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Research Article

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Histological examination showed an increase in crypt cells but no decrease in the length of the villi. Cellular migration along the villi, as well as activity of uridine kinase in intestinal mucosa, was increased in colchicine-treated rats. It was concluded that colchicine did not depress intestinal invertase, lactase, and alkaline phosphatase by decreasing cellular renewal, but rather it exerted its effect directly on the differentiated cells of the villus.

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Effect of Colchicine on Intestinal Disaccharidases: Correlation with Biochemical Aspects of Cellular Renewal

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A B S T R A C T There was a significant depression of the activities of intestinal lactase, invertase, and alkaline phosphatase in rats given drinking water containing 2.5 mg of colchicine per 100 ml. Activities of intestinal maltase, aspartate transcarbamylase, and dihydroorotase were not affected by the drug. Injection of colchicine (1 mg/kg) caused depression of intestinal invertase activity within 8 hr. Investigation of the effect of colchicine on the disaccharides in vitro demonstrated that invertase and maltase were not affected by concentrations up to 125 mg/100 ml. Intestinal lactase was inhibited by concentrations exceeding 5 mg/100 ml. Calculation of the concentration of colchicine present in the intestine, after a single injection, indicated that the *in vivo* effect of colchicine was not due to simple enzyme inhibition.

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INTRODUCTION

Intestinal disaccharidases are intimately associated with the region of the brush border of mature, nondividing,

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differentiated cells of the villus and are absent from the proliferating cells of the crypts (1-3). Malabsorption of disaccharides resulting from a hereditary deficiency of these enzymes has been noted in man (4-8) and certain species of sea lions (9). Exogenous factors, such as infection, parasitic infestations, hormones, diet, and drugs are also capable of having a deleterious effect on intestinal disaccharidases (10). The specific biologic mechanisms by which these exogenous factors produce a decrease in enzymatic activity have not been elucidated.

Colchicine is an excellent representative exogenous factor, since it is well known to be associated with malabsorption. It has been shown that this drug, when given in large doses, can cause an almost complete loss of intestinal villi, with concomitant steatorrhea and xylose malabsorption, as well as a depression in activity of disaccharidases in the intestine of man (11) and rat (12, 13). Colchicine has been considered to be a potent mitotic inhibitor causing an arrest of cell division in metaphase, possibly by interference with formation of the spindle fibers (14). It has been generally assumed that gastroenterologic symptomatology and pathology encountered with large doses of this drug are probably the result of inhibition of growth of cellular elements of the crypt (15, 16).

In this study, colchicine was administered orally to rats, and the intestinal mucosa was examined to determine whether the effects were exerted primarily on the proliferative progenitor cells in the crypts, or directly on the differentiated cells of the villus which contain the disaccharidases.

It was concluded from these investigations that a small constant dose of colchicine can exert an effect directly on the differentiating cells of the villus, resulting in a decrease in the activity of some disaccharidases with no deleterious effect on the dividing cells of the crypt.

METHODS

Litters of newly weaned Wistar rats 21–25 days of age were randomly divided into control and colchicine-treated groups and fed a standard laboratory chow (Purina) ad lib. Unless otherwise stated, the colchicine-treated group was offered 2.5 mg of colchicine per 100 ml in the drinking water, resulting in an average intake of colchicine of about 4–5 mg/kg per day. Approximately 2 wk after the animals were started on the drug, they were killed by decapitation, and the proximal $\frac{1}{3}$ of the small intestine distal to the ligament of Treitz (jejunum) was removed and flushed with ice-cold 0.15 M KCl. The whole mucosa was collected by gentle scraping of the intestine with a steel spatula. A 3% homogenate of jejunal mucosa was prepared in 0.15 M KCl in a Teflon glass homogenizer and assayed for activity of lactase, invertase, and maltase by the glucose oxidase method (17). Alkaline phosphatase was assayed using *p*-nitrophenyl phosphate as the substrate (18). An aliquot of each homogenate was centrifuged at 10,000 $\times g$ for 10 min at 5°C, and the supernatant fluid was assayed for aspartate transcarbamylase by the method of Koritz and Cohen (19), as modified by Gerhart and Pardee (20).

Mucosa to be assayed for dihydroorotase activity was prepared as a 10% homogenate in 0.15 M KCl, centrifuged in the cold at 10,000 $\times g$ for 10 min, and assayed by the method of Yates and Pardee (21).

A 5% homogenate of intestinal mucosa was prepared in 0.07 M Tris buffer at pH 7.4 and centrifuged at 40,000 $\times g$ for 1 hr at 5°C; the supernatant fluid was assayed for uridine kinase activity. An aliquot of 0.05 ml of the supernatant fluid was added to 0.2 ml of an incubation medium, similar to that of Sköld (22), containing 15 μ moles ATP, 10 μ moles Mg⁺⁺, 10 μ moles Tris buffer, pH 7.4, and 0.1 μ mole uridine containing 0.05 μ Ci of uridine-2-¹⁴C (New England Nuclear Corp., Boston, Mass.). At zero time and after 1 hr of incubation at 37°C, 0.05-ml aliquots were placed directly into tubes containing 4 ml of boiling water, maintained for 3 min, and then cooled. Under the conditions of the assay, the reaction was linear with enzymatic concentration for at least 5 hr.

Phosphorylated uridine compounds were collected by pouring the contents of the cooled tubes over three 2.5-cm discs of DEAE-cellulose paper (Whatman DE-81) retained in a microanalysis filter holder. The discs were washed four times with 5-ml aliquots of distilled water to remove free uridine and then dried under a heat lamp. The dried discs were placed in vials containing toluene-scintillation fluid and counted in a Packard model 2010 liquid scintillation spectrometer at 70% efficiency. Under the conditions of this assay, over 98% of the phosphorylated uridine compounds was retained on the discs, and more than 99% of the non-phosphorylated uridine was washed off the discs.

All enzyme activities were related to the protein concentration determined by the method of Lowry, Rosebrough, Farr, and Randall (23).

Sections of the intestine were taken just beyond the ligament of Treitz and were fixed in 10% formalin for histological examination or for preparation of radioautographs according to the method of Kopriwa and Leblond (24). The height of the villi and depth of the crypts were expressed as the number of cells lining one side of an average villus or crypt column. These values were determined by counting the number of cells in 10 properly oriented villi and crypts (25).

Since colchicine is known to arrest cells in metaphase, the measurement of mitotic index was not used to express the

rate of cell proliferation. Instead, an indication of the rate of cellular proliferation was obtained radioautographically. The number of crypt cells incorporating thymidine into nuclear material was determined 3 hr after intraperitoneal injection of tritiated thymidine (2Ci/mmole; New England Nuclear).

12 days after initiation of colchicine, tritiated thymidine (2 Ci/g body weight) was injected intraperitoneally into rats. Jejunal samples were taken at approximately 10-hr intervals up to 50 hr, and the rates of cellular migration along the length of the villi were related to the distance traveled by the leading edge of the tritium-labeled cells. These distances were measured in cell spaces in order to avoid artifacts caused by shrinkage of the tissue during preparation of radioautographs.

RESULTS

Effect of dose. In preliminary experiments, the effects of dose, duration of exposure, and route of administration of colchicine on intestinal mucosal function were determined using the activity of invertase as a marker.

Colchicine was added to the drinking water at a concentration of 2.5 mg/100 ml, and the daily intake of the rats was approximately 4–5 mg/kg per day. Although the animals had mild diarrhea, they continued to eat well and gain weight. Activity of intestinal invertase gradually decreased over a 6 day period to approximately 60% of control values, and it remained depressed for at least 30 days if the rats continued to ingest colchicine in their drinking water.

When the concentration of colchicine in the drinking water was increased so that the daily intake exceeded 6 mg/kg per day, the rats developed severe diarrhea and

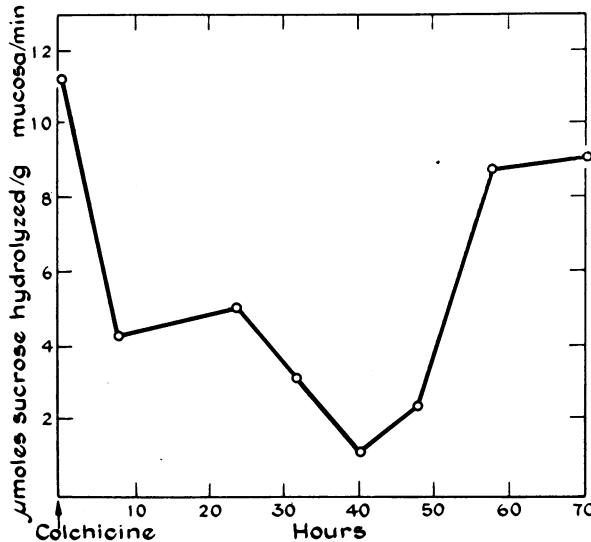


FIGURE 1 Activity of invertase after a subcutaneous injection of colchicine (1 mg/kg or ca. 0.1 mg/rat). Each point is an average of values obtained from two individual rats.

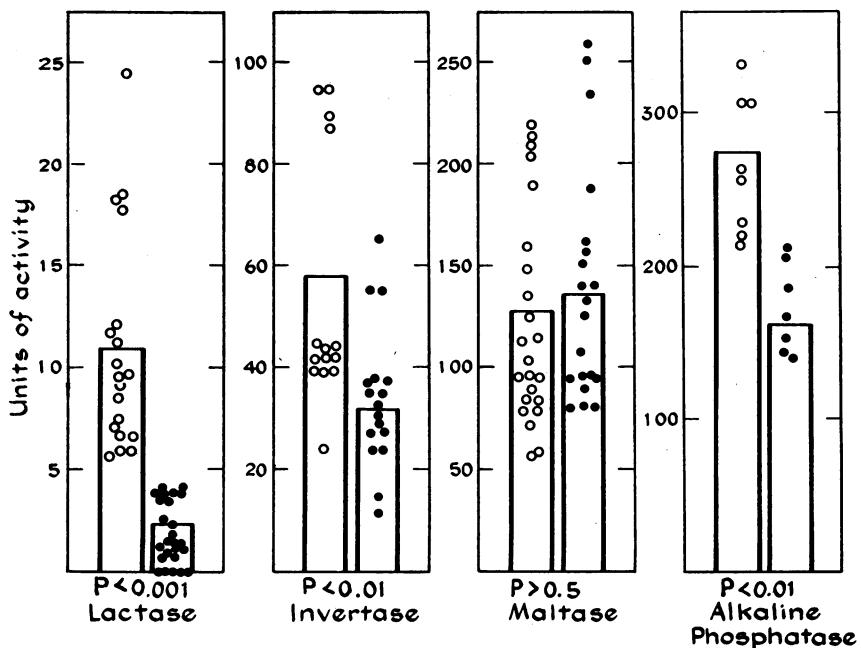


FIGURE 2 Activity of various intestinal enzymes in rats receiving orally 4-5 mg colchicine/kg per day. ○ signifies control animals, and ● signifies the animals receiving colchicine. Each symbol indicates duplicate determinations in a separate animal. The top of the bar represents the mean of all the determinations and the P value is indicated below. The activities of the enzymes are expressed as μ moles of substrate hydrolyzed per minute per gram protein.

died. Their mucosa demonstrated severe villus destruction and atrophy. If the daily intake of colchicine was 3 mg/kg per day or less, there was no significant depression in activity of intestinal invertase, and the animals remained completely asymptomatic.

Since daily ingestion of 4-5 mg/kg per day of colchicine consistently produced a mild diarrhea, and a decrease in the activity of invertase, but did not make the animals severely ill, this dose was given for a period of 10-15 days in all subsequent experiments, at which time the animals were killed and the studies performed.

To assess the rapidity of action of colchicine on the activity of intestinal invertase, weaned rats were given a single subcutaneous injection of 1 mg of colchicine per kg of body weight and then killed at various intervals. In 8 hr the activity of invertase was depressed and reached a minimum in 40 hr. The activity remained depressed for 48 hr, and returned to control values within 60 hr (Fig. 1). The animals were well for the first 24 hr after injection, but they became listless, anorectic, and had loose stools at 40 hr. By 55-60 hr, the animals were more active, and their stools and food intake returned to normal. Microscopically, the jejunum appeared normal 24 hr postinjection, but at 40-48 hr, there was mild alteration in the mucosa with increased inflammatory cell infiltration and goblet cell production.

Injection of nursing mother rats with the same dose of colchicine resulted in the death of the suckling animals within 24 hr. If the pups were injected directly with 1 mg/kg, death resulted. Injection of 0.1 mg/kg caused the pups to become listless, and many died, indicating that suckling rats are much more sensitive

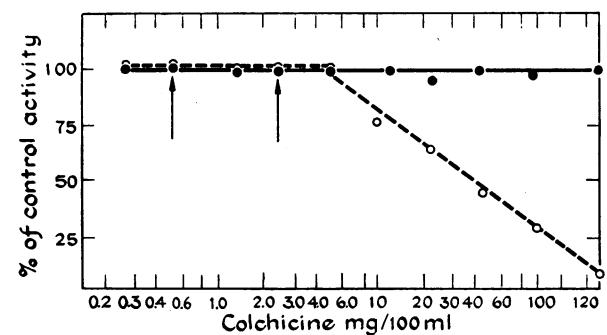


FIGURE 3 The in vitro effect of colchicine on some disaccharidases. ●—● represents the data obtained with maltase or invertase. ○—○ is lactase. These data were derived from 3% homogenates. The same results were obtained with a 60-fold purified preparation of lactase. The dose of colchicine used in drinking water is represented by the arrow on the right. The estimated concentration of colchicine in the intestine, after injection, is indicated by the arrow on the left.

to the toxic effects of colchicine than are weaned rats.

Activity of mucosal enzymes. Rats receiving 4-5 mg of colchicine per kg per day in the drinking water had a 40% depression of invertase activity ($P < 0.01$) and an 80% depression of lactase activity ($P < 0.001$), but no depression of the maltase activity in the jejunal mucosa when compared with normal littermates. Alkaline phosphatase activity was depressed 40% in the intestinal mucosa as previously reported using histochemical methods (26) (Fig. 2). When colchicine was removed from the drinking water, activities of invertase, lactase, and alkaline phosphatase in the jejunal mucosa returned to normal within 48 hr.

There was no evidence of enzymatic inhibition upon mixing preparations from control and colchicine-treated rats. When colchicine was added to the homogenate in a concentration equal to that in the drinking water (2.5 mg/100 ml), it had no effect on the activity of lactase, invertase, or maltase. If the concentration of colchicine in the reaction mixture exceeded 5 mg/100 ml, the activity of lactase decreased until at a concentration of 125 mg/100 ml the activity was only 16% of normal. Invertase and maltase were not affected by the very high concentrations of the drug (Fig. 3).

The activities of aspartate transcarbamylase and dihydroorotase in jejunal mucosa were not altered by treatment with colchicine. In contrast, uridine kinase activity was elevated in the jejunal mucosa of colchicine-treated rats as compared with the controls (Table I).

Mucosal morphology and cellular turnover. In the intestine of animals receiving oral colchicine there was a slight increase in the number of goblet cells and an increased number of inflammatory cells in the lamina propria, otherwise, mucosal architecture was not altered in the jejunum. The heights of the villi were normal, but the average depth of the crypts increased from 36 to 48 cells. The per cent of crypt cells incorporating tritiated thymidine into nuclear material was similar in

TABLE I
Enzymes of Pyrimidine Biosynthesis*

| | Activity in jejunal mucosa† | |
|----------------------------|-----------------------------|--------------------|
| | Control | Colchicine-treated |
| Dihydroorotase | 54 ± 2.1 | 55 ± 4.3 |
| Aspartate transcarbamylase | 18 ± 0.5 | 20 ± 4.1 |
| Uridine kinase | 1.7 ± 0.3 | 2.3 ± 0.6§ |

* The data are reported as SEM. Each mean was obtained from data acquired from 12 separate animals.

† μ moles of product formed per min/g protein.

§ Significant increase $P < 0.01$.

TABLE II
Villi and Crypts in Jejunum of Control and Colchicine-Treated Rats

| | Height villi (cells) | Depth crypt (cells) | Number of animals | Labeling index* | Number of animals |
|--------------------|----------------------|---------------------|-------------------|-----------------|-------------------|
| Control | 86 ± 3.2 | 37 ± 3.6 | 12 | 36% | 6 |
| Colchicine-treated | 84 ± 3.1 | 49 ± 2.1 | 12 | 37% | 8 |
| P | >0.5 | <0.001 | | >0.5 | |

* Per cent of crypt cells incorporating thymidine- 3 H 3 hr after injection.

control animals (35.9%) and littermates treated with colchicine (36.7%, Table II).

The increased depth of the proliferating cell compartment (crypts) was correlated with a markedly increased rate of cellular migration along the villi of colchicine-treated rats as compared with control littermates (Fig. 4). 30 hr after injection of tritiated thymidine, the leading edge of labeled cells had almost reached the tips of the villi in the treated rats, while in the control animals the leading edge of labeled cells had migrated only about $\frac{1}{3}$ to $\frac{1}{2}$ of the distance along the villi.

DISCUSSION

Colchicine was used as a prototype of the various exogenous factors affecting intestinal function in the hope that these experiments might elucidate the biological mechanisms responsible for acquired deficiency of intestinal disaccharidases.

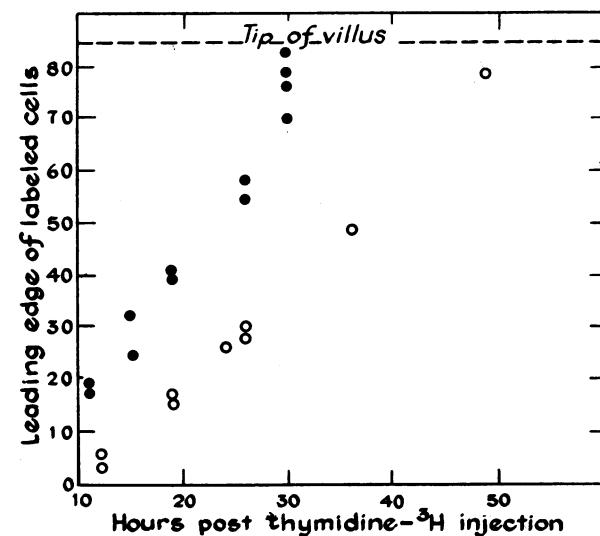


FIGURE 4 Rate of migration of intestinal cells along the villus. O represents control animals, and ● represents colchicine-treated animals. The tip of the villus is designated.

Colchicine has been known to cause diarrhea and had been used as a poison for at least 2000 yr before it was introduced for treatment of acute gout by von Störk in 1763 (27). The mitotic arresting effects of colchicine were first described in the intestinal mucosa of dogs in 1889 by Pernice, and it apparently acts by interfering with spindle formation (14).

Diarrhea is a frequent side effect of colchicine therapy, and depression of intestinal disaccharidase activities and decreased absorption of xylose, fat, and vitamin B₁₂ have been noted in man (11, 28). Decreased absorption of simple sugars as well as depressed activity of intestinal maltase have been demonstrated in rats after three daily injections of colchicine (1 mg/kg) (12, 13). However, these animals were ill; they had gross villus atrophy associated with listlessness and diarrhea, and many of them died 72 hr after the drug was initiated. The intestinal pathology after large doses of colchicine is probably related to cessation of cellular proliferation and has been compared to that observed in acute radiation injury of the bowel (29).

Previous animal experiments in which intestinal function was investigated after administration of colchicine employed near lethal doses. With the dosage used in our experiments, the animals continued to gain weight and closely approximated the conditions seen after oral colchicine therapy in humans, where mild diarrhea and depression of activities of intestinal disaccharidases have been observed.

Intestinal lactase activity was most severely depressed after chronic colchicine administration, activity of invertase was less severely depressed, and maltase activity was unaltered. Alkaline phosphatase, another enzyme located in the brush border region, was also depressed, as has previously been reported (26). Intestinal disaccharidase deficiencies caused by other exogenous factors show a similar pattern in that lactase activity is most frequently depressed, invertase less likely to be depressed, and maltase activity least likely to be depressed (30).

It is difficult to interpret our finding that activity of maltase was unaffected by colchicine. There are, however, several enzymes which contribute to the total activity of maltase. At least one of these enzymes is located in the lysosomal fraction of the cell and not in the brush border¹ and, therefore, might not be affected by ingestion of colchicine. Also, maltase activity varies greatly from animal to animal, even in littermates, and is affected by the type and amount of food ingested by the animal (31). Future experiments should attempt to delineate the effect of colchicine on each of the enzymes contributing to the over-all maltase activity.

¹Koldovsky, O. Personal communication.

With the amount of colchicine used in our investigations, intestinal morphology was not markedly altered, although there was a mild increase in inflammatory and goblet cells as has been noted in humans who have depressed intestinal disaccharidase activities while on colchicine (11). The labeling index was the same in normal and colchicine-treated animals, indicating that colchicine did not alter the rate of DNA synthesis (Table II). Although the lengths of the villi were unchanged in the two groups, the depths of the crypts were increased (Table II), and the rate of cellular migration along the villus was more rapid in the colchicine-treated animals (Fig. 4). These findings are similar to those reported for suckling animals treated with cortisone (25) and in germfree animals after colonization (32). Activities of aspartate transcarbamylase and dihydroorotate, two enzymes of the *de novo* pathway of pyrimidine biosynthesis and ultimately RNA and DNA synthesis, as well as activity of uridine kinase, a salvage pathway enzyme, were determined in intestinal mucosa of control and treated rats. Activities of the two *de novo* pathway enzymes were unchanged, but activity of uridine kinase, the salvage pathway enzyme, was significantly increased ($P < 0.01$) in colchicine-treated rats (Table I).

The minimal changes in intestinal histology, the normal uptake of tritiated thymidine by crypt cells, the normal or increased activity of enzymes important in RNA and DNA synthesis, and the measurement of the rate of cellular migration along the villus, indicate that the depression in activity of brush-border enzymes was not the result of interference with cellular proliferation in the crypts.

There was no effect on enzymatic activity by either adding a preparation of intestine from animals poisoned with colchicine, or by adding colchicine directly to the control preparation in a quantity equivalent to that added in drinking water (2.5 mg/100 ml). Although at concentrations over 5 mg/100 ml activity of lactase is inhibited, a concentration 50 times that given in the drinking water would be required to depress the activity of lactase to the extent seen after the oral administration of colchicine.

Previous investigators (33) have demonstrated that 4 hr after injection of 1 mg of colchicine-¹⁴C into a mouse, approximately 25% of the drug was located in the intestinal tract. We have shown that intestinal invertase is markedly depressed within 8 hr by a single injection of 1 mg/kg body weight (Fig. 1). Assuming that the intestine represents 5-7% of the total body weight, the concentration of colchicine in intestinal mucosa could be approximately 0.5 mg/100 g. Neither invertase nor maltase is inhibited *in vitro* by concentrations of colchicine as high as 125 mg/100 ml. Lactase,

the disaccharidase most sensitive to colchicine *in vivo*, is not inhibited *in vitro* by 5 mg of colchicine per 100 ml, a concentration 10 times greater than that calculated to be present in the intestine after a single injection (Fig. 3).

Although there are no specific data, it is possible to conclude that *in vivo* the drug may act somewhere in the differentiative mechanism concerned with the synthesis of lactase, invertase, and alkaline phosphatase, rather than directly on the enzymes. This conclusion does not satisfactorily explain the different intensity of effect of the drug on various disaccharidases. The rapid depression of invertase activity within 8 hr after parenteral doses of colchicine is further evidence that the drug affects the differentiated cells directly and does not act primarily on the dividing crypt cells, since within this time the cells would have barely left the crypt. Thus far, electron microscopic studies have not been helpful in clearly delineating any major morphologic difference between the villus epithelium of the control and colchicine-treated animals.*

The frequency of recognition of secondary or acquired intolerance to disaccharides is rapidly attaining major proportions in adult and pediatric medicine. This situation, in addition to the recent finding of a common occurrence of disaccharide intolerance in Negroes and Orientals, makes it important to acquire more information concerning those factors affecting biochemical differentiation in intestinal epithelial cells.

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