Treatment of Rats with Dexamethasone or Thyroxine Reverses Zinc Deficiency-Induced Intestinal Damage[1,2]

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ABSTRACT Structural and functional damage to the intestine and the potential beneficial effects of dexamethasone (Dex) and thyroxine (T4) were examined in zinc-deficient rats. Rats were assigned to zinc deficient (ZD), control (C) or pair-fed (PF) groups and fed for 40 d a zinc deficient (1 mg/kg) diet (ZD rats) or a similar diet supplemented with 50 mg Zn/kg (C and PF rats). Some rats of the ZD group were treated for the last 10 d with low (250 mg/kg) or high (5 mg/kg) doses of Dex or with T4 (100 mg/kg). Serum corticosterone of T4-treated ZD rats did not differ from untreated ZD rats. Serum T4 of T4-treated ZD rats did not differ from C rats. ZD rats developed ulcerations, inflammation and edema in the small intestine, particularly in the jejunum. PF rats did not show mucosal changes relative to C rats. ZD rats showed significantly lower crypt cell production rate (CCPR) and labeling index (LI) in the three intestinal regions, and lower cell migration rate and higher turnover time in the duodenum relative to C rats. Sucrase and maltase activities of ZD rats were significantly lower than C rats in the three mucosal regions. Treatment with the low dose of Dex resulted in fewer ulcerations compared with ZD rats. In rats administered the high dose of Dex or T4, all morphological alterations disappeared; the CCPR, LI, cell migration rate, cell turnover time and disaccharidase activities did not differ from C rats. In conclusion, Dex and T4 exert beneficial effects on zinc deficiency-induced intestinal alterations in rats. J. Nutr. 127: 1807–1813, 1997.

KEY WORDS: • *zinc deficiency* • *dexamethasone* • *thyroxine* • *intestine* • *rats*

Deficiency of zinc is frequently observed in human populations. Zinc deficiency can be due to inadequate dietary intake, decreased absorption, increased requirements, decreased utilization, increased loss or genetic disease (Bettger and O'Dell 1993, Brewer 1995, Sandstead 1995, Vallee and Falchuk 1993). Deficiency of zinc can be observed in many disease states such as alcoholism, liver disease, sickle cell anemia, renal disease and gastrointestinal disorders (Cho 1991, Okada et al. 1995, Vallee and Falchuk 1993).

The intestine is one of the tissues most sensitive to zinc deficiency. Flattening of villi, decreased numbers of crypts, inflammatory cell infiltration of the lamina propria and lesions of intestinal mucosa have been reported in both experimental animal and human zinc deficiency (Elmes and Jones 1979, Southon et al. 1984, Vallee and Falchuk 1993). These morphological alterations are associated with a loss of enterocyte absorptive function. Several studies have shown an impairment of disaccharidase activity after short or long periods of zinc deficiency (Gebhard et al. 1983, Park et al. 1985). However, no effect of zinc deficiency on intestinal architecture and enzyme activity was reported by other authors (Naveh et al. 1990, Zarling et al. 1985).

The biochemical basis of zinc deficiency pathology has not yet been elucidated. Zinc is essential in many biochemical processes and may have a relevant part in the control of both cell proliferation and cell loss (Bettger and O'Dell 1993). Zinc is essential for enzymes involved in DNA synthesis and mitosis (Wu and Wu 1987), is a component of many transcription factors and proteins that control the cell cycle (Endicott et al. 1995, Shields et al. 1996) and can inhibit apoptosis (Lazebnik et al. 1993, Zalewski et al. 1991). Zinc deficiency affects cell cycle progression (Chester 1989, Prasad et al. 1996), although an increase in cell death without alterations in cell cycle has also been reported in embryonal zinc deficiency (Rogers et al. 1995).

Rat enterocytes are continuously exfoliated from the tips of the villi and are replaced with new cells migrating out of the crypts to reach the villus apex in 2–3 days (Leblond 1981). Reduced crypt cell proliferation may imply a disruption of mucosal structure and function. Only a few studies have investigated whether zinc deficiency could depress the proliferation of mucosal cells. A lower rate of crypt cell division has been shown in jejunum of deficient rats (Southon et al. 1985). However, no direct evidence was reported to indicate whether the reduced mucosal cell proliferation was associated with a decreased cell migration to the villus tip. Moreover, no investigations have been conducted to study whether the three regions of intestine are equally affected by zinc deficiency.

The possibility of repair of damaged tissues in zinc deficiency has been poorly examined. Zinc compounds are used to treat patients with intestinal disorders and a zinc deficient status. However, especially with long term therapy, zinc may

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inhibit copper absorption and influence the metabolism of iron, calcium, and HDL cholesterol (Brewer 1995, Fox 1989, Hooper et al. 1980.)

Dexamethasone (Dex)[4] and thyroxine (T4) are regulators of different intestinal activities, both in normal and pathological conditions. Several studies have shown that glucocorticoids can modulate the proliferation of intestinal epithelial cells. Some authors found a stimulatory effect (Herbst and Koldovsky 1972, Tutton 1973), whereas others reported an inhibitory effect (Wright 1978). Up to now, the role of glucocorticoids in the regulation of epithelial cell proliferation has not been well defined. Thyroid hormones are also regulators of cell proliferation, and their stimulation of epithelial cell production has been demonstrated (Carriere 1966, Tutton 1976).

Both of these hormones can also influence the activity of intestinal brush border enzymes. A role of glucocorticoids and thyroid hormones in the maturation of intestinal enzymes has been reported in several studies (Kedinger et al. 1980, Nanthakumar and Henning 1993, Yeh et al. 1991). Moreover, a synergistic effect of Dex and T4 on enzyme ontogeny has been shown, although no effect was detected when T4 was administered alone (McDonald and Henning 1992).

In this study we investigated whether changes in crypt cell proliferation, cell migration along the villus and turnover time, and enterocyte digestive functions are involved in zinc deficiency-induced damage in the three regions of intestine. Furthermore, we determined whether treatment with Dex or T4 would reverse changes in these parameters.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Charles River, Como, Italy) weighing 150–160 g were divided into three groups: *1*) a zinc deficient (ZD) group of 96 rats fed a purified diet containing 1 mg Zn/kg diet; *2*) a control (C) group of 40 rats fed the ZD diet supplemented with zinc carbonate to raise the zinc concentration to 50 mg Zn/kg diet; and *3*) a pair fed (PF) group of 8 rats fed the C diet but in an amount equivalent to that consumed by the ZD rats, as control for inanition associated with zinc deficiency. The ZD and C rats had free access to food. All rats were fed their respective diets for 40 d. During the last 10 d of dietary treatment, daily intraperitoneal injections of hormone were administered to some rats of the ZD and C groups. Dex was prepared in ethanol and diluted in saline vehicle (9 g NaCl/L). A low dose of Dex [250 μg/kg body wt (0.62 μmol/kg)] was administered to 16 rats, and 26 rats received a high dose [5 mg/kg body wt (12.5 μmol/kg)]. T4 was dissolved in NaOH, diluted in saline vehicle, and administered to 26 rats at a dosage of 100 μg/kg body wt (0.13 μmol/kg). A subset of control rats received these Dex or T4 treatments or the same volume of vehicle. Preliminary experiments indicated that lower doses of Dex and T4 had no effect (data not shown).

All rats were housed in stainless-steel cages in a room maintained at 23°C with a 12-h light:dark cycle. Deionized, distilled water was freely available to all rats. Body weight was measured weekly. The composition of the ZD diet is shown in **Table 1**. The diets were purchased from Ditta Rieper (Vandoies, Italy).

All experiments were approved by the Animal Care and Ethics Committee of the Istituto Nazionale della Nutrizione, Rome, Italy and conformed to published guidelines (National Research Council 1985).

Tissue preparation. At the end of the experimental period, rats were anesthetized with an intraperitoneal injection of pentobarbital (10 mg/kg). Blood was collected by intracardiac puncture for determination of serum zinc concentration. The small intestine was removed, pieces of duodenum, jejunum and ileum (~2.5 cm) were dissected for histology, and the remainder was washed in cold PBS and stored at −80°C for biochemical analysis.

[4] Abbreviations used: BrdU, 5-bromo-2-deoxyuridine; C, control; CCPR, crypt cell production rate; Dex, dexamethasone; ICI, inflammatory cell infiltration; LI, labeling index; PF, pair-fed; T4, thyroxine; ZD, zinc deficient.

TABLE 1

Composition of the zinc deficient diet[1]

Ingredient	Concentration
	g/kg diet
Egg white solids	220
Cornstarch	293
Sucrose	300
Cellulose	40
Soybean oil	70
Mineral mixture[2]	35
Vitamin mixture[3]	10
DL-methionine	4

[1] In the Zn adequate diet, $ZnCO_3$ (0.09 g/kg) replaced an equal amount of cellulose.
[2] Mineral mixture was formulated according to AIN (1993).
[3] Vitamin mixture was formulated according to AIN (1993).

Morphological studies. The pieces of duodenum, jejunum and ileum were immersed in Bouin's fixative for 12 h, washed in PBS for 24 h, embedded in paraffin at 58°C and sectioned at 7 μm. After Mallory staining, sections were analyzed under a light microscope (Leitz, Germany). Histopathological observations were made on at least four sections of the different regions of the intestine. The variables selected to establish the extent of the most evident tissue modifications were ulcerations, edema, inflammatory cell infiltration (ICI) and dilatation of blood vessels. A score from 0 to 3 was assigned to the histological variables in relation to the severity of disease (0 was normal and 3 was severely damaged). The villus length was measured with a micrometer.

Crypt cell production rate (CCPR) and labeling index (LI). Rats were injected intraperitoneally with vincristine (1 mg/kg body wt) to arrest cells in metaphase. After 6 h the intestine was removed, and sections of duodenum, jejunum and ileum were prepared as described above. The CCPR was estimated in 20 crypts by the mean number of nuclei arrested in metaphase per crypt column divided by the time of vincristine exposure (cells $\cdot$ crypt^{-1} $\cdot$ h^{-1}). Crypt column was defined by the number of epithelial nuclei. The LI, i.e. the proportion of cells entering methaphase, was calculated as (number of metaphase-arrested cells in crypt column $\cdot$ 100)/(number of cells in crypt column).

Cell migration rate and turnover time. Rats were injected intraperitoneally with 100 mg/kg body wt of 5-bromo-2-deoxyuridine (BrdU; Sigma, St. Louis, MO) in saline. After 4 and 24 h, the small intestine was dissected, and pieces of duodenum, jejunum and ileum were embedded in OCT compound (TAAB Laboratories, Berkshire, UK). Tissue sections were prepared in a cryostat (5 μm thick), fixed in 70% ethanol and hydrolyzed in a 95:5 (v/v) solution of formamide:sodium citrate buffer pH 7.4 (0.3 mol/L) for 1 h at 95°C as previously reported (Hamman et al. 1992). BrdU labeling was then detected with an immunohistochemical assay using a BrdU labeling and detection kit (Boehringer Mannheim Biochemica, Milano, Italy). Twenty well-oriented villi per specimen were examined for villus height and distance from the base of the crypts to the leading edge of labeled cells. The migration rate (μm/h) was calculated as (distance at 24 h − distance at 4 h)/20, according to Batt and Peters (1976). The cell turnover time (h) was calculated as the ratio of villus height to migration rate (Batt and Peters 1976).

Enzyme and protein assays. Sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) activities were assayed in intestine by the method of Messer and Dahlqvist (1966). Protein concentration was measured by the method of Lowry et al. (1951).

Serum corticosterone and thyroxine analysis. Corticosterone and thyroxine were assayed in serum with an immunochemical assay using a Corticosterone-DA 125I RIA kit and a Thyroxine CT 125I RIA kit (ICN Biomedicals, Costa Mesa, CA), respectively.

Zinc analysis. The zinc concentrations of diets and sera were determined by flame atomic absorption spectrophotometry using a SpectrAA-400 model atomic absorption spectrometer (Varian, Sun-

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TABLE 2

Body weight and serum zinc concentration of control (C), zinc deficient (ZD), and pair-fed (PF) rats and the effect of dexamethasone (Dex) or thyroxine (T4) treatment on ZD rats[1]

	Body weight	Serum zinc
	g	*μmol/L*
C	364 ± 37[a]	19.58 ± 1.99[a]
ZD	189 ± 21[b]	4.59 ± 0.46[b]
PF	233 ± 2[b,c]	20.19 ± 1.99[a]
ZD + Dex, 250 μg/kg	198 ± 22[b]	4.74 ± 0.61[b]
ZD + Dex, 5 mg/kg	185 ± 20[b]	4.89 ± 0.75[b]
ZD + T4	196 ± 21[b]	5.97 ± 0.61[b]

[1] Values are means ± SEM, $n \geq 24$ for all rats except $n = 8$ for PF rats and $n = 16$ for Dex (250 μg/kg) rats. Within a column, values with different superscripts are significantly different. For body weight, [b] $P < 0.01$ and [c] $P < 0.05$; for serum zinc, [b] $P < 0.001$

nyvale, Melbourne, Australia). The diets were first lyophilized and mineralized with nitric acid in the presence of hydrogen peroxide. The accuracy of the method was verified using the appropiate NBS Standard reference (Washington, DC).

Statistical analysis. Data are the mean ± SEM of at least 6 experiments. The significance of the difference was evaluated by one-way ANOVA with a Fisher's protected least significant difference post hoc test (Winer 1971). Differences with P values < 0.05 were considered significant.

RESULTS

Effect of zinc deficiency and treatment with Dex or T4 on body weight and serum zinc concentration. As previously reported (Mengheri et al. 1995), zinc deficiency had a dramatic effect on body weight; ZD rats weighed 48% less than the C rats (**Table 2**). Mean body weight of the PF rats was lower than that of C rats, but it was not significantly different from the other groups. Serum Zn concentration of the ZD rats was significantly lower than that of C or PF rats, which did not differ from one another. Treatment of ZD rats with Dex or T4 did not affect body weight or serum zinc concentration. Body weight and serum zinc concentration of hormone-treated C rats did not differ from C rats (not shown).

Evaluation of mucosal damage and repair. The dramatic morphologic changes of intestinal mucosa after 40 d of zinc deficiency as compared to normal mucosa are shown in **Figure 1.** Histologic scores of ZD rats and of ZD rats treated with Dex or T4 are listed in **Table 3.** In ZD rats the jejunum was the most affected region, presenting widespread ulcerations, severe edema, dense ICI and severe dilatation of blood vessels. The assigned score was 3. Similar damage was also present in the duodenum, which showed a lower number of ulcerations, severe edema, moderate ICI and severe dilatation of blood vessels, with a score of 2–3. The ileum was less damaged, presenting few ulcerations, moderate edema, mild ICI and moderate dilatation of blood vessels, with a score of 1–2. The length of the villi in all the three regions of intestine was unaffected by zinc deficiency. The intestines of the PF rats did not show any alterations relative to C rats and were not further analyzed.

The histologic examination of intestine at the beginning of hormone treatment, after 30 d of zinc deficiency, (data not shown) revealed the presence of few ulcerations, moderate edema and ICI in the duodenum and ileum with a score of 1–2, whereas the jejunum was already severely damaged. In ZD rats administered the low dose of Dex, only a small effect was seen in the duodenum and ileum where ulcerations were scored lower than in untreated ZD rats. Treatment of ZD rats with the high dose of Dex or with T4 had extraordinary effects since no intestinal alterations were observed. The mucosa of the C rats treated with the two hormones did not show any change (data not shown).

Serum corticosterone and thyroxine. The serum corticosterone concentration in the ZD rats was significantly greater than in the C rats (**Table 4**). Administration of T4 had no significant effect on circulating corticosterone. Serum T4 concentration was not significantly affected by zinc deficiency. Moreover, serum T4 in the T4-treated ZD rats was higher than in the ZD rats but not different from that of the C rats. Thus, the dose of T4 used in this study is physiologic, as also reported by others (McDonald and Henning 1992).

Cell kinetics. The CCPR and LI were significantly affected by zinc deficiency (**Fig. 2**). As compared to C rats, the CCPR of the ZD rats was >30% lower in the three regions of small intestine, whereas the LI was 38% less in duodenum and ileum or 28% less in jejunum. The low dose of Dex had little effect only in the duodenum an ileum, although both the CCPR and LI were significantly different from those of the C rats. In rats treated with the high dose of Dex or with T4, the CCPR and LI were not different from the C rats in all regions of the small intestine. The cell migration rate and turnover time are reported in **Figure 3.** These variables were affected in the ZD rats only in the duodenum, with lower migration rate and greater turnover time than in C rats. In the ZD rats treated with the high dose of Dex or with T4, the migration rate and cell turnover time did not differ from the C rats.

Enzyme activities. The sucrase and maltase activities in all three regions of intestine were significantly lower in the ZD than in the C rats (**Fig. 4**). The most dramatically affected was the jejunal sucrase activity. Treatment of the ZD rats with the high dose of Dex or with T4 reversed the negative effect of zinc deficiency on these activities, resulting in activities that did not differ from the C rats in the three intestinal regions.

DISCUSSION

Treatment with Dex and T4 is extremely effective in repairing the dramatic alterations of the intestinal mucosa induced by zinc deficiency. Several studies of the effect of Dex

TABLE 3

Histologic score of zinc deficient (ZD), pair-fed (PF) and ZD rats treated with dexamethasone (Dex) or thyroxine (T4)[1]

	Duodenum	Jejunum	Ileum
C	0	0	0
ZD	2–3[2]	3	1–2[3]
PF	0	0	0
ZD + Dex, 250 μg/kg	1–2[3]	3	1
ZD + Dex, 5 mg/kg	0	0	0
ZD + T4	0	0	0

[1] A score from 0 to 3 was assigned according to the presence and severity of ulcerations, edema, inflammatory cell infiltration (ICI) and dilatation of blood vessels. (0 = normal, 1 = mild alterations, 2 = moderate alterations and 3 = severe alterations).

[2] Means moderate presence of ulcerations and ICI while other histologic variables were severely altered.

[3] Means mild presence of ulcerations and ICI while other histologic variables were moderately altered.

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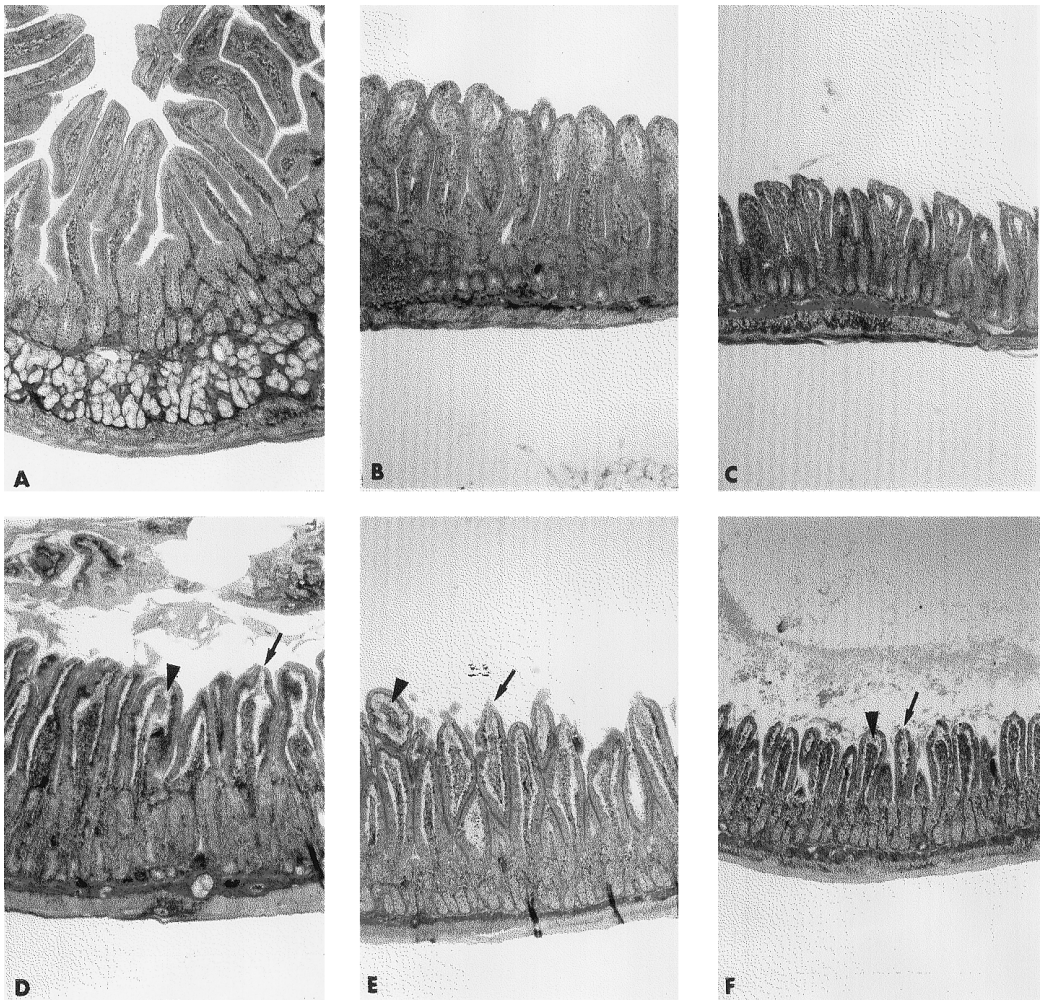

FIGURE 1 Histological photographs showing the alterations in the three regions of small intestine caused by zinc deficiency as compared to normal mucosa. *A-C*: normal mucosa of control (C) rats. *D-F*: mucosa of zinc deficient (ZD) rats presenting ulcerations, edema, inflammatory cell infiltration and dilatation of blood vessels. *A* and *D*: duodenum; *B* and *E*: jejunum; *C* and *F*: ileum. The severity of ulcerations was different in the three regions, and the most affected was the jejunum. The arrows and arrowheads indicate sites of ulcerations and edema, respectively.

that have been conducted on both experimental animals and human intestinal diseases have given contradictory results (Breese et al. 1994, Lionetti et al. 1993). Moreover, there are no data on the use of a physiological dose of T4 to protect intestine from damage. To our knowledge, this is the first time that Dex and T4 have been shown to induce a complete recovery of intestinal damage caused by zinc deficiency.

It has been demonstrated that administration of T4 to intact animals elevates the concentration of serum corticosterone by increasing the concentration of corticosteroid-binding globulin (D'Agostino and Henning 1982). Therefore, the results obtained in this study in ZD rats administered T4 could be ascribed to an increase in circulating corticosterone. However, only a small and insignificant increase was detected in serum corticosterone of the T4-treated ZD rats compared with the ZD rats, indicating that the beneficial effects exerted by T4 are likely not mediated by corticosterone. The lack of an increase in circulating corticosterone after T4 administration can be explained by the inability of the ZD rats to increase the production of corticosteroid-binding globulin or by a negative feedback regulation due to the high serum corticosterone concentration of the ZD rats.

Zinc deficiency induced several ulcerations in the three tracts of the small intestine. These could be the consequence of increased exfoliation of cells that are not adequately substituted by new cells originating from crypts because of a reduced cell production and migration to villus tip. Previous studies have shown reduced cell production in jejunal crypts of zinc deficient rats that possibly were responsible for the morphological and structural changes observed (Southon et al. 1985). In this study we have found that the CCPR and LI were markedly lower in zinc deficient rats. However, the extent of difference between ZD and C rats was similar in the three tracts of

TABLE 4

Serum corticosterone and thyroxine (T4) concentrations in control (C), zinc deficient (ZD) and T4-treated ZD rats[1]

	Corticosterone	T4
	pmol/L	*nmol/L*
C	0.37 ± 0.05[a]	84.9 ± 9.0[a,b]
ZD	0.82 ± 0.10[b]	63.1 ± 10.3[a]
ZD + T4	0.99 ± 0.07[b]	103.0 ± 15.4[b]

[1] Values are means ± SEM, $n = \geq 6$. Within a column, values with different superscripts are significantly different ($P < 0.01$).

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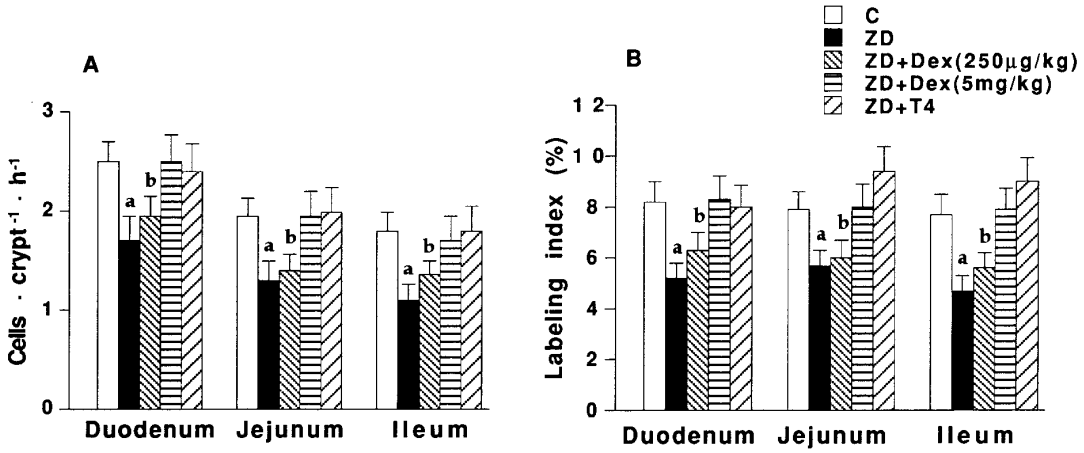

FIGURE 2 Cell kinetics in the different regions of small intestine of control (C), zinc deficient (ZD) and ZD rats treated with dexamethasone (Dex) or thyroxine (T4). Values are means ± SEM, $n \geq 6$. ZD rats were injected with low (250 μg/kg) or high (5 mg/kg) doses of Dex or with T4 (100 μg/kg) for the last 10 d of the experimental period. To measure crypt cell production rate (CCPR) and labeling index (LI), rats were injected with vincristine (1 mg/kg) and killed 6 h later. A: CCPR, expressed as number of cells arrested in metaphase per crypt column divided by the time of vincristine exposure. *B*: LI representing the proportion of cells entering metaphase. Within each region, significant differences from C rats are represented, [a] $P < 0.01$, [b] $P < 0.05$.

intestine or, in the case of LI, less evident in the jejunum. In addition, both the migration rate and the cell turnover time were significantly affected by zinc deficiency only in the duodenum. Since the epithelium of the jejunum is the most damaged by zinc deficiency, our results suggest that the changes in cell kinetics contribute to villus ulcerations but are not the only cause.

Intestinal cell renewal may be influenced by a variety of factors. Hormones, such as corticosteroids and T4, induce changes in the proliferation of intestinal crypt cells. In this study, treatment of ZD rats with the low dose of Dex resulted in fewer ulcerations and higher CCPR, although not significantly, in the duodenum and ileum relative to C rats. Following T4 or the high dose of Dex, the CCPR and LI did not differ from those of the C rats in all three intestinal regions. Therefore, these data suggest that the mucosal lesions can be repaired by Dex and T4 through increased epithelial cell proliferation. Our data are in agreement with previous studies showing that glucocorticoids stimulate the proliferation and migration of enterocytes of both suckling and adult rats (Herbst and Koldovsky 1972, Tutton 1973) and that adrenalectomy reduced the epithelial cell migration rate of fetal sheep and adult rats (Trahair et al. 1987, Tutton 1973). Moreover, studies conducted both in vivo and in vitro showed a stimulation of rat intestinal cell proliferation by T4 (Carriere 1966, Tutton 1976), although it was not clear whether this effect was mediated by an increase in corticosterone level (D'Agostino and Henning 1982). However, it has also been reported that glucocorticoids inhibit mitotic activity of crypt cells of adult rat jejunum (Wright et al. 1978). This discrepancy could be explained by the different dose and length of Dex treatment or, more likely, by the different physiological conditions of the animals.

The cell migration rate and cell turnover time in the duodenum contributed to the intestinal healing induced by the two hormones. A higher cell migration rate and lower turnover time could also be expected in the most damaged jejunum, although these variables were not altered by zinc deficiency. The fact that the cell migration rate and turnover time did not change in the jejunum may indicate that the increase in

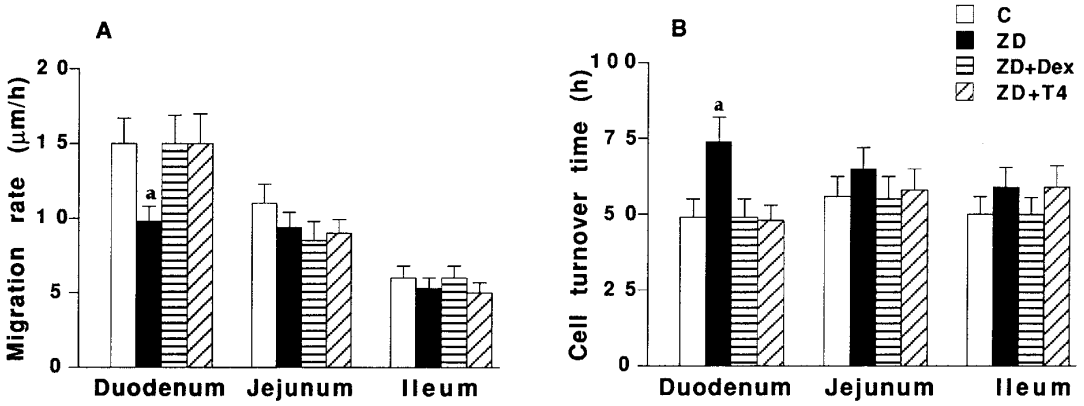

FIGURE 3 Migration rate and cell turnover time in the different regions of small intestine of control (C), zinc deficient (ZD) and ZD rats treated with dexamethasone (Dex) or thyroxine (T4). Values are means ± SEM, $n \geq 6$. Dex (5 mg/kg) or T4 (100 μg/kg) were administered as daily intraperitoneal injections to ZD rats for the last 10 d of the experimental period. Rats were injected with 5-bromo-2-deoxyuridine (100 mg/kg) and killed 4 or 24 h later. The migration rate (*A*) was calculated as (distance at 24 h − distance at 4 h)/20 h. The cell turnover time (*B*) is expressed as the ratio of villus height to migration rate. Within each region, significant differences from C rats are represented, [a] $P < 0.01$.

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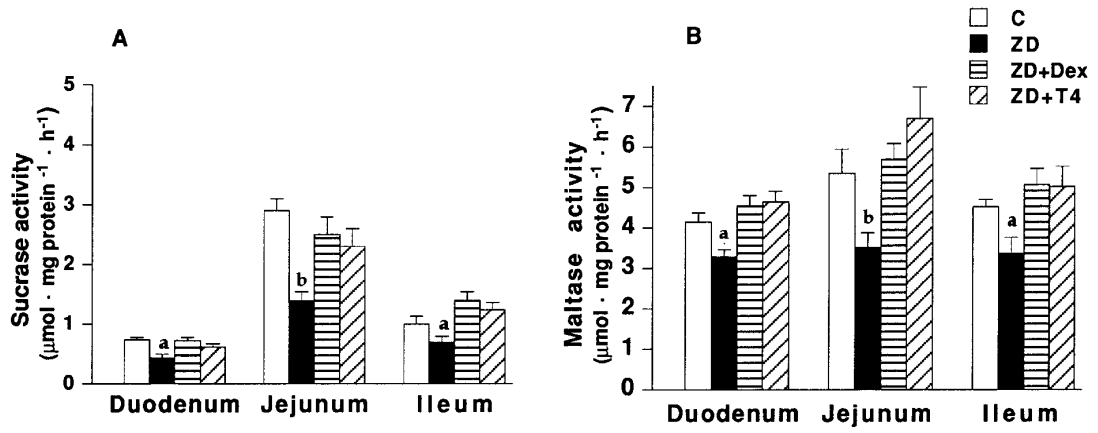

FIGURE 4 Activities of sucrase (*A*) and maltase (*B*) in the three regions of small intestine of control (C), zinc deficient (ZD) and ZD rats treated with dexamethasone (Dex) and thyroxine (T4). Values are means ± SEM, $n \geq 6$. Dex (5 mg/kg) or T4 (100 μg/kg) were injected to ZD rats for the last 10 d of the experimental period. Within each region, significant differences from C rats are represented, $^{a}P < 0.05$, $^{b}P < 0.01$.

CCPR is sufficient to replace dead cells at villus tip or mechanisms other than changes in cell kinetics are involved in the ulceration healing. It has recently been suggested that deficiency of zinc causes a desensitization to external signals, and consequently a cascade of events take place that result in functional changes at cellular level (Bettger and O'Dell 1993). It is possible that Dex and T4 can also act by blocking one particular event of the cellular sequela thus preventing cellular injury. Support of this hypothesis is given by the disappearence not only of the ulcerations but also of all the morphological alterations induced by zinc deficiency after Dex or T4 treatment.

The morphological changes induced by zinc deficiency are associated with a decrease of functional activities as indicated by the reduced disaccharidase activities in all the three regions of intestine. Previous studies have found an impairment of disaccharidase activities after a severe zinc deficiency (Gebhard et al. 1983). On the contrary, Naveh el al. (1990) reported that feeding rats a zinc deficient diet did not result in a reduced disaccharidase activity in the jejunum. However, in their study the duration of zinc deficiency was shorter than in our study and the intestinal morphology was not analyzed to indicate whether intestinal alterations were present.

In hormone treated ZD rats, the disaccharidase activities were significantly greater than those of untreated ZD rats. Different effects of Dex and T4 on intestinal enzyme activities have been previously shown in developing and adult intestine. Studies conducted in adrenalectomized rats indicated that T4 does not influence the intestinal development of sucrase and maltase in the absence of glucocorticoids (Martin and Henning 1982). More recent studies have shown a synergistic effect of T4 and Dex on enzyme ontogeny in rat small intestine (McDonald and Henning 1992, Yeh et al. 1991) and a partial stimulation by Dex alone (McDonald and Henning 1992). On the other hand, it has been shown that glucocorticoids have no effect on intestinal enzymes such as sucrase, maltase, lactase and acid β-galactosidase of adult rats (Henning and Leeper 1982, Nanthakumar and Henning 1993). The present results show that in particular conditions such as zinc deficiency, the disaccharidase activities of adult rats can respond to Dex, indicating that the lack of glucocorticoid responsiveness occurs in normal control conditions but not necessarily in nonphysiological or pathological conditions.

The mechanisms by which Dex and T4 influence the intestinal enzymes must be determined. Glucocorticoids and thyroxine regulate the transcription of several genes, therefore transcriptional regulation of disaccharidases may occur either directly or, more likely, through activation of other regulatory genes, as also suggested in other studies (Nanthakumar and Henning 1995). In fact, the promoter region of human and mouse sucrase-isomaltase genes do not contain the glucocorticoid response elements.

In conclusion, we have shown dramatic structural and functional alterations in all three regions of intestine induced by zinc deficiency that are repaired by Dex or T4 treatment. Although the same results were obtained after the two treatments, further studies are necessary to understand whether a common pathway exists and what molecules are involved in the repair processes of Dex and T4. It should also be considered that, since the receptors for both glucocorticoid and thyroid hormones are zinc-finger proteins, the function of these hormones can be altered or inhibited by zinc deficiency and that treatment with Dex and T4 can result in a better utilization or activation of glucocorticoid receptors. A final consideration is that the effects of Dex and T4 may be not specific to zinc related damage, but represent more generalized hormonally induced repair mechanisms.

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