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Intestinal Mucosal Changes Following Induced Hypothyroidism in the Developing Rat*

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Summary. In this study, morphological changes which occur in the small intestine of hypothyroid rats are described. Hypothyroidism was induced during the first 24 h postnataly. – Samples from duodenojejunal and ileocecal junctions were taken, and histologic, histomorphometric, and ultrastructural studies were performed. The most prominent feature was the decrease of the villous height and total mucosal thickness. At electron microscopic level, microvillous height was also markedly decreased. These changes probably represent a direct effect of hormonal deprivation upon the intestinal mucosa, combined with the effect that hypothyroidism may have on the villi.

Key words: Experimental cretinism – Small intestine – Histomorphometry – Ultrastructure.

Introduction

It has been known for many years that hypothyroidism produces a diffuse metabolic upset whose nature is not completely clear at present (Sokoloff et al., 1968).

As far as the intestinal tract is concerned, some biochemical studies have demonstrated a decrease in the absortion of hexoses, both in human hypothyroidism and in hypothyroidectomized animals (Althausen, 1949; Althausen and Stockholm 1938; MacGregor, 1964). Some isolated cases in which hypothyroidism was asso-

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ciated with malabsorption syndromes have also been described (Green and Wollaeger, 1960; Morson and Dawson, 1972). However we have not been able to find any studies describing the morphological changes which may occur in the small intestine either in human hypothyroidism or in thyroidectomized animals.

In the present study, we describe the histologic and electron microscopic changes that take place in the small intestine of the developing rat after induced hypothyroidism (experimental cretinism).

Material and Methods

Hypothyroidism was induced in 18 male Wistar rats by a single intraperitoneal injection of 100 microcuries of I-131. Radioactive iodine was injected during the first 24 h postnatally, according to Goldberg and Chaikoff, 1949. Animals received ad libitum and iodine poor diet (a variant of the Remington diet) and distilled water (Escobar del Rey et al., 1968).

Six groups of rats, 4 per group (3 thyroidectomized and 1 control of the same sex and age) were formed. In order to prevent the changes which occur due to the great difference in weight reached for injected and control rats of the same age, a second control of the same weight was used. Controls received the same diet as thyroidectomized rats supplemented with potasium iodine (1 microgram per gram of dry diet).

Hypothyroid and control rats of each group were sacrificed at ages ranging from 15 to 180 days (see Tables 1 and 2). The first 6 cm of jejunum and the last 6 cm of ileum were taken for hostology. Small fragments of mucosa were selected from these areas for electron microscopy. The tissue was placed on a cork and immersed in formol with Millonig buffer (Carson et al., 1973). Before paraffin embedding, tissue was properly oriented. Sections were stained with Haematoxylin and eosin, P.A.S., Alcian-blue, colloidal iron and Aldehyde-fuchsine.

Villous height and total mucosal thickness were measured in both controls and thyroidectomized rats from 20 consecutive well oriented villi in each section (Wall et al., 1970). Villous height was measured from the tip of the villi to the mouth of the crypt, and total mucosal thickness was measured from the tip of the villi to the middle point of the muscularis mucosae. The height of the epithelial cells which line the villi was also measured at the level of the middle third of both sides of the villi.

Measurements of the height and width of the microvilli which line the epithelial cells of the middle third of the villi were performed in electron micrographs in control and hypothyroid rats. Quantitative methods were carried out according to Calot (1970), and Rios (1970), in order to compare the data obtained from thyroidectomized and control rats.

Histological serial sections of the neck region were made to confirm the absence of thyroid tissue.

Results

Hypothyroid rats presented a marked decrease in size and weight in relation to controls of the same age. They also showed a slowing down in their movements and difuse alopecia, which became more prominent as the age of the animals increased.

Histological Studies. Histological study showed all thyroidectomized animals to have a total abscence of thyroid parenchyma with preservation of the neighbouring structures (parathyroid, striated muscle, etc).

Intestinal changes were restricted to the mucosa, and were similar in all hypothyroid rats. These changes were unrelated to the age of animals. There was a marked decrease in the villous height and total mucosal thickness in both jejunum and ileum (Fig. 1). The muscular layer showed the same thickness in both groups, hypothyroid and controls.

Special staining showed no deposits of neutral and/or acid mucopolisaccharides in the intestinal tract of thyroidectomized rats.

Statistical Studies. Villous height and total mucosal thickness were measured in hypothyroid and control rats. The numerical data obtained were processed in order to carry out a statistical evaluation (Figs. 2 and 3). The following hypotheses were stated:

1. There were no significant differences in the villous height and total mucosal thickness between jejunum and ileum.

2. There were no significant differences in the villous height and total mucosal thickness in relation to the age of animals.

3. There were significant differences in the villous height and total mucosal thickness between hypothyroid and control rats.

In order to examine the first hypothesis we have used the χ^2 significance test, the result proving that the difference between jejunum and ileum was not significant. Therefore, both series of data (i.e., heights of jejunum and ileum) may be considered as belonging to the same population.

$$\chi^2 = \frac{\sum (X_0 - X_e)^2}{X_e} = 8.11; \quad \text{for } n-1 \text{ degrees of freedom } P < 0.1.$$

With respect to the second hypothesis, we calculated the tendency and regression of the data obtained; the resulting equations were as follow:

Villous height of hypothyroid rats: $y = 330.73 - 0.0043\, t$.

Mucosal thickness of hypothyroid rats: $y = 474 + 0.0022\, t$.

Villous height in controls: $y = 455.45 + 0.191\, t$.

Mucosal thickness in controls: $y = 642.98 + 0.532\, t$.

The slopes of regression lines are very close to zero. This means that there is no variation in the data with respect to the age of animals.

In order to check up the third hypothesis we established a confidence for the mean of the hypothyroid series, and we observed that control values are out of the interval, and values of the hypothyroid series were found outside. This prove that these are mutually excluding samples, and therefore they are different. The size of the interval is $3G$, where

$$G = \frac{\sqrt{(X_1 - \bar{X})^2}}{N}.$$

Thus, we may conclude that both villous height and total mucosal thickness of jejunum and ileum show quantitatively significant differences between both populations, controls and hypothyroids.

We did not find differences in the height of the epitelial cells between hypothyroid and control rats.

Electron Microscopic Study. Electron microscopic studies of epithelial cells of intestinal mucosa showed that the microvillous height was decreased approximately 20% in all hypothyroid rats in relation to controls. The width of microvilli showed no differences between both groups of animals (Fig. 4). We have not found significant alterations in nuclei, endoplasmic reticulum, etc.

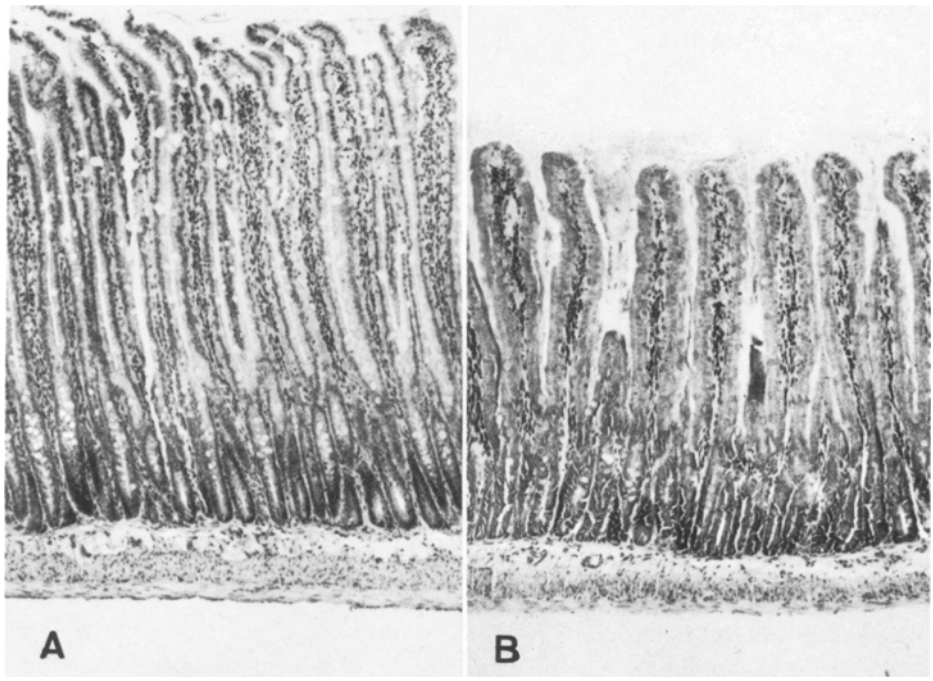

Fig. 1 A and B. Jejunum from control (**A**) and hypothyroid (**B**) rats stained with H.E., obtained at the same magnification. Hypothyroid rat shows a marked decrease of villous height and total mucosal thickness

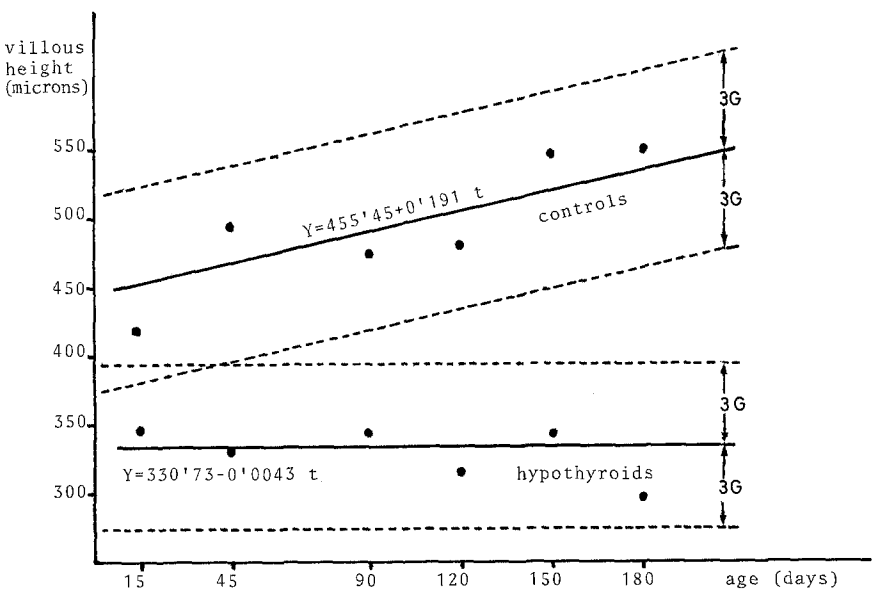

Fig. 2. Relationship between age of animals expressed in days, and villous height expressed in microns. Each dot-represents the mean value for each group of animals sacrified at the same age. Continous lines are mean regression lines for controls and hypothyroid rats. Interrupted lines-indicate confidence limits

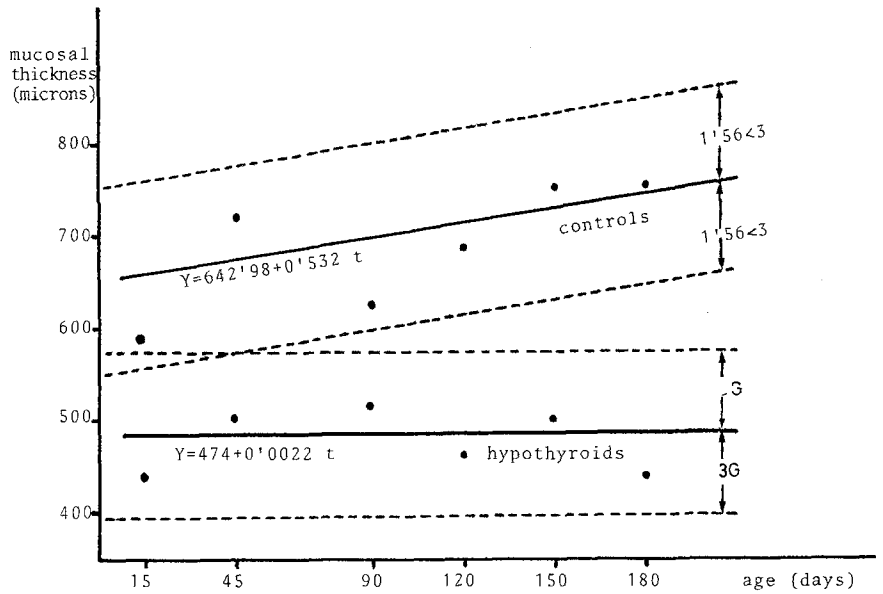

Fig. 3. Relationship between age of animals expressed in days, and total mucosal thickness expressed in microns. Regression lines, confidence limits, and mean values of rats sacrified at the same age are plotted as in Figure 2

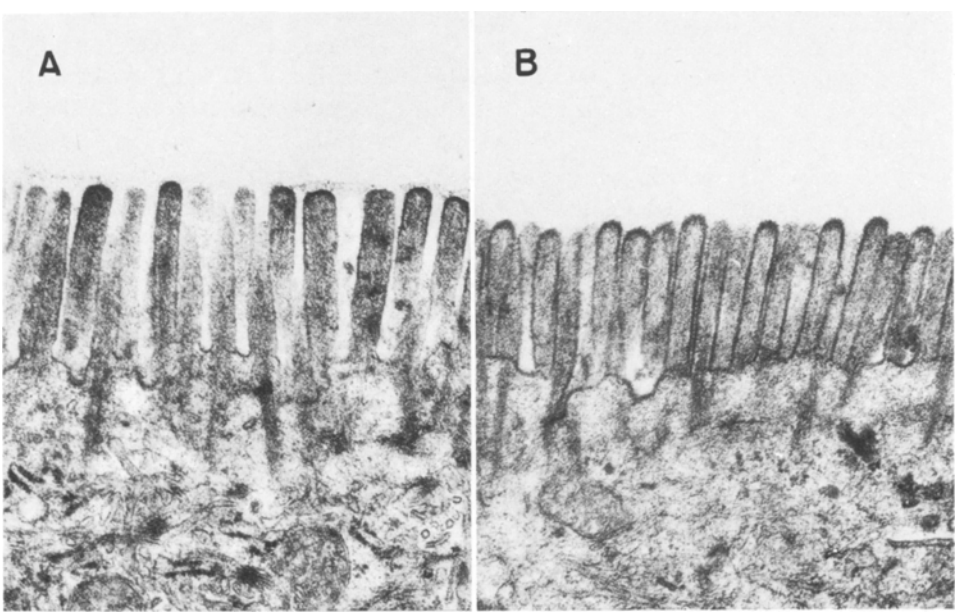

Fig. 4 A and B. Electronmicrograph of epithelial surfaces from control (**A**) and hypothyroid (**B**) rats obtained at the same magnification. Hypothyroid rat shows a marked decrease of microvillous height

Discussion

This study shows that the changes which occur in the small intestine in hypothyroid growing rats consist basically in a decrease of villous height and total mucosal thickness.

Our results show that there are no significant differences in the villous height and total mucosal thickness in relation to age. However Kapadia and Baker (1976), found that villous height increases rapidly in normal rats during the first month, and then shows some fluctuation thereafter. Thus there is a disagreement in the data obtained from 15 day old rats between our results and those of Kapadia and Baker (1976). This may be due to the greater number of normal rats studied in each age group by these workers compared with the number of our controls. Nevertheless, we do not believe that these differences will interfere with our results when comparing hypothyroid and control rats.

Several years ago it was shown that lactation in the rat is accompanied by intestinal hypertrophy, and that this hypertrophy could be prevented by restricting the diet (Campbell and Fell, 1964; MacGregor, 1964). These findings suggest that the cause of hypertrophy during lactation is hyperphagia rather than the increase in metabolic demand which occurs at this time. However, Wall et al. (1970) observed that hyperthyroid rats, which showed villous hypertrophy, doubled their food intake.

We know that hypothyroidism produces hypophagia and constipation (Mac Gregor, 1964) and, as we have shown, a decrease in villous height and total mucosal thickness. It is therefore possible that the changes observed in the small intestine could be due in part to the hypophagia induced by hypothyroidism. We do not believe that the composition of the diet is likely to be responsible for these changes because both groups of animals, hypothyroid and controls, received the same diet. Nevertheless, hypothyroid rats decreased their food intake by about one half in relation to controls.

We think that the size, weight, and age of animals are unrelated to the height attained by intestinal villi. On the other hand, the differences between hypothyroid rats and controls of the same age (which showed a greater weight) were identical to the differences observed between hypothyroid and control rats of the same weight. We therefore believe that a more direct effect of thyroid hormones on the intestinal mucosa must be considered.

Extensive enzymatic changes in experimental hypothyroidism have been described (Wolff and Wolff, 1964); these changes include a decrease in the hexokinase activity of the intestinal mucosa of the rat (Nishikawara, 1961; Nishikawara and Gabrielson, 1961). The mechanism by which the thyroid hormone produces these negative effect on protein synthesis remains unknown (Sokoloff et al., 1968; Tata et al., 1963).

In summary, we consider that the morphological changes observed in the small intestine of hypothyroid developing rats represent a combination of a direct effect of hypothyroidism on the epithelil cells of the intestinal mucosa, and the effects that hypophagia has on the villi.

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