Inhibition of the Cortisone-Evoked Increase of Intestinal Sucrase by Actinomycin D[1] (36621)

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(Introduced by A. S. Goldman)

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There has been considerable interest in the development of hydrolytic enzyme systems in the small intestinal mucosa of various mammals in recent years. Sucrase activity in the small intestine of suckling rats was demonstrated to be virtually absent during the first 2 weeks of postnatal life. An increase in sucrase activity occurs during the third postnatal week (1–3). This maturation is subject to hormonal control since hydrocortisone administration causes a premature increase in the sucrase activity (1) and adrenalectomy of suckling rats will delay the increase of sucrase activity beyond the third postnatal week (4). A similar advancement in the increase of sucrase activity following cortisone has been reported to occur in the small intestine of suckling mice and of the chick embryo (5, 6).

In further evaluation of this hormonal effect, Moog (5) found the administration of actinomycin D did not prevent the precocious increase in sucrase activity evoked by cortisone. In addition, in her experiments with suckling mice she found that actinomycin D alone caused an enhancement of jejunal sucrase activity. Based on these and other experiments in which inhibitors of protein synthesis such as puromycin, cycloheximide and ethionine caused a similar increase in sucrase activity, Moog (5) concluded that the cortisone-evoked increase of sucrase activity is probably produced by a rearrangement of preexisting protein molecules rather than *de novo* synthesis of enzyme protein. In similar studies using the small intestine of the chick embryo, Brown (6) concluded that the actinomycin D evoked increase of sucrase activity could be attributed to the interference in the production of a naturally occurring inhibitor or repressor of sucrase activity.

The present communication summarizes the results of our studies in suckling rats in which actinomycin D was found to inhibit the cortisone-evoked increase of sucrase activity.

Materials and methods. Pregnant Charles River rats were obtained and gave birth in our own animal house. On the second day after birth, the size of the litter was reduced to 8–9 pups. At the start of the experiment, animals in each litter were divided into four groups: controls, those injected with actinomycin D, cortisone, or both actinomycin D and cortisone. Animals were sacrificed by decapitation and the small intestine was excised. The duodenum was discarded. The remaining small intestine was divided along its length into three segments (7), the first being called the jejunum and the last the ileum. After flushing the segments with ice-cold 0.9% NaCl, the segments were homogenized in bi-distilled water in a Potter-Elvehjem homogenizer using a Teflon piston.

Assay of sucrase activity was performed according to Dahlqvist (8) in a slightly modified form. In each assay the reaction mixture contained 1 ml of homogenate and 1 ml of substrate–buffer mixer (0.056 *M* solution of sucrose in 0.1 *M* Na maleate buffer, pH 5.8). The reaction was stopped by placing the samples in the boiling water bath for 2 min. The liberated glucose was determined

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TABLE I. Effect of Cortisone and Actinomycin D on Sucrase Activity in the Jejunum of Suckling Rats.[a]

Expt.	After the first injection of cortisone (hr)	Control	Actinomycin	Cortisone	Actinomycin + cortisone
1	48	0.08 ± 0.001 (3)[b]	0.04 ± 0.005 (3)	1.85 ± 0.42 (3)[c]	0.56 ± 0.13 (3)[e]
2	55	0.02 ± 0.003 (4)	0.14 ± 0.015 (4)[c]	1.87 ± 0.23 (4)[c]	0.45 ± 0.091(6)[e]
3	68	0.04 ± 0.005 (5)	0.14 ± 0.042 (5)[d]	2.30 ± 0.26 (6)[c]	1.00 ± 0.23 (6)[e]

[a] Cortisone acetate was administered always im in doses of 5 mg/100 g body weight. In Expt. 1, animals received cortisone and actinomycin D (13 μg/100 g of body wt, ip) on days 8 and 9 of their postnatal life simultaneously with cortisone acetate. Similar results were obtained with a higher dose of actinomycin D (25 μg/100 g body wt).

In Expt. 2, animals received cortisone and actinomycin D (25 μg/100 g body wt, ip) on days 12, 13 and 14, application of actinomycin preceded that of cortisone by 5 hr. Similar results were obtained when actinomycin was given subcutaneously.

In Expt. 3, animals received cortisone and actinomycin D (25 μg/100 g of body wt, sc) on day 12, the injection of actinomycin D preceded the injection by 5 hr, on day 13 a second injection of actinomycin D (12.5 μg/100 g of body wt, sc) was given. Similar results were obtained when only one injection of actinomycin was given or when actinomycin D and cortisone were given simultaneously.

[b] Activity of sucrase is expressed as mean ± SEM, micromoles of liberated glucose per milligram of protein per 60 min. Number of animals used is given in parentheses.

[c] This value is significantly different from value found in control group: $p < .01$; [d] $p < .05$.

[e] This value is significantly different from value found in cortisone-treated rats $p < .01$.

with Tris–glucose–oxidase reagent prepared according to Dahlqvist from Glucostat (Worthington). When low sucrase activity was encountered in the mucosal homogenates, it became necessary to use concentrated homogenates which, in turn, inhibit the glucose oxidase reaction. To compensate for this inhibition, boiled homogenates with the same protein concentration as used in the assay were added to the glucose standards (9). Sucrase activity was assayed during linear conditions in respect to time and amount of protein homogenate. The details of actinomycin D (Calbiochem, Los Angeles, CA) and cortisone acetate (Upjohn, Kalamazoo, MI) administration are given in the legend to the Table I. Student's t test was employed to test the significance of the results.

Results. Various experimental conditions were used and these data are summarized in Table I. In agreement with previously published data, little sucrase activity was detected in either 10-, 14- or 15-day-old control animals. The administration of cortisone acetate always resulted in an elevation of sucrase activity when compared to aged matched controls (1, 10). In experiments where 12-day-old rats were treated with actinomycin D only, a slight increase of activity was observed (0.14 μmole vs. 0.02–0.04 μmole of liberated glucose/mg of protein/60 min in control rats).

Under various experimental conditions, actinomycin D always produced an inhibition of the cortisone-evoked increase of sucrase activity. These values were always at least 50% lower than those obtained from cortisone treated animals alone. The inhibition of the cortisone-evoked increase by actinomycin D was not dependent on the method of its administration, *i.e.*, the same inhibitory effect was seen with either subcutaneous or intraperitoneal injection. The inhibition was also seen regardless of whether cortisone or actinomycin D was injected repeatedly or given to the animal only on one occasion. The inhibition was also present regardless of the age of the animals when the treatment was started, *i.e.*, 8- and 12-day-old animals responded similarly. In addition, actinomycin

D also prevented the precocious increase of sucrase activity in the ileum (not shown in Table I).

Discussion. The present results further characterize the phenomenon of cortisone-evoked increase of sucrase activity in the small intestine of immature animals. This cortisone evoked increase of sucrase appears to be a general phenomenon, since it occurs both in mammals (1, 4, 5) and avians (6).

Whereas in mice the increase was explained by rearrangement of preexisting protein molecules (5), our experiments have shown that activation of preexisting protein can only account for a small part of the evoked increase. Under our experimental conditions *de novo* protein synthesis prevailed. The conditions of this experiment in mice (5) are closely comparable to one of our experiments (Expt. No. 3 and its variation; see footnote to the Table I). In similar experiments in rats Gallagher and Mearrick (11) support our findings. However, insufficient details were published in this abstract to allow complete comparison.

Thus the only significant difference between our experiments and those of Moog (5) is that the present study employed suckling rats instead of mice. Although the small intestine of rats and mice shows similarities in the development of various functions there might exist some differences in the developmental pattern of sucrase activity (12). Since changes during the postnatal development in mice have not been studied in detail, we offer this only as a possible explanation for the discrepant results.

Summary. Administration of actinomycin D inhibits the cortisone-evoked increase of sucrase activity in the jejunum and ileum of suckling rats. An interpretation of the present study suggests that *de novo* protein synthesis was inhibited and prevented the cortisone-evoked increase of sucrase activity.

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