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Induction of Invertase Activity by Hydrocortisone in Chick Embryo Duodenum Cultures.* (31563)

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Moog(1) demonstrated that hydrocortisone initiated the precocious development of alkaline phosphatase activity of embryonic duodenum in tissue culture. These observations have been confirmed and extended in our laboratory with studies on alkaline phosphatase, invertase, and lactic dehydrogenase in the chick embryo. Alkaline phosphatase and invertase but not lactic dehydrogenase responded to hydrocortisone(2). This response is defined in our experiments as an increase in the enzyme specific activity. It was dependent on the age of the embryonic tissue as well as the time of exposure to the hormone.

Experiments reported here on invertase were selected in an attempt partially to define the action of this steroid on the duodenum of the chick embryo.

Methods. Preparation of cultures. The duodenums of chick embryos, ages 14, 16 and 19 days were cultured as described previously(2). Duodenal fragments of each embryo were distributed on Millipore (.45 μ) membranes in 2 Falcon culture vessels, only one of which contained 0.5 μg of hydrocortisone. The untreated half of the duodenum served as a control. The duodenal tissue (1-2 mm) was positioned so that the serosal side contacted the Millipore membranes. All cultures were incubated in a 5% CO_2-air humidified chamber at 37°.

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Assay procedures. The tissues were homogenized in 1.4 ml of 0.1% Brij at 4°C to dissociate membrane enzymes. Complete homogenization was assured using a motor driven Kontes glass homogenizer. All homogenates were centrifuged for 10 minutes at 900 *g* and the supernatant decanted for protein and invertase analyses. Invertase determinations on 0.4 ml aliquots were made according to the procedure of Dahlqvist(3) using glucostat special reagent to measure the amount of glucose released. Zero time blank values and glucose released in the absence of substrate were subtracted as controls for glucose in the tissue. Proteins were precipitated by the Somogyi procedure(4). Specific activities were expressed as micrograms of glucose released per mg of protein per hour. Protein determinations were made according to the procedure of Lowry *et al*(5) using 0.05-0.1 ml aliquots, with bovine serum albumin as a standard.

Materials. Millipore® filters were obtained from the Millipore Corp. Brij 35; Pierce Chemical Co., Rockford, Ill. Glucostat Special: Worthington Biochemical Corp., Freehold, N. J. Tris buffer (Tris-Trizma Base); Sigma Chemical Co., St. Louis, Mo. Sucrose: M. A. Special Enzymatic Grade, Mann Research Laboratories, Inc., New York. Iodoacetate Acid; Fluka, A. G., Buchs, S. G., Switzerland. Organ culture tissue flasks were obtained from Falcon Plastics. Solu-Cortef (hydrocortisone-sodium hemisuccinate) Upjohn Co., Kalamazoo, Mich.

Fourteen-day embryonated Plymouth Rock-White Rock chicken eggs were obtained from the Durham Farmers' Exchange. The eggs were placed in a 37° incubator until the desired day of the experiment. Beak and toe measurements were used to verify embryonic age(6).

Results. The invertase specific activities were determined for the duodenum and jejunum of 19-day embryos (Fig. 1). Though the specific activities of the jejunum were higher than those of the duodenum (average values of 96 *vs* 64 μg of glucose per mg of protein per hour), the latter was selected for these experiments because of its clear demarcation, which starts at the pylorus, ends at the gall bladder, and encloses the pancreas. In addition, the invertase activity of random sections of equal halves of the duodenum showed little variation and therefore provided ideal paired controls.

TABLE I. Invertase Activity in the Developing Chick Duodenum.

Age of embryo	Specific activity, μg glucose released per mg protein per hr
14 days	24.1 ± 4.9
15 "	24.0 ± 12.2
16 "	42.2 ± 3.3
17 "	46.8 ± 9.3
18 "	58.4 ± 13.2
19 "	69.7 ± 10.7
21 "	92.4 ± 26.5
3-5 hr after hatching	49.9 ± 9.3

Four determinations of invertase activity were made in each age group except for 3 determinations at 21 days. For 14- and 15-day-old embryos, 3 duodenums were pooled for each determination. For 16- and 17-day groups, 2 duodenums were used and 1 each was used for the remainder.

Specific activities of invertase in the developing chick embryonic duodenum are shown in Table I. Activity was low before the 16th day, after which time it increased progressively until the day of hatching. There was an abrupt fall after hatching. The invertase activity of the duodenum of 3-5-hour hatched chicks was less than that of the 18-day embryo. No feed was offered post hatching.

The results of experiments designed to compare tissue cultures (clear bars) with *in vivo* tissue of equivalent chronological age are shown in Fig. 2. It will be noted that the invertase activities of 14- and 16-day explants increased in tissue culture (A_2 *vs* A_1, B_2 *vs* B_1). However, this increase was not so great as would occur naturally *in vivo* (A_3 *vs* A_2 and B_3 *vs* B_2). The activity of tissues from a 14-day-old embryo group grown in culture increased from a mean of 27.4 to 49.4 in 5 days whereas the 19-day-old embryo duodenum has a value of 69.7. Siimilar relationships hold for duodenums from a 16-day-old embryo. On the other hand, tissue from a 19-day-old embryo maintains activity for 3 days better than the duodenum *in vivo* which shows a drop in activity after birth (C_3 as contrasted with C_2).

Fig. 3 shows the effect of exposure to hydro-

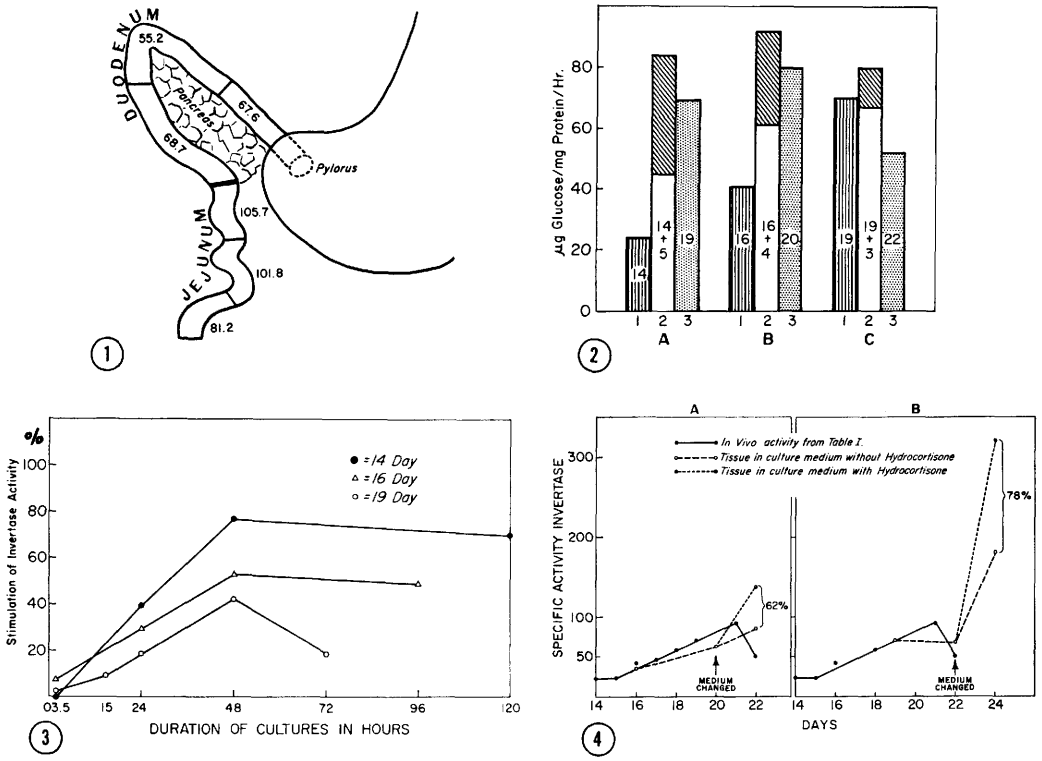

FIG. 1. A schematic diagram showing the anatomical distribution of invertase activities from selected sections of the duodenum and jejunum of 19-day-old chick embryos. The numbers represent the specific activities of the various segments assayed by procedures described in text.

FIG. 2. Comparison of duodenal invertase activity *in vivo* and after culture with and without hydrocortisone.

In each age group (A, B, C) are charted the duodenal enzyme activities of: 1) Embryos of stated age; 2) Duodenums of equal age cultured for 3-5 days without (clear) and with hydrocortisone (shaded); and 3) Embryos chronologically equal to No. 2. Figures in bars represent age in days.

FIG. 3. Figure 3 shows percentage response of invertase activity in 14 (●—●), 16 (△—△) and 19 (○—○) day duodenums cultured for 3.5 to 120 hr in continuous presence of hydrocortisone (0.5 µg/ml) as described in text. Each point represents 6-8 determinations using the duodenums from 1-4 embryos for each determination.

FIG. 4. Figure represents the corticoid induced stimulation of invertase activity after three to four days culture without cortisone.

Tissue cultures were initiated with sixteen- and nineteen-day explants and cultured in the absence of hydrocortisone for four and three days respectively. At the arrow, the cultures were divided and maintained for an additional two days in the presence and in the absence of cortisone. The percentages represent the inductive response to hydrocortisone.

cortisone (HC) on the invertase activity of embryonic tissue obtained from 14-, 16- and 19-day-old embryos. Exposure for 3½ hours was insufficient to stimulate enzyme activity in these cultures. The response became evident after 24 hours and was maximal at 48 hours. Fig. 3 also demonstrates the decrease in stimuation by hydrocortisone with increasing age of the embryos from which the tissues were taken. The response to hydrocortisone is therefore dependent not only upon the duration of exposure but also upon the age of the tissues tested. This relationship is graphically depicted also in Fig. 2 where the shaded portions (A_2, B_2 and C_2) indicate the increased enzyme activity when the culture medium contains hydrocortisone. Both relative and absolute increases in activity decrease with the age of the embryo from which the tissues were taken.

TABLE II. Effect of Substrate and Hydrocortisone on Invertase Activity.

Exp	No sucrose	Sucrose, 2 mg/ml	Avg difference between pairs, %
1. 16-day tissue, no hydrocortisone (3.5 hr)	45.3 52.0	42.6 54.5	— .7
2. 16-day tissue + .5 μg/ml hydrocortisone (3.5 hr)	41.7 54.1 39.1 42.1	64.3 67.6 41.9 48.0	+25.0
3. 19-day tissue + .5 μg/ml hydrocortisone (3.5 hr)	79.6 65.7 73.1 80.9	82.9 57.2 73.5 71.9	— 4.9
4. 19-day tissue + .5 μg/ml hydrocortisone (24 hr)	84.6 88.8 65.5 81.9 74.7 64.4	75.4 73.4 79.8 78.0 76.8 82.0	+ 3.2

16- and 19-day duodenal tissue was prepared for culture and exposed to conditions as shown. Controls contained no sucrose in the medium. At times indicated the cultures were harvested and assayed for invertase activity.

Each duodenum served as its own control, one-half being used for experiment and one-half for control. Because the experiments were done on different days with different batches of eggs, the different groups are not comparable; *e.g.*, Group 4 with Group 3.

The effect of substrate on 16- and 19-day tissues exposed to HC for only 3½ hours is shown in Table II. In the absence of the steroid, sucrose alone was ineffective in stimulating invertase activity. In its presence, however, the substrate results in a 25% increase of invertase activity in the 16-day tissues. The 19-day explants were refractory even when this tissue was exposed to sucrose and HC for 24 hours.

Experiments were then undertaken to determine whether tissues grown for several days in the absence of hydrocortisone would maintain their sensitivity to this agent. Fig. 4 demonstrates that this is the case. Tissues from 16- (Fig. 4A) and 19- (Fig. 4B) day-old embryos were cultured for 4 and 3 days respectively in medium without hydrocortisone. At this time, the cultures were divided and transferred to fresh medium for an additional 2 days, with or without hydrocortisone. Each half of the duodenum served as its own control. Under these conditions, there was a 62% stimulation of activity by hydrocortisone in the 16-day embryo tissue and 78% increase in the 19-day embryo tissue. These results are in sharp contrast to previous observations. Fig. 3 shows only a 49% and 18% response respectively for these tissues when exposed directly and continuously to the hormone for 3 to 4 days.

A surprising result of this experiment was that changing the medium alone (without hydrocortisone) caused a marked increase in invertase activity over that expected from tissues cultured for similar periods of time without changing the medium. For example, the specific activity of 19-day-old embryonic tissue grown with the change in medium but without hydrocortisone showed a specific activity of 180.3, (Fig. 4B). Comparison with Fig. 2 (C_2) shows that 19-day-old embryonic tissue grown for 3 days showed an activity of 67.4 without cortisone and 79.8 with cortisone. This effect was much less pronounced in the case of the 16-day-old embryonic tissue grown as stated above (medium changed after 4 days). The specific activity was 84.7,—a figure to be compared to 61.6 of 16-day-old tissue grown for 4 days as shown in Fig. 2 (B_2).

Discussion. The rapid advance in knowledge concerning the mechanisms involved in protein synthesis and its genetic control has made the study of the regulation of specific enzyme formation of great interest. It has been suggested that hormones may act as specific inducers of genetic transcription for the synthesis of a number of enzymes (7,8). In addition, recent work has further suggested the possibility that some hormones may also have an action on some step in protein synthesis beyond the level of genetic transcription(9).

Our previous experiments(2) have demonstrated that hydrocortisone selectively stimulated the specific activities of alkaline phosphatase and invertase, whereas lactic dehydrogenase appeared to be refractory. The viability of these tissues in culture was evident by light microscopy(2) and, as demonstrated herein, by the capacity to respond

to hormone induction after a culture period of 3-4 days in its absence.

Present experiments suggest in addition that hydrocortisone stimulation of the specific activity of invertase is dependent on: 1) age of the initial tissue explant; 2) duration of exposure to the hormone; and 3) pretreatment of the tissue before hormone application. Maximum stimulation was observed after exposure to the hormone for 48 hours. In general, the extent of stimulation varied inversely with age of tissue beginning with the 14-day-old embryo. Without pretreatment, maximal responses were obtained with 14-day tissues and minimal responses occurred with 19-day tissue. If the tissues were first cultured for 3 to 4 days in the absence of the hormone and then placed in fresh medium and cultured for an additional 48 hours in its presence, however, this age dependent response was lost and even 19-day tissue cultures demonstrated a response similar to 14-day tissues. These preliminary experiments suggest the presence of an inhibitor which diffuses into the medium and is lost when the medium is changed. It is unlikely that these results could be explained by replenishment of essential nutrients alone since tissue *in vivo* does not attain these levels of activity (solid line in Fig. 4). These results may account for some observations reported by Moog(1) for HC induced alkaline phosphatase activity which increased when the cultures were transferred to balanced salt solution. The nature of this inhibitor is currently under investigation.

Summary. Embryonic duodenal tissue cultures respond to hydrocortisone with an increase in the specific activity of invertase. This response is dependent on: 1) the age of the initial tissue explant; 2) the duration of exposure to hormone; and 3) the pretreatment of the tissue before hormone application. These experiments suggest the presence of an inhibitor for the induction of invertase activity.

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The Role of Androgens in Differentiation of the Mammary Gland In Male Mouse Fetuses. (31564)

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Teats do not occur in normal male rats whereas they do develop in male rat fetuses under the influence of antiandrogens. Male mammary bud also showed greater glandularity under the influence of antiandrogens than is seen in normal male animals(1,2). Since the embryonic development of the mammary gland in the mouse is better known than that of any other animal species(3,4) we have extended our observations to the mouse. This is also appropriate because the glandular bud in the male mouse fetus is inhibited more markedly by fetal androgens than is the case in any other murine animal. Concentration of periglandular mesenchyma causes by 15th day of the embryonal development either the