longer lifetimes in the atmosphere and assuming that the atmospheric aerosols are only weakly absorbing, we can only guess that small values of $(1 - \omega)/\omega\beta$ would be favored and that generally cooling would be expected. Also, the present data on climatic trends do not offer any clues to the effect of aerosols (*5*). We hope that the present gaps in our knowledge will be shortened in the near future by measurements of the aerosol parameters. In fact, someday their effect may be measured directly when the changes in the albedo of the earth-atmosphere system are remotely monitored by satellites (*11*).

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7. Since $I_+(\tau)$ and $I_-(\tau)$ are assumed to be isotropic over the upward and downward hemispheres, respectively, integrating Eq. 1 over μ gives Eqs. 2 and 3 with

$$\beta\omega = \frac{1}{2}\int_0^1 d\mu \int_0^1 d\mu' p(\mu, -\mu')$$

Thus $\beta\omega$ is proportional to the average backscattering cross section of the aerosol.
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Intestinal Lactase Activity in the Suckling Rat: Influence of Hypophysectomy and Thyroidectomy

Abstract. *Intestinal lactase activity, which is high in the infant rat intestine but falls to a low level by the end of the third week, fails to decline in animals hypophysectomized at the age of 6 days. Treating these animals with thyroxine lowers lactase activity to the control level at 24 days, but cortisone is only partly effective. Thyroidectomy at 6 days also results in persistence of high lactase activity; thyroxine again is more effective than cortisone in reducing activity. The thyroid gland appears to play a previously unsuspected role in intestinal maturation.*

The intestines of infant mammals are rich in a neutral β-galactosidase (lactase) that is abundant at birth but generally falls to a low level at about the time of weaning (*1*). In mice and rats, lactase activity begins its decline during the second week, reaching the low adult level in the fourth week (*2–5*). This pattern is the reciprocal of the patterns of increase of maltase activity, which is low in murine rodents during the first 2 weeks, and of sucrase, which is absent (*3, 5–7*). Sucrase and maltase activity may be elicited or enhanced precociously by administration of glucocorticoids (*5–7*), but such treatment does not bring about a premature depression of lactase activity (*6, 8*). Evidently the aspect of intestinal maturation that entails the decrease of lactase is controlled by some mechanism other than (or in addition to) the pituitary-adrenal system. This other mechanism is apparently not the loss of substrate when the young no longer consume milk; although the onset of lactase decline may be postponed by a few days in nurslings forced to subsist solely on maternal milk (*9*), attempts to stave off the decrease of the enzyme by supplying substrate orally or intraperitoneally have generally been unsuccessful (*2, 4, 10*).

Although the adenohypophysis influences the maintenance of the adult intestine (*11*) and promotes the growth of the infant intestine (*12*), little is known about the influence of this gland on the specific maturational events that occur in the mouse and rat intestine during the third postnatal week. In studies now under way in our laboratory, we have observed that the hypophysis influences lactase activity at least in part by way of the thyroid gland.

In these experiments we used NLR rats bred in our laboratory. At 6 days of age or later the infants were hypophysectomized under cold anesthesia by the parapharyngeal approach, as described by Walker *et al.* (*13*). Each operation was completed in less than 10 minutes. Litters of ten were used, of which six were hypophysectomized, two were subjected to the same operation with the exception of removal of the gland, and the others remained intact. They were killed at 20, 24, or 28 days of age, at which times the heads and the tracheas with adherent thyroids were fixed in Bouin's fluid for histological examination. Of 257 rats hypophysectomized at 6 days of age, 151 survived to 20, 24, or 28 days of age (when they were killed); 84 of these were found to be completely free of pituitary fragments.

A 5-cm jejunal segment, taken midway along the length of the small intestine, was used for lactase assay. The content was removed by gentle pressure. The piece was weighed, stored in 0.15*M* NaCl at −24°C, and subsequently homogenized in 0.15*M* NaCl in a Ten Broeck grinder. Lactase activity of total homogenates was determined by Dahlqvist's technique (*14*). The substrate mixture consisted of 0.3*M* lactose (Sigma) in 0.1*M* maleate buffer at *p*H 6.0 (*2, 3*). One-tenth of a milliliter of homogenate (2.5 mg/ml) was added to 0.1 ml of substrate mixture and incubated for 60 minutes at 37.5°C; the reaction was then stopped by adding 2 ml of tris-glucose oxidase reagent (Worthington Biochemical Corporation). Protein was determined by the Lowry technique (*15*). Sucrase and maltase activity were also assayed in all homogenates (*16*). Activity is reported in units that represent micromoles of substrate hydrolyzed per milligram of protein per hour. Results were evaluated statistically by Student's *t*-test.

In rats hypophysectomized at 6 days of age, lactase activity at 20 days is as high as in intact animals at 6 days (Fig. 1A). Thereafter activity declines somewhat, but at 28 days of age is still higher than in 2-week-old infants. The experimental animals reported in Fig. 1A are those in which histological examination of the base of the skull showed that the operation was complete; in those in which fragments of anterior pituitary were found, lactase activities were lower than in the complete cases, but significantly above those of intact controls. At 24 days the mean value for sham-operated animals was identical to that for intact

specimens. At 20 days the mean of the sham-operated was higher (4.83 ± 0.63 versus 3.96 ± 0.24 units), but the difference was not significant ($P > .1$).

Hypophysectomy at 10 days of age also resulted in above-normal lactase activities at 24 days. When the operation was performed at 14 or 16 days, the effect was to halt further decline of activity to 24 days (Fig. 1B).

Administration of a single dose of cortisone acetate (Merck Sharp & Dohme) to hypophysectomized animals at 19 or 21 days of age did not influence lactase (or duodenal alkaline phosphatase) after 3 days. If cortisone acetate was given from 19 to 21 days of age (50 μg per gram of body weight daily, intraperitoneally) lactase activity at 24 days was at a level intermediate between those of untreated hypophysectomized subjects and intact 24-day-old controls. This result was apparently not due to inadequacy of the hormonal stimulus, for in the same animals sucrase and maltase activities, which we have found to be very deficient in hypophysectomized animals, rose to normal levels (*16*).

In the chick embryo, the efficacy of cortisone in eliciting phosphatase increase is dependent on the presence of thyroxine (*17*). This fact suggested that the failure of lactase to respond fully to corticoid stimulation in our experiments might be due to an inadequate supply of thyroid hormone, for growth and differentiation of the thyroid are inhibited by hypophysectomy at 6 days of age (*12, 16*). To test this possibility, we injected cortisone acetate (50 μg/g per day, in saline suspension, intraperitoneally) or DL-thyroxine (Sigma; 1 μg/g per day, dissolved in 0.01*M* NaOH, intraperitoneally) from 19 to 22 days of age, and collected the jejunum at 24 days. Hypophysectomized controls received equal volumes of saline or NaOH. After cortisone, lactase activity remained above the normal 24-day value (Fig. 1C), despite the administration of four doses of the hormone in this case (compared with three doses in the experiment described above), but thyroxine alone reduced lactase activity to the control level (Fig. 1C).

This result suggested thyroidectomy as a logical next step. The operation was carried out on 36 rats at 6 days of age. Under cold anesthesia, the thyroid was dissected out through a midventral incision after separation of the sternohyoid muscle along the midventral line. At the end of the experiment the trachea was fixed in Bouin's fluid for evaluation of the completeness of the operation by histological inspection. In each litter used, some control animals were subjected to all operative steps except removal of the gland.

Jejunal lactase was as high in 24-day-old rats that had been thyroidectomized at 6 days of age as in those that had been hypophysectomized at the same age (Fig. 1D). Administration of thyroxine (1 μg/g) daily from 19 to 22 days of age also depressed lactase activity (Fig. 1D). Administration of cortisone acetate (50 μg/g) daily over the same period also depressed lactase activity (Fig. 1D), but the level in the cortisone-treated animals was significantly higher than in those given thyroxine ($P < .005$). Activity at 24 days was lower in the sham-operated rats than in intact rats, the difference being significant ($P < .025$). Activity in untreated thyroidectomized animals was the same as in those given saline or NaOH.

Both hypophysectomized and thyroidectomized rats at 24 days of age weighed only about 60 percent as much as their intact littermates at the same age, the growth-retarding effect of the former operation being slightly greater. The growth of the small intestine was more severely affected than that of the whole body, the relative weight of the organ at 24 days being in turn about two-thirds of that in normal littermates (*16*). The administration of cortisone or thyroxine according to the regimens described above did not exert any marked effect on intestinal weight or structure. On histological preparations of proximal jejunum from 24-day-old hypophysectomized or thyroidectomized subjects, the intestinal wall measured 0.15 mm from the serosa to the mouths of the crypts. There was little variation in this dimension from animal to animal, whether or not cortisone or thyroxine had been given. The villi were of equal length (about 0.4 mm) in hormone-treated and untreated animals. Hence the lowering of specific lactase activity elicited by administration of hormones cannot be regarded as an artifact resulting from

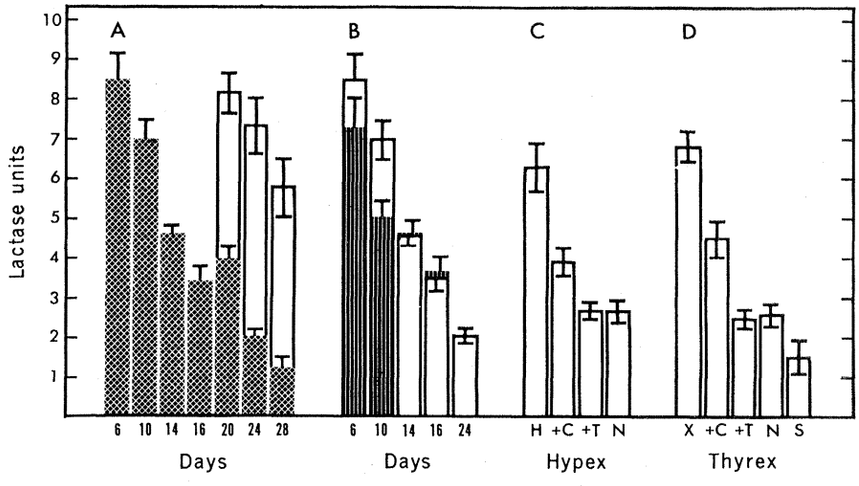

Fig. 1. Lactase activity in rat jejunum. (A) Activity in intact animals from 6 to 28 days (crosshatched columns) and in animals hypophysectomized at 6 days of age (clear columns). There were six to ten rats in each control group, and six to nine in each operated group. Vertical lines indicate standard error of the mean. (B) Activity at 24 days in rats hypophysectomized at 6, 10, 14, or 16 days of age. In each of the first four columns, the clear portion shows lactase activity in intact animals on the day shown on the abscissa; the striped portion shows activity at 24 days in animals operated on the day shown on the abscissa. The fifth column represents activity in intact rats at 24 days. There were six in each group of intact animals, nine hypophysectomized at 6 days, seven at 10 days, and five each at 14 and 16 days. (C) Activity at 24 days in six intact rats (*N*) compared with that in seven rats hypophysectomized (*Hypex*) at 6 days (*H*), 11 hypophysectomized and given cortisone (*C*), and seven hypophysectomized and given thyroxine (*T*). See text for details. $P < .005$ for H versus $H + C$; H versus $H + T$; and $H + C$ versus $H + T$. (D) Activity at 24 days in eight intact (*N*) and four sham-operated rats (*S*) compared with that in ten rats thyroidectomized (*Thyrex*) at 6 days (*X*), seven thyroidectomized and given cortisone (*C*), and six thyroidectomized and given thyroxine (*T*). See text for details. $P < .005$ for N versus $X + C$; X versus $X + C$; and $X + C$ versus $X + T$.

the presence of different amounts of lactase-free tissues under different experimental regimens.

The finding that hypophysectomy in early infancy retards the decline of lactase activity that normally occurs in the juvenile animal is not unexpected. Despite the failure to accelerate lactase decrease by exogenous glucocorticoids (*6, 8*), it is well established that the pattern of intestinal maturation, in which decrease of lactase activity is a correlated element, is under the control of the pituitary-adrenal system (*1, 18*). That the pituitary may affect intestinal maturation in mammals by way of the thyroid is, however, somewhat surprising. Except for a recent report that thyroxine elicits premature cessation of uptake of macromolecules in intact rats (*19*), the thyroid hormone has not previously been implicated in the differentiative events that normally precede weaning in mammals (*20*).

Since glucocorticoids are undoubtedly essential in promoting intestinal differentiation, it might be assumed that thyroid hormone exerts its influence by stimulating glucocorticoid secretion (*21*). Obviously, thyroxine could not do so by way of the pituitary in our experiments, since it elicits lactase decrease in hypophysectomized rats. Probably thyroxine does not act by directly stimulating the adrenal cortex to increase its output either, for the thyroid hormone is more effective in depressing lactase in hypophysectomized rats than is cortisone. There is in fact little evidence to support the view that thyroxine may exercise a direct effect on the adrenal cortex (*22*); but the question has not been investigated in infant animals.

Another possibility is that the effects of thyroxine interact with those elicited by glucocorticoids within the intestinal epithelium itself. Such a relationship appears to obtain in both the chick embryo (*17*) and adult rat (*23*), in which cortisone fails to stimulate increase of alkaline phosphatase unless thyroxine is available. The situation in the suckling rat is different, however. Lactase remains high in the 24-day-old thyroidectomized animals; it is significantly depressed by administration of cortisone, but is lowered to the normal level at 24 days only if thyroxine is given. This result probably does not mean that thyroxine alone is involved in lactase decline, for the decrease of the enzyme is said to be delayed in adrenalectomized animals (*20*), a finding which we have confirmed. Our preliminary studies on adrenalectomized rats indicate that thyroxine is only partially effective in reducing lactase activity in the absence of adrenocorticoids.

That the thyroid hormone should play a critical role in the development of the intestine in the preweaning period is consistent with what is known of the influence of this hormone on the development of the brain. Although thyroprivia has no deleterious effects during the first 10 days in the rat, the hormone is essential for normal differentiation of the cerebellum between 10 and 21 days (*24*). Hamburgh *et al.* (*25*) have in fact ascribed a "clock" function to the thyroid in regulating brain differentiation in the precise period in which intestinal lactase activity is declining.

The decrease of lactase activity in the suckling period is in contrast to the concurrent increase of numerous other enzymes in the intestine and in other organs (*26*). Is the reduction of lactase a biochemical reminiscence of the role of thyroxine in amphibian metamorphosis, in which this hormone regulates the regression of the intestine, among other catabolic events? This view predicts that the decline of lactase is due to a transient enhancement of the degradation rate, a possibility that is not necessarily at variance with the fact that the turnover rate of the enzyme surviving in the adult intestine is similar to that of maltase or sucrase (*27*).

It may in fact be appropriate to describe the developmental alterations that occur in the intestinal epithelium in the third postnatal week not as differentiation or maturation, but rather as metamorphosis. The intestinal epithelium of the neonatal rat is highly differentiated to carry out the digestion and absorption of milk. The changes of the late suckling period represent a redifferentiation in which the epithelium loses the structural and histochemical qualities appropriate to the pinocytotic functioning of infancy (*28*), and at the same time acquires the characteristics required for the type of membrane digestion (*29*) that predominates in the adult. That thyroxine should play a vital part in such a metamorphic program would not be surprising. This view of thyroid involvement in intestinal development is, moreover, not inconsistent with our finding that the hormone is also required for the increase of alkaline phosphatase activity in hypophysectomized animals (*16*), for it is well known that thyroxine regulates numerous anabolic as well as catabolic events in amphibian metamorphosis (*30*).

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