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Prenatal Induction of Sucrase Activity in Rat Jejunum

By PAUL CELANO, JOCELYN JUMAWAN, CAROLYN HOROWITZ, HERBERT LAU and OTAKAR KOLDOVSKY
The Joseph Stokes Jr. Research Institute, Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia, 34th and Civic Center Boulevard, Philadelphia, PA 19104, *and the Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA* 19104, *U.S.A.*

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Daily application of cortisone acetate (10mg/100g body wt.) or L-tri-iodothyronine (20μg/100g body wt.) to female rats in the last (third) week of pregnancy elicits a precocious appearance of jejunal sucrase in their foetuses.

Sucrase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) is found in microvilli (brush border) of the enterocytes of adult mammals. During the suckling period in most mammals its activity is low, and in rats it is practically absent (Koldovsky, 1969, 1972). The activity increases during weaning. Studies in rats have shown the important role of the adrenals and thyroid in the increase during weaning. Adrenalectomy (Koldovsky *et al.*, 1965; Galand & Jacquot, 1970) and thyroidectomy (Koldovsky *et al.*, 1975) delay the increase. Exogenous corticoids (Doell & Kretchmer, 1964; Koldovsky *et al.*, 1965; Herbst & Koldovsky, 1972) or thyroxine (Koldovsky *et al.*, 1974; Yeh & Moog, 1975) can evoke a precocious appearance of sucrase in suckling rats. The question has arisen whether sucrase activity could also be induced precociously during the foetal period. Previous studies performed in our laboratory have shown that, although the sucrase activity could be induced very soon after birth, the sensitivity of the sucrase-producing system to corticoids was substantially lower during the early suckling period than at its end (Herbst & Koldovsky, 1972).

Experiments were therefore performed in which the effect of application of cortisone acetate and L-tri-iodothyronine to pregnant rats during the last (third) week of pregnancy on jejunal sucrase activity of their foetuses was followed.

Materials and Methods

Animals of Charles River strain CD (Wilmington, DE, U.S.A.) were used. Pregnant rats were shipped from the producer by air on day 14 of pregnancy and arrived at our animal house on day 15. On day 16, experiments were started. Rats were assigned, by chance, to different groups. They were weighed daily and injected according to the schedule given in Table 1. Controls were injected with appropriate solvents. Experiments were terminated on day 22 of pregnancy (this is the day when the litters in this strain are born in the late afternoon or at night) by decapitation of the mother. Foetuses were obtained by caesarian section.

Foetuses from each uterine horn were treated as one sample. After their placentas were removed, foetuses were counted, cleaned and weighed; their viability was also noted. Foetuses were then killed by decapitation and jejuna (the first proximal third of the jejunoileum; Koldovsky & Chytil, 1965) from foetuses from one horn were pooled and frozen (–40°C). Assays for sucrase activity and protein were performed within 1 week of collection of intestine.

Cortisone acetate (Merck, Sharp and Dohme, Rahway, NJ, U.S.A.) was injected intramuscularly at a dose of 10mg/100g body wt. (0.2ml of solution/100g body wt. daily in the morning). Controls received 0.9% NaCl intramuscularly (0.2ml/100g body wt.). L-Tri-iodothyronine (free acid; Sigma Chemical Co., St. Louis, MO, U.S.A.) was injected subcutaneously at different doses (see Table 1) dissolved in 0.005M-NaOH in a total fluid volume of 0.2ml/100g body wt. Controls received 0.005M-NaOH.

Assay of sucrase activity was performed as described by Dahlqvist (1964). In each assay the reaction mixture contained 1 vol. of homogenate and 1 vol. of substrate buffer mixture (56mM-sucrose in 0.1M-sodium maleate buffer, pH5.8). The reaction was stopped by placing the samples in a boiling-water bath for 2min. The liberated glucose was determined with Tris/glucose oxidase reagent prepared as described by Dahlqvist (1964) from Glucostat Special (Worthington Biochemical Corp., Freehold, NJ, U.S.A.). When low sucrase activities were assayed in concentrated mucosal homogenates, a standard glucose curve was run in which the standards contained boiled homogenate of the same concentration as in the assay, since concentrated mucosal homogenates inhibit the glucose oxidase reaction (Asp *et al.*, 1967).

Sucrase specific activity is expressed as μmol of glucose liberated/60min per mg of protein. Protein

Table 1. *Effect of injection of cortisone acetate and* L-*tri-iodothyronine into pregnant females on various parameters of pregnant females and their foetuses*
Results are expressed as the mean of each group ± S.E.M. Control and experimental groups are described in the Materials and Methods section. Groups (D) and (E) are presented as supportive evidence only. The number of determinations in each group is shown in parentheses. *P* values refer to the level of statistical significance of difference between control and experimental group calculated by using the *t* test. N.S., Not significant, ($P > 0.02$); N.C., significance not calculated.

	Pregnant females			Foetuses			
	Body wt. (g)		Gain in body wt. (g) between days 16 and 22	Total litter weight (g)	No. of foetuses/litter	Mean body wt. (g)	Jejunal sucrase activity (μmol/60 min per mg of protein)
Group	On day 16	On day 22					
(A) Controls (pooled)	259.2 ± 3.1 (9)	329.4 ± 5.9 (9)	71.9 ± 5.0 (9)	56.0 ± 3.8 (9)	10.1 ± 0.8 (9)	5.5 ± 0.1 (17)	0.013 ± 0.002 (17)
(B) Cortisone acetate (daily, 10 mg/100 g body wt.)	275.2 ± 5.0 (6)	314.0 ± 8.2 (6)	39.3 ± 4.0 (6)	51.5 ± 3.2 (12)	11.0 ± 0.5 (6)	4.7 ± 0.1 (12)	0.050 ± 0.006 (12)
	$P < 0.02$	N.S.	$P < 0.001$	N.S.	N.S.	$P < 0.001$	$P < 0.001$
(C) L-Tri-iodothyronine (daily, 20 μg/100 g body wt.)	271.7 ± 1.2 (3)	331.0 ± 9.5 (3)	59.3 ± 9.8 (3)	55.0 ± 3.6 (3)	11.0 ± 0.6 (3)	5.0 ± 0.2 (6)	0.446 ± 0.095 (6)
	$P < 0.02$	N.S.	N.S.	N.S.	N.S.	$P < 0.001$	$P < 0.001$
(D) L-Tri-iodothyronine (daily, 200 μg/100 g body wt.; two rats only)	270 (295, 245)*	339 (384, 294)	69 (89, 49)	52.5 (68.0, 37.0)†	8.5 (11.0, 6.0)	6.1 ± 0.2 (4)	1.040 ± 0.208 (4)
	N.C.	N.C.	N.C.	N.C.	N.C.	$P < 0.05$	$P < 0.001$
(E) L-Tri-iodothyronine 200 μg/100 g body wt. on days 16 and 19 of pregnancy only, one rat only)	280	327	47	49	9	5.3 (5.0, 5.6)	0.230 (0.210, 0.250)

* Individual body weights of two rats used.
† Foetuses recently dead.

was determined as described by Lowry *et al.* (1951), with standards of bovine serum albumin. Statistical significance of differences was estimated by using Student's *t* test. All chemicals used were of reagent grade.

Results and Discussion

All results are summarized in Table 1, which also gives details of arrangements of L-tri-iodothyronine experiments. Since no differences were noted between the two types of controls (injected with 0.9% NaCl or 0.005M-NaOH), results from controls were pooled and are treated as one group.

All foetuses were found alive and their appearance did not differ from controls, except in the experiments with the highest dose of L-tri-iodothyronine (200μg/100g body wt. daily for 6 days). In this experiment the foetuses were not moving, and apparently had died recently because they were large and their appearance was 'fresh'. Their abdominal cavity contained a dark-red gelatinous substance.

We base our conclusions on results obtained on groups treated with cortisone acetate and with lower doses of L-tri-iodothyronine (20μg/100g body wt. daily for 6 days). Results for the other L-tri-iodothyronine-treated groups (D and E in Table 1) are given only as supportive evidence.

Gain in body weight in pregnant females treated with lower doses of L-tri-iodothyronine used in these experiments did not differ significantly from controls during the experiment. Treatment with cortisone, however, did cause a significantly lower gain in body weight as compared with controls. Although the number of foetuses per litter as well as the total weight of the litter did not differ significantly in experimental groups from controls, the mean body weight of foetuses of females treated with L-tri-iodothyronine (20μg/100g body wt. daily for 6 days) and cortisone was significantly lower than that of controls.

Treatment of pregnant females with cortisone and L-tri-iodothyronine evoked a precocious increase of sucrase activity in foetal jejunum, thus showing that the sucrase-producing system in foetal enterocytes is already capable (competent) of reacting to the stimulation. Although in our experiments we have seen a more pronounced effect of L-tri-iodothyronine than of cortisone acetate, the arrangement of these experiments does not show that L-tri-iodothyronine is more effective.

There are only three reports on the effect of adrenals on the development of alkaline phosphatase in the small intestine of mammalian foetuses. Verne & Hebert (1949) and Ross & Goldsmith (1955) have shown histochemically an increase in alkaline phosphatase after the application of adrenal-cortex extract to the rat embryo or of cortisone to the pregnant rat. Bearn (1973) reported that decapitation *in utero* of foetal rabbits abolished the increase in alkaline phosphatase. Administration of corticotropin to these foetuses reverses the effect of decapitation on the enzyme.

On the other hand, the effect of hormones on the prenatal development of liver and lung has attracted considerable attention. Prenatal application of steroids accelerates the functional development of foetal liver (e.g. Jacquot *et al.*, 1973) and lung (Taeusch *et al.*, 1972; Gross *et al.*, 1975; Lefebvre *et al.*, 1976). Similarly thyroxine applied directly to foetuses induces foetal liver enzymes (Schapiro & Percin, 1966; Greengard & Dewey, 1968) or accelerated maturation of lungs (Wu *et al.*, 1973). According to literature reviewed by Fisher (1975), the extent of transplacental transfer of thyroid hormones is significant but limited. Nevertheless, our experiments, as well as those of Schapiro & Percin (1966), which also used large doses of thyroid hormones, suggest than an increase in maternal concentration of thyroid hormones could be followed by a transfer thereof to the foetus in effective doses.

This study has demonstrated the inducibility of sucrase activity in the foetal period. Further experimentation is needed to elucidate the mechanisms leading to the observed phenomenon.

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