Preliminary Communications

Reversal of Folate Malabsorption in Tropical and Non-tropical Sprue by Calf Jejunum

Brit. med. J., 1968, **3**, 472–474

Summary: In eight patients with folic acid malabsorption the malabsorption was reversed only when a combination of either 250 mg. of lyophilized human jejunal juice or 5 g. of lyophilized calf jejunum with 5 mg. of synthetic folic acid was fed; calf liver was ineffective. The factors in calf jejunum that re-established folic acid absorption were heat-stable and did not form polyglutamyl folates on previous incubation with folic acid.

Introduction

Folic acid malabsorption sometimes occurs in sprue (Baker *et al.*, 1964; Klipstein, 1966). In this disease jejunal villous atrophy may underlie folate malabsorption by decreasing or eliminating factors which make folic acid absorbable. Thus folate malabsorption may be a reflection of multiple anatomic and enzymatic defects in diseased intestinal mucous membrane (Baker *et al.*, 1964; Veeger *et al.*, 1965; Klipstein, 1966).

This paper describes the use of lyophilized whole-calf jejunum to promote folic acid absorption in eight selected sprue patients having folate malabsorption. Lyophilized normal human jejunal juice also stimulated folic acid absorption in one of these patients, the only one receiving this additional treatment. Jejunum was tried because it seemed a likely source of folate absorption stimulators.

Materials and Methods

Control Subjects.—These were 25 healthy members of the laboratory staff; none had any history of intestinal disease or folate deficiency.

Nutritional Folate-deficient Subjects.—These were 25 patients without history of intestinal disease; all had folate deficiency.

Patients Selected for Study.—The following criteria guided selection of patients. Only untreated patients having diarrhoea with steatorrhoea, no evidence of parasitic infestations, with histological evidence of severe jejunal atrophy, and abnormal absorption of folic acid were studied. Thirty-three patients were screened; eight who met these criteria were selected from this group. Folate absorption tests were promptly started on admission of all 33 patients. For those showing defective folate absorption the tests were continued for two to seven days. None of the eight patients selected were given any vitamins before this study, nor did they receive any special diets during its course.

Folate Absorption Studies.—Subjects were given 5 mg. of synthetic folic acid (pteroylmonoglutamic acid) orally; 5 ml. of blood was drawn into tubes containing 0.2 ml. of 25% sodium citrate from an antecubital vein before and 2, 4, and 24 hours after ingestion of folic acid (Baker *et al.*, 1965). The plasma removed by centrifugation was assayed for total folates with *Lactobacillus casei* (Baker *et al.*, 1965; Baker and Frank, 1967). *L. casei* was used in preference to *Streptococcus faecalis*. This method obviates the need to presaturate the tissues with folic acid before carrying out the tests. With *Str. faecalis* there is little detectable folic acid in the serum or plasma before test doses are given (Chanarin *et al.*, 1958); this was confirmed by ourselves (Baker *et al.*, 1965) and others (Herbert and Zalusky, 1962), hence the *L. casei* method proves more sensitive for the measurement of plasma folates.

Human Jejunal Juice and Calf Jejunum.—Jejunal juice was obtained from a patient without intestinal disease undergoing laparotomy. About 20 cm. of jejunum immediately distal to the ligament of Treitz was isolated between occlusive clamps. It was washed through with saline, the washings were discarded, and the accumulated jejunal juice was collected during the laparotomy. This method has been used for studying in vivo intestinal transport of various metabolites (Duthie, 1967).

The jejunal juice (*p*H 8.1) was lyophilized, yielding 550 mg. of material. Aliquots (250 mg.) were resuspended in 25 ml. of distilled water, homogenized, and fed to the aforementioned patient.

Calf jejunum was obtained in an abattoir within 10 minutes after the calves were slaughtered. The veterinarian excised 100 cm. of jejunum, beginning immediately distal to the ligament of Treitz. Ten specimens were transported in ice. Each specimen was freed from adherent fat and ligaments, flushed clean of its contents with ice-cold saline, homogenized in a blender, and lyophilized within an hour after collection. For folate absorption studies 5 g. of lyophilized intestine was suspended in 50 ml. of distilled water and homogenized in a blender; the *p*H was 6.2. The jejunum preparations were treated as follows: (*a*) incubated with 5 mg. of folic acid for four hours at 37° C. in a water-bath; (*b*) autoclaved for 30 minutes at 15 lb./sq. in. (1 kg./sq. cm.) before addition of the 5 mg. of folic acid and then incubated with folic acid for four hours; (*c*) autoclaved for 30 minutes at 15 lb./sq. in. and not incubated with the added 5 mg. of folic acid. The *p*H did not change. Human jejunal juice was treated in the same manner as the calf jejunum "*a*" preparation. One folate absorption study involved orally administered lyophilized calf liver; this preparation was treated like the calf jejunum "*a*" preparation to test its promotion of folic acid absorption.

Results

The results are given in the Table. As we have shown (Baker *et al.*, 1964), normal and nutritionally folate-depleted subjects have increased plasma folate during the two- to four-hour intervals after oral folic acid administration; folate deficiency does not adversely affect folic acid absorption (see Table).

The eight patients chosen for study had a folate deficiency; their plasma level was less than 3.5 mμg./ml. (Baker and Frank, 1967). Absorption of 5 mg. of folic acid was subnormal in these eight patients (see Table); none had more than slight increases of plasma folate two and four hours after oral administration of folic acid. Cases 1 and 2, both from New York, had a gluten enteropathy; they later responded to a gluten-free diet. The other six were from Puerto Rico and had tropical sprue; a gluten-free diet did not cause remissions.

When Case 1 was given the incubated calf jejunum/folic acid combination, the folic acid absorption pattern returned to normal. In Case 2, in which human jejunal juice was tested as well, the administered 250 mg. of lyophilized juice had 0.13 μg. of total folate activity; it was not effective in raising plasma folate without added folic acid. When the juice was incubated with 5 mg. of folic acid, then fed, it produced a normal folic acid absorption pattern in the plasma. Five grammes of calf jejunum, containing 0.24 μg. of folate activity, with no added folic acid, did not raise the plasma folate in all these patients. When this patient was fed calf jejunum incubated with 5 mg. of folic acid the plasma values indicated normal folic acid absorption. Folic acid malabsorption without any added calf jejunum was still evident in Case 2 two days after the absorption of folic acid with added calf jejunum (Day 7 of the

study), the indication being that the added calf jejunum was still necessary at this time to promote folic acid absorption.

Cases 3 and 4 had normal folic acid absorption only after ingesting the incubated calf jejunum/folic acid preparation ; on Day 4 of the study Case 4 still did not absorb folic acid without the added calf jejunum. Plasma folate levels in Cases 5, 6, and 7 showed that all absorbed folic acid, even when given the *autoclaved* jejunum/folic acid preparation, either incubated or not incubated with folic acid (see Table). Case 7 was still unable to absorb folic acid on Day 6 of the study without the addition of the calf jejunum/folic acid preparation, indicating that the malabsorption was not yet reversed three days after the absorption of folic acid with added calf jejunum. Case 8 had a normal folic acid absorption pattern only when given autoclaved or non-autoclaved calf jejunum, both incubated with folic acid. On Day 4 of the study 5 g. of lyophilized liver, containing 75 μg. of total folates, did not increase plasma folates ; when incubated with folic acid, liver, unlike calf jejunum, did not produce a normal folic acid absorption pattern as reflected in the plasma.

An 8–12% reticulocytosis was seen in these patients within 5 to 10 days after administration of the test preparation ; this response was probably due to the absorbed folate. We also noted that two weeks after the last treatment with calf jejunum Cases 3, 4, and 8 absorbed folic acid without added calf jejunum.

Folic Acid Absorption with Calf Jejunum, Human Jejunal Juice, and Calf Liver

Case No.	Diagnosis	Day of Study	Material Fed	Plasma Folate Level (mμg./ml.) Pretreatment	2 Hours after Administration	4 Hours after Administration	24 Hours after Administration
Normal subjects			Folic acid	5–24	55–600	59–480	6–34
Nutritional folate-deficient subjects			Folic acid	1·2–3·2	92–600	78–440	3–15
1	Non-tropical sprue	1	Folic acid	2·5	3·4	4·2	3·2
		2	Calf jejunum + folic acid, incubated	3·2	80	78	5·6
2	Non-tropical sprue	1	Folic acid	1·0	4·0	4·4	2·6
		2	250 mg. jejunal juice, incubated	2·6	2·6	1·8	2·1
		3	250 mg. jejunal juice + folic acid, incubated	2·1	96	58	2·5
		4	Calf jejunum, incubated	2·5	2·6	1·9	1·9
		5	Calf jejunum + folic acid, incubated	1·9	270	345	4·1
		7	Folic acid	1·0	3·8	4·4	3·6
3	Tropical sprue	1	Folic acid	1·0	2·4	3·7	2·0
		2	Calf jejunum + folic acid, incubated	2·0	53	133	3·0
4	Tropical sprue	1	Folic acid	1·0	4·1	3·1	2·0
		2	Calf jejunum + folic acid, incubated	2·0	126	136	2·9
		4	Folic acid	2·1	4·8	5·1	3·4
5	Tropical sprue	1	Folic acid	2·5	5·1	4·2	3·2
		2	Calf jejunum + folic acid, incubated	3·2	114	116	3·1
		3	Autoclaved calf jejunum + folic acid (no incubation)	3·1	139	90	3·8
6	Tropical sprue	1	Folic acid	1·0	1·0	3·6	4·0
		2	Autoclaved calf jejunum + folic acid, incubated	4·0	86	102	2·0
		3	Autoclaved calf jejunum + folic acid (no incubation)	2·0	69	84	7·9
7	Tropical sprue	1	Folic acid	2·0	7·1	8·1	3·6
		2	Autoclaved calf jejunum + folic acid, incubated	3·6	156	100	3·4
		3	Autoclaved calf jejunum + folic acid (no incubation)	3·4	89	100	16
		6	Folic acid	3·8	17	29	2·9
8	Tropical sprue	1	Folic acid	1·0	11	18	2·1
		2	Calf jejunum + folic acid, incubated	2·1	96	127	3·6
		3	Autoclaved calf jejunum + folic acid, incubated	3·6	56	90	2·8
		4	Liver	2·8	2·1	2·6	2·4
		5	Liver + folic acid, incubated	2·4	30	18	1·6

Discussion

Folic acid absorption was remarkably stimulated by calf jejunum or human jejunal juice and folic acid combination in patients having folic acid malabsorption despite the persistence of the folate malabsorption. How folic acid absorption is so facilitated is conjectural. The factor is heat-stable ; autoclaving the calf jejunum elicited the same response as a non-autoclaved preparation, indicating that the factor is not wholly enzymatic. In-vitro incubation for favouring complexing of jejunal factors and folic acid also seems unnecessary (Cases 5, 6, and 7). Based on differential microbiological folate assays (Baker and Frank, 1967), unpublished observations indicate that calf jejunum and folic acid do not form folylpolyglutamates.

Presumably, folic acid absorption depends on factors produced in the normal jejunum ; some folic acid malabsorbers temporarily lack folate absorption factors during the time of severe jejunal disease (Klipstein, 1968). It is tempting to draw an analogy between folic acid absorption factors and jejunum with vitamin B_{12} absorption and intrinsic factor. In jejunal mucosal atrophy, as seen in these and many other sprue patients, folic acid absorption factors may be depleted or their secretion blocked. When these factors are supplied exogenously —for example, as calf jejunum or human jejunal juice—normal folic acid absorption is regained (see Table). This suggests that, in man, folic acid is absorbed by a special mechanism like those known for transport of B_{12}, amino-acids, pyrimidines, monosaccharides, and certain electrolytes (Burgen and Goldberg, 1962).

Since folic acid malabsorption is reversed by a combination of calf jejunum and folic acid, it remains to be seen whether this combination also re-establishes normal absorption of other nutrients as well as normal intestinal mucosal architecture and histochemistry. This is especially significant because we noted that folic acid was absorbed normally without added calf jejunum in three patients two weeks after the study was stopped. We are not certain whether the calf jejunum or the absorption of folic acid in combination with calf jejunum permitted this reversal ; the latter is probably more significant.

We chose patients with malabsorption of pharmacological doses of folic acid ; hence it was obvious that in these patients small oral doses of folic acid would be therapeutically ineffective. These patients had previously shown a lack of haematological response to folate-rich foods—for example, milk, eggs, and green leafy vegetables. It was therefore not surprising that crystalline folic acid gave no therapeutic response. More work still remains to be done to explain the mechanism of absorption of food folates versus synthetic folic acid in normal subjects and patients with the malabsorption syndrome.

Part of this work was supported by Grants AM 04917, AM 04437, FR-63-05, and AM 04903 from the National Institutes of Health and the National Vitamin Foundation.

H. BAKER, PH.D.,
Associate Professor of Medicine.
O. FRANK, PH.D.,
Assistant Professor of Medicine.
H. ZIFFER, M.D.,
Clinical Assistant Professor of Medicine.
S. FEINGOLD, B.S.,
Research Assistant, Department of Medicine.
Division of Hepatic Metabolism and Nutrition, New Jersey College of Medicine and Dentistry and East Orange Veterans Administration Hospital, East Orange, New Jersey.
A. A. CINTRON-RIVERA, M.D.,
Associate Professor of Medicine, Department of Medicine and the Clinical Research Center, University of Puerto Rico School of Medicine, San Juan, Puerto Rico.

REFERENCES

Baker, H., and Frank, O. (1967). In *The Vitamins*, edited by P. Gyorgy and W. N. Pearson, 2nd ed., vol. 7, p. 269. New York.
Baker, H., *et al.* (1964). *J. Amer. med. Ass.*, **187**, 119.
Baker, H., *et al.* (1965). *Amer. J. clin. Nutr.*, **17**, 88.
Burgen, A. S. V., and Goldberg, N. J. (1962). *Brit. J. Pharmacol.*, **19**, 313.
Chanarin, I., Anderson, B. B., and Mollin, D. L. (1958). *Brit. J. Haemat.*, **4**, 156.
Duthie, H. L. (1967). *Brit. med. Bull.*, **23**, 213.
Herbert, V., and Zalusky, R. (1962). *J. clin. Invest.*, **41**, 1263.
Klipstein, F. A. (1966). *Bull. N.Y. Acad. Med.*, **42**, 638.
Klipstein, F. A. (1968). *Gastroenterology*, **54**, 275.
Veeger, W., ten Thije, O. J., Hellemans, N., Mandema, E., and Nieweg, H. O. (1965). *Acta med. scand.*, **177**, 493.

Medical Memoranda

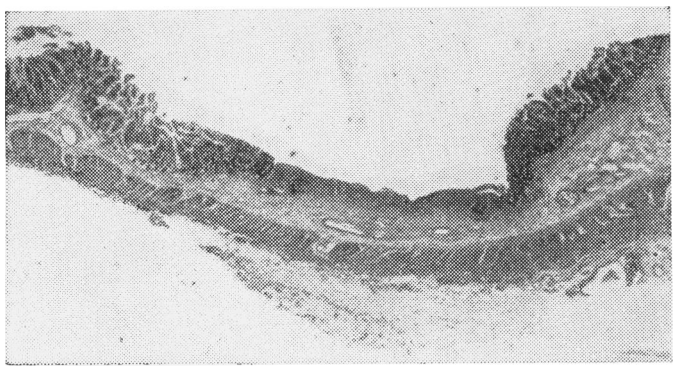
Primary Non-specific Ulcer of Ileum Presenting with Massive Rectal Haemorrhage

Brit. med. J., 1968, **3**, 474

Primary ulcer of the small intestine occurring beyond the duodenum is rare. It has to be differentiated from multiple ulcers occurring in Crohn's disease and from ulcers due to uraemia, typhoid, tuberculosis, and other specific infections. Baille first reported a case of primary ulcer of the ileum in 1805. Reviews published by Morrin (1931), Evert *et al.* (1948), and Morlock *et al.* (1956) include 147 cases. There are also a number of single case reports, and a total of some 175 cases have been reported. The present case report describes an ulcer of the ileum in a youth of 16 who was seen and treated at the Friarage Hospital, Northallerton. This case differed from most of the recorded cases with ulcers of the small intestines in that the presenting symptom was a massive rectal haemorrhage.

CASE REPORT

A youth aged 16 was admitted to hospital, having lost about 3 pints (1,700 ml.) of blood per rectum one hour previously. There was no history of abdominal pain or indigestion, or of his having taken any drugs like aspirin or potassium chloride in the recent past. He was found to be shocked, with blood pressure of 90/60 mm. Hg and a pulse rate of 100. Clinical examination showed no abnormality of the abdomen, and proctoscopically there was no local cause for the bleeding. Transfusion of 2 pints (1,140 ml.) of blood did not produce much improvement and the loss of fresh blood per rectum continued. Laparotomy was carried out through a right paramedian incision. The stomach, duodenum, jejunum, and proximal ileum were collapsed and empty. The large bowel contained blood but no bleeding site was seen. A segment of terminal ileum about 3 ft. (90 cm.) from the ileocaecal valve was distended with blood. At the apex of this segment there was an ulcer with an eroded blood vessel at its centre which was clearly the cause of the haemorrhage. A segmental resection and end-to-end anastomosis were carried out. The patient made an uneventful postoperative recovery and was discharged home on the fourteenth postoperative day. He has since been seen at the follow-up clinic and has remained symptom-free.



Section through the ileum showing the ulcer. The ulcer is a superficial erosion which does not extend deeper than the mucosa. Several small arteries are seen in the submucosa and normal ileal mucosa to the edge of the erosion. (Haematoxylin and eosin. ×3.8.)

Examination of the resected loop showed a superficial ulcer in the mucosa 2 by 1 cm. with a small bleeding vessel in the floor. The appearance of the rest of the resected loop was normal. No diverticulum was present. Section of the ulcer (see Fig.) showed that it was superficial; the mucosa had disappeared but the submucosa was intact. There was little evidence of inflammation and none of a specific infection. Several small arteries about 500 microns in diameter were present in the submucosa. Because the ulcer was in the part of the ileum where a Meckel's diverticulum might occur a careful search was made for ectopic gastric mucosa such as is sometimes found in Meckel's diverticulum, but none was found. None the less, because of the resemblance of the ulcer to a superficial gastric erosion, the pathologist thought it possible that there had been a small area of ectopic gastric mucosa which had subsequently been entirely eroded.

COMMENT

Simple ulcers of the small intestine have occurred at all ages from 1 to 77 years, the average being 47 years. Seventy-five per cent. occurred in males. The origin is obscure. The causes that have been suggested include inflammation of bacterial origin, damage by a foreign body subsequently passed, and local ischaemia of the mucosa owing to vascular changes in the mesentery. Few of the symptoms and signs in the reported cases have been directly attributable to the ulcers, though in a few cases there was colicky abdominal pain. Usually the ulcers remained unsuspected until operation had been carried out for complications—these were perforation (85%), intestinal obstruction (10%), or haemorrhage (5%). In some of the reported cases the ulcer was discovered only at necropsy. In the cases where bleeding occurred it was nearly always in the form of melaena rather than an overt loss of fresh blood from the rectum. Watson (1963), reviewing the more recent case reports, stated that with earlier surgical intervention local perforation with obstruction has replaced acute perforation as the complication most often encountered. The treatment is resection of the involved segment with end-to-end anastomosis, and earlier resort to surgery has reduced the high mortality (60%) reported by Morrin (1931) to about 11% (Morlock *et al.*, 1956).

I wish to express my thanks to Mr. F. Ellis, under whose care this patient was admitted, for permission to publish the details and for his guidance in the preparation of this paper, and to Dr. P. N. Coleman for his help with the histopathology.

M. J. SHAH, F.R.C.S.,
Surgical Registrar, the Friarage Hospital, Northallerton, Yorkshire.

REFERENCES

Evert, J. A., Black, B. M., and Docherty, M. B. (1948). *Surgery*, **23**, 185.
Morlock, C. G., Goehrs, H. R., and Docherty, M. B. (1956). *Gastroenterology*, **31**, 667.
Morrin, F. J. (1931). *Irish J. med. Sci.*, p. 198.
Watson, M. R. (1963). *Arch. Surg.*, **87**, 600.