EXPERIMENTAL MALABSORPTION WITH JEJUNAL ATROPHY INDUCED BY COLCHICINE

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INTEREST in the jejunal mucous membrane has greatly increased since Paulley (1954) described atrophic changes in the villi in cases of idiopathic steatorrhoea. Similar changes have been described in a number of other conditions, including coeliac disease, tropical sprue, reticulum cell sarcoma of the small bowel, anaplastic carcinoma of the jejunum, and in treatment with neomycin. All of these conditions have been associated with some degree of malabsorption (Shearman, Girdwood, Wynn Williams and Delamore, 1962).

Various attempts have been made to produce malabsorptive syndromes experimentally : Woll and Oleson (1951), and Vitale, Zamcheck, Digiorgio and Hegsted (1954) demonstrated atrophic changes in the jejunal villi of rats, following administration of the folic acid antagonist, 4-aminopteroyl glutamic acid (aminopterin). Butterworth, Perez-Santiago, Martinez and Santini (1959) demonstrated a reduced absorption of d-xylose in dogs, following administration of aminopterin and 4-amino-N-10 methylpteroylglutamic acid (methotrexate). Jacobson, Chodos and Faloon (1960) described an experimental malabsorption in man, induced by neomycin.

In a study of the effect of colchicine on normal and neoplastic tissues in mice, Clearkin (1937) reported visible effects in the cells of the jejunal crypts within 4 hr. of an injected dose. LeBlond and Stevens (1948) used colchicine as a mitotic poison to estimate the rate of renewal of intestinal epithelium of the rat. It was therefore decided to test the effect of repeated doses of colchicine in the hope of producing a sprue-like picture.

MATERIALS AND METHODS

Male Wistar rats of between 100 and 300 g. weight were used. They were maintained on Purina Lab Chow, *ad libitum*. Colchicine was administered by subcutaneous injection, daily doses varing from 50–200 μg. per 100 g. body weight. The stock solution was 20 mg. colchicine in 100 ml. of normal saline.

D-xylose excretion test

A dose of 32 mg. of d-xylose in 1·6 ml. of water was given either by intragastric tube or by intraperitoneal injection. This dose was chosen as being the equivalent in the rat of the 25 g. dose in man. The rats were placed in individual metabolic cages. The separated urine was collected for 8 hr. Bacterial destruction of xylose was prevented by adding 5 ml. of glacial acetic acid to the collecting flasks. The xylose content of the urine was estimated by the method of Roe and Rice (1948), as modified by Clark (1962). To avoid error from retained xylose at least 24 hr. were allowed to elapse between tests on individual rats.

· Animals were killed on the fourth or fifth day. The small bowel was rapidly removed, and fixed in formalin. After fixation, the bowel was rolled into spools and sectioned as described by Zamcheck (1960).

RESULTS

Daily doses of 70 to 100 µg. of colchicine per 100 g. body weight

Within 24 hr. the rats became listless. By 48 hr., some animals had diarrhoea and at 72 hr., all had diarrhoea. A brown crust formed around the eyes. Death occurred in 3 to 5 days. The results of the d-xylose excretion test are seen in the Table. Following oral administration of xylose, there was a progressive reduction

TABLE.—*Urinary Xylose Excretion*

(Percentage of a 32 mg. dose)

	Route of administration			
	Oral		Peritoneal	
	No.	(Mean ± S.D.)	No.	(Mean ± S.D.)
Controls . .	44	23·4 ± 7·5	37	60·0 ± 5·6
Colchicine (100 µg. per 100 g. body wt/day)				
A. Immediate effect .	16	20·0 ± 4·3 ($t = 1·71, P < 0·1$)	..	..
B. 24 hr. . . .	8	21·0 ± 7·8 ($t = 1·37, P < 0·2$)	..	..
C. 48 hr. . . .	10	14·6 ± 6·9 ($t = 3·35, P < 0·001$)	..	..
D. 72 hr. . . .	13	12·5 ± 4·3 ($t = 6·60, P < 0·001$)	11	54·0 ± 19·8 ($t = 1·64, P < 0·1$)

in the urinary xylose excretion, but there was no effect on excretion of xylose given by intraperitoneal injection.

Histological examination of the small bowel mucous membrane showed progressive atrophy. The normal appearance is seen in Fig. 1. The villi are tall and pointed. At 24 hr. the villi are slightly shortened. As described by LeBlond and Stevens (1948) there was an increase in the number of mitoses in the crypts. By 48 hr. (Fig. 2), the villi were still shorter. The epithelium was often seen to separate from the lamina propria and fusion of adjacent villi was occasionally seen. By 72 hr. there was gross villous atrophy (Fig. 3). Histological examination of heart, liver, kidney and spleen showed no lesions.

Daily doses of 200 µg. per 100 g. body weight

In 3 rats this high level produced identical functional and morphological changes. Death occurred earlier.

Daily doses of 60 µg. per 100 g. body weight

Doses of 60 µg. produced variable results. Three out of 11 rats showed reduced xylose excretion. Histologically the villi of the small bowel showed changes varying from an almost normal appearance to one of gross villous atrophy. Correlation between xylose excretion and morphology was not complete.

Daily doses of 40 *μg. per* 100 *g. body weight*

Using 6 rats this dose was maintained for 13 days. They lost about 10 per cent of their body weight during this period. Xylose excretion was 27 ± 1 per cent of the administered oral dose. This is not significantly different from normal ($t = 0·816$, $P < 0·5$). The small intestine was microscopically normal. The only lesions seen were hyperplasia of the sternal bone marrow and myeloid metaplasia in the spleen.

Immediate effect of colchicine

The excretion of oral xylose given simultaneously with colchicine was $20·1 \pm 8·6$ per cent of administered dose. Although this is slightly lower than normal, the difference is not statistically significant ($t = 1·71$, $P < 0·2$).

DISCUSSION

In rats, repeated doses of 70 to 200 μg. of colchicine per 100 g. body weight produced atrophy of the small bowel mucosa, and malabsorption of xylose. The histological appearance of the small bowel bears a striking resemblance to that seen in jejunal biopsies from patients with various malabsorptive conditions.

Use of intraperitoneal injection of xylose has demonstrated that colchicine depresses absorption rather than excretion of this sugar. The progressive decrease in absorption correlates well with the atrophic changes of the small bowel mucous membrane. We did not demonstrate an immediate toxic effect of colchicine on xylose absorption. Large doses of folic acid did not prevent the changes.

Since the aetiology of tropical sprue is unknown, it is interesting that similar lesions may be produced by the natural alkaloid, colchicine. The normal source of colchicine is the root of the autumn crocus (*Colchicum autumnale*); it is also present in various other members of the family *Liliaceae*. None of these plants is known to be used for culinary purposes. Because of the report (*Chemical Abstracts*, 1953) that *Crocus sativus* contained colchicine, and because Saffron, derived from this plant , is widely used for colouring food in various countries, including many of those where tropical sprue is endemic, we investigated the toxic effects of this substance. We found no effect on the small bowel mucosa. Subsequently, we discovered that the report in *Chemical Abstracts* appeared to be a mistranslation of the original paper of Santavy and Bartek (1952).

Our experiment may throw some light on the pathogenesis of villous atrophy in malabsorptive conditions. Padykula, Strauss, Ladman and Gardiner (1961), and Yardley, Bayless, Norton and Hendrix (1962) have noted increased mitotic counts in non-tropical sprue. They interpret this to mean an increased rate of cell loss and subsequent renewal. This is disputed by Creamer (1962), who

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EXPLANATION OF PLATE

FIG. 1.—Normal rat jejunum. H. and E. × 50.
FIG. 2.—Effect of colchicine for 48 hr. Note that the villi are shortened and blunted. H. and E. × 50.
FIG. 3.—Effect of colchicine for 72 hr. There is gross villous atrophy. H. and E. × 50.

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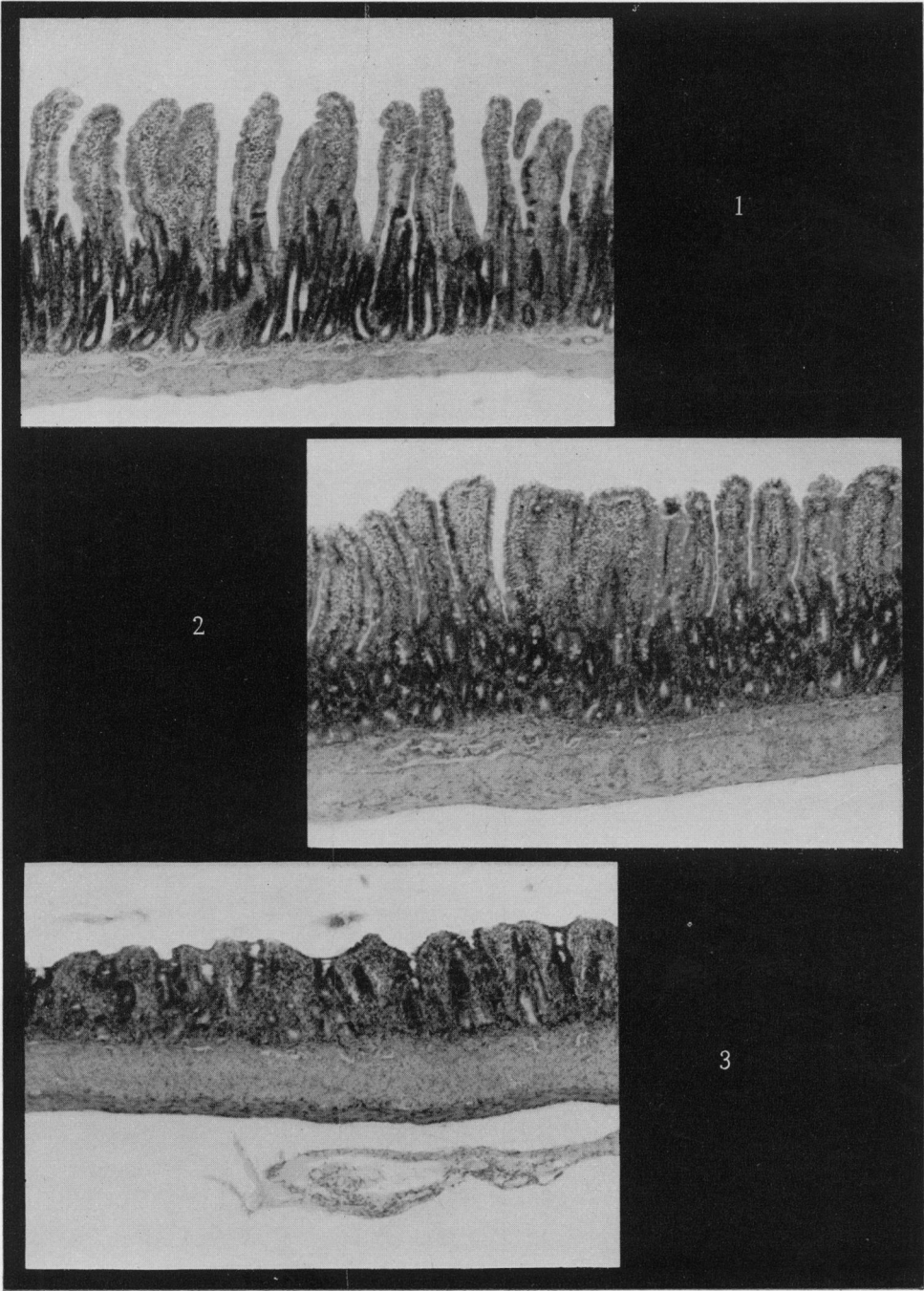
claims that the mitotic rate is less than normal when expressed as mitoses per hundred crypt cells. He considers that there is a maturation arrest of the crypt cells, the vast majority of which die *in situ*.

Since colchicine, a mitotic poison, has produced a picture similar to sprue it is suggested that the initial lesion in sprue is an arrest of mitosis in the crypt cells. This concept would explain the increased number of mitoses in association with a diminished number of epithelial cells in the jejunal mucous membrane.

SUMMARY

Villous atrophy of the jejunum has been produced in rats by repeated doses of colchicine. This condition was morphologically similar to sprue and there was malabsorption of xylose. It is postulated that in sprue the initial lesion may be an arrest of mitosis in the crypt cells.

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