invertase in the duodenum is the contrast it makes with the pattern of alkaline phosphatase[7]. Between

17 and 19 days the two enzymes rise approximately in parallel. At 19 days, however, invertase declines to a stable low level as phosphatase activity enters on a period of rapid increase that carries it to a peak more than 20 times its 19-day level. Assuming that the invertase is a component of the microvilli in the chick embryo, as it is in young mammals[19], and as phosphatase is in the chick embryo[7], one may conclude that the microvilli, at the time that they are undergoing the form changes delineated by OVERTON AND SHOUP[1], are becoming richer in one constituent enzyme activity and poorer in another. DOELL, ROSEN AND KRETCHMER[19] have demonstrated a similar differentiation in the infant rat jejunum, in which the microvilli lose lactase activity as they gain invertase.

The factors controlling the patterns of invertase accumulation in the chick intestine remain to be elucidated. It may be suspected that the secretions of the adrenal cortex play a role, for glucocorticoids strongly accelerate the total pattern of duodenal differentiation[20], including increase of phosphatase activity[21], in the chick embryo. In the rat injection of hydrocortisone elicits precocious development of invertase activity[19,22]. If cortical hormones similarly affect invertase activity in the chick, the evidence suggests that they may evoke responses of opposite sign in the duodenum and jejunum.

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1 J. OVERTON AND J. SHOUP, *J. Cell Biol.*, 21 (1964) 75.
2 A. BROWN, *J. Cell Biol.*, 12 (1962) 623.
3 S. L. CLARK, *Am. J. Anat.*, 109 (1961) 57.
4 J. OVERTON, A. EICHOLZ AND R. K. CRANE, *J. Cell Biol.*, 26 (1965) 693.
5 D. MILLER AND R. K. CRANE, *Biochim. Biophys. Acta*, 52 (1961) 293.
6 A. M. UGOLEV, N. JESUITOVA AND P. DELAEY, *Nature*, 203 (1964) 879.
7 F. MOOG, *J. Exptl. Zool.*, 115 (1950) 109.
8 L. MENDEL AND P. MITCHELL, *Am. J. Physiol.*, 20 (1907) 81.
9 S. AURICCHIO, A. RUBINO AND G. MURSET, *Pediatrics*, 35 (1965) 944.
10 R. DOELL AND N. KRETCHMER, *Federation Proc.*, 22 (1963) 495.
11 J. WELSH AND A. WALKER, *Proc. Soc. Exptl. Biol. Med.*, 120 (1965) 525.
12 A. DAHLQVIST, *Nature*, 190 (1961) 31.
13 A. DAHLQVIST, *Biochem. J.*, 86 (1963) 72.
14 A. DAHLQVIST, *Biochem. J.*, 78 (1961) 282.
15 J. SUMNER, *J. Biol. Chem.*, 65 (1925) 393.
16 A. DAHLQVIST, *Acta Chem. Scand.*, 14 (1960) 1797.
17 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR, R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
18 R. DOELL AND N. KRETCHMER, *Biochim. Biophys. Acta*, 62 (1962) 353.
19 R. DOELL, G. ROSEN AND N. KRETCHMER, *Proc. Natl. Acad. Sci. U.S.*, 54 (1965) 1268.
20 F. MOOG, in D. RUDNICK, *Cell, Organism, and Milieu*, Ronald Press, New York, p. 121.
21 F. MOOG AND D. RICHARDSON, *J. Exptl. Zool.*, 130 (1955) 29.
22 R. DOELL AND N. KRETCHMER, *Science*, 143 (1964) 42.

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