Opposite effects of one and three injections of cortisone or thyroxine on intestinal lactase activity in suckling mice[1]

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Summary. A single injection of cortisone or thyroxine to 8-day-old suckling mice initiates a temporary decrease of lactase activity. On the contrary, 3 injections of cortisone or thyroxine provoke a significant increase of lactase activity. It appears that the mechanism which controls the postnatal development of lactase in suckling animals is more complex than expected.

In mice and rats, lactase (neutral β-galactosidase) activity is abundant at birth and falls to a low level at the time of weaning. The decrease of lactase is in contrast to the concurrent increase of the other brush border enzymes[2-4]. It is well established that the pattern of intestinal maturation is under the control of glucocorticoid hormones and of thyroxine [2, 3, 6-10]. Yeh and Moog[6] have observed that jejunal lactase fails to decline in rats hypophysectomized at 6 days of age. They have shown that administration of thyroxine or cortisone to these animals from 19 to 22 days of age depresses lactase activity, but the level in the cortisone-treated animals was higher than those given thyroxine. On the other hand, Koldovsky and Sunshine[7] reported that administration of cortisone to intact suckling rats did not bring about a premature depression of jejunal lactase activity. A similar behaviour of lactase was observed in vitro after the addition of hydrocortisone[8].

The purpose of the present study was to examine the response of lactase, in the different parts of the small intestine, to the administration of cortisone or thyroxine to intact suckling mice.

Materials and methods. Swiss ICR mice were used. At 8 days of age, the animals were injected i.p. with DL-thyroxine (Sigma) dissolved in 0.005 N NaOH, or with cortisone acetate (Merck Sharp and Dohme) suspension diluted in saline. Dosages were 1 µg thyroxine or 25 µg cortisone per g b.wt/day. Controls received equivalent amounts of the vehicules only. Immediately following decapitation, the intestine was removed, measured and cut into 3 equal parts. Each part was weighed and homogenized in 99 vol. of ice-cold redistilled water. The homogenates were used immediately for enzymatic determinations. Lactase was assayed according to a modification by Lloyd and Whelan[11] of Dahlqvist's method[12], and proteins were determined according to Lowry et al.[13].

Results and discussion. As shown in figure 1, the activity of lactase is falling throughout the intestine 24 h after a single injection of cortisone. However, during the 2nd and the 3rd day, no significant difference can be observed between the cortisone-treated animals and the controls, except for the distal third where an unexplained increased lactase activity is noted during the 3rd day. A single injection of thyroxine

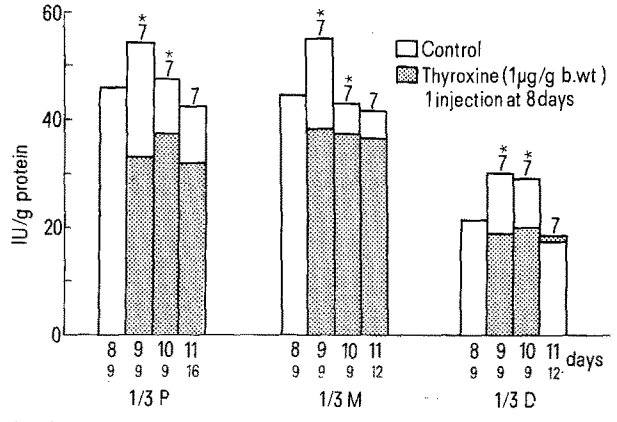

Fig. 2. Influence of a single injection of thyroxine on lactase activity. Symbols as in figure 1. *24 h after the injection (1/3 P and 1/3 M, $p<0.0025$; 1/3 D, $p<0.0125$); 48 h after the injection (1/3 P and 1/3 D, $p<0.005$; 1/3 M, $p<0.05$).

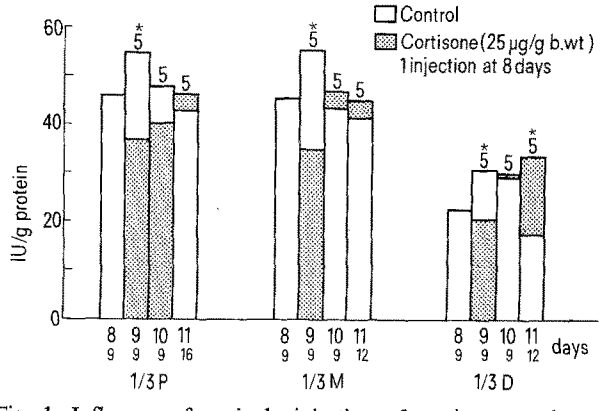

Fig. 1. Influence of a single injection of cortisone on lactase activity. Results are reported for proximal thirds (1/3 P), middle thirds (1/3 M), and distal thirds (1/3 D). Small numbers below each day and above each bar represent, respectively, the number of intestines assayed for the controls and the cortisone-treated animals. *Differences between controls and treated-animals (1/3 P and 1/3 M, $p<0.005$; 1/3 D, $p<0.025$).

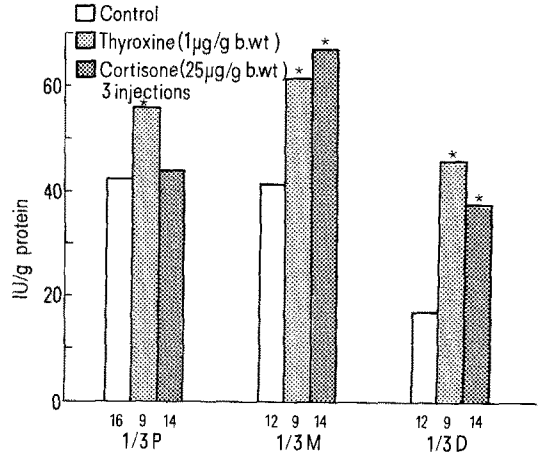

Fig. 3. Influence of thyroxine or cortisone on lactase activity. The hormonal treatments started at 8 days and the suckling mice received 1 injection per day for 3 days. The animals were sacrificed 24 h after the last injection. Symbols as in figure 1. *Differences between controls and treated-animals: thyroxine (1/3 P, $p<0.05$; 1/3 M, $p<0.0005$), cortisone (1/3 M, $p<0.0125$; 1/3 D, $p<0.0005$).

also initiates the decrease of lactase along the entire small intestine (figure 2). However, thyroxine appears to be more effective in initiating the decrease of lactase than is cortisone, as the decreased activity can be observed for 48 h following the single injection. This conclusion is in accordance with that of Yeh and Moog[6]. The temporary depression of lactase observed after a single injection of hormone could be related to the inadequacy of the hormonal stimulus, even though a single injection of cortisone is able to provoke a precocious appearance of sucrase and an increase of maltase activity[3]. In order to verify this, the response of lactase has been studied after 3 injections of hormone. An opposite effect of cortisone is noted (figure 3). Indeed, a significant increase of lactase activity is recorded in the middle and distal thirds, cortisone being without effect in the proximal third. 3 injections of thyroxine (figure 3) also stimulate the lactase activity, even in the proximal third. The opposite effects of 1 and 3 injections of hormone on intestinal lactase activity in intact suckling mice is surprising. Lactase seems to be the only brush border enzyme to respond in that way. Indeed, both 1 or 3 injections of cortisone induce a premature increase of the other brush border enzymes[2, 3, 10]. All the factors possibly involved in the regulation of intestinal maturation are not known. In hypophysectomized or thyroidectomized suckling rats, repeated injections of cortisone or thyroxine do decrease lactase activity. It appears that hypophysectomy or thyroidectomy may affect the development of at least one unknown factor, and the administrated hormone is then able to provoke the decrease of lactase activity. The mechanism which regulates the postnatal development of lactase appears to be different from that of the other brush border enzymes and more complex than expected.

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Degradation of bacterial lipopolysaccharide by gut juice of the snail *Helix pomatia*

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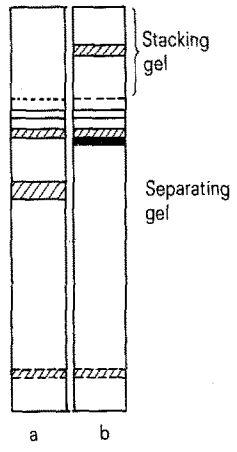
Summary. Lipopolysaccharides from several bacteria were selectively degraded by gut juice of the snail *Helix pomatia* with extensive loss of anticomplementary activity and changes in the electrophoretic pattern in polyacrylamide gels. The gut juice had little effect on ketodeoxyoctonate content or immunodominant sugars. The lipid A moiety of the lipopolysaccharide appeared to be the main site of attack.

In contrast to the voluminous literature on the alteration or degradation of bacterial lipopolysaccharides by chemical methods, there are only a few reports on biological systems which degrade lipopolysaccharides. These include phages[1,2], bacterial isolates[3,4], the amoebae of the cellular slime mould *Dictyostelium discoideum*[5] and mammalian tissue[6]. As part of a survey on the biodegradation of lipopolysaccharides in nature we have examined the gut juice of the snail *Helix pomatia* which is known to contain many degradative enzyme activities[7].

Methods. snail gut juice was obtained from Sigma Chemical Co., St. Louis, USA (β-glucuronidase from *Helix pomatia* crude soln. No. G-0876). Lipopolysaccharides (extracted by the Westphal procedure) from *Escherichia coli* strain $O_{55}B_5$, *E. coli* strain $O_{111}B_4$ and *Shigella flexneri* were purchased from Difco Laboratories, London, England. Lipopolysaccharide from *Salmonella minnesota* strain 9700 was prepared in this laboratory by the method of Westphal et al.[8]. This procedure was also applied to mixtures of snail gut juice plus lipopolysaccharide to analyze degradation of the lipopolysaccharide. Ketodeoxyoctonate was determined by a modification[9] of the thiobarbituric acid method[10] with ketodeoxyoctonate-1,4-lactone (from British Drug Houses, Poole, England) as standard. The lactone was found to yield an absorbance value equivalent to that given by ketodeoxyoctonate when allowance was made for the difference in mol.wt. For convenience, analytical results were expressed as ketodeoxyoctonate. Slab gel electrophoresis with a discontinuous SDS buffer system was carried out by the method described elsewhere[11] using lipopolysaccharide in place of protein. The bands were made visible by the periodic acid-Schiff procedure[12]. For haemagglutination-inhibition tests, serial dilutions of antigen (i.e. potentially



Line drawing of the band patterns observed after SDS-polyacrylamide gel electrophoresis of *E. coli* strain $O_{55}B_5$ lipopolysaccharide, *a* alone and *b* after incubation with snail gut juice as described in the table.