

## Normal Absorption in Thai Subjects with Non-specific Jejunal Abnormalities\*

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There is a growing belief that subclinical tropical sprue is widespread in tropical countries (Klipstein, 1967). This opinion is based on studies of asymptomatic adults in India (Baker *et al.*, 1962), Pakistan (Russell *et al.*, 1966 ; Lindenbaum *et al.*, 1966b), and Thailand (Sprinz *et al.*, 1962), in whom jejunal histological abnormalities and impaired xylose absorption were frequently found. Recent studies in Haiti by Klipstein *et al.* (1966) show that this condition is very common in individuals complaining of one or more symptoms of the disease. The ease with which the same histological and biochemical abnormalities could be demonstrated in asymptomatic subjects led these workers to speculate that tropical sprue is a disease with a wide range of expression and that the overt cases represent only a fraction of the total number affected.

Because a jejunal lesion has been found commonly in asymptomatic subjects in Thailand (Sprinz *et al.*, 1962), we undertook to study its functional expression with a wider variety of tests than previously employed in order to assess the lesion as a possible precursor of tropical sprue.

### Methods

Forty volunteer subjects, primarily from two villages on the outskirts of Bangkok, gave informed consent and were admitted to hospital for the studies to be described. There were 22 females aged 16-72 years and 18 males aged 13-45 years. History and physical examination were within normal limits. The following were performed in every subject: complete blood counts, total serum protein, protein electrophoresis, serum phosphorus, calcium, iron, and beta-carotene. Urinalysis and determination of blood urea nitrogen and serum creatinine were also carried out in all subjects and found to be normal by North American standards. Chest x-ray examinations, performed in all subjects, were also normal.

Five-hour urinary D-xylose excretion and two-hour serum xylose levels were measured in fasting subjects. All subjects received 25 g. of xylose, except for three who received 0.5 g./kg. (13.7, 22.0, and 22.6 g.). Urine and serum specimens were kept frozen after collection. The xylose determination (Roe and Rice, 1948) was performed within 24 to 48 hours after completion of the test.

Vitamin-B<sub>12</sub> absorption was measured by the Schilling (1953) technique, <sup>57</sup>Co vitamin B<sub>12</sub> (Racobalamin) with intrinsic factor being used. Vitamin-A absorption was tested in fasting subjects by the oral administration of a dose of 260,000 international units mixed in corn oil. Serum levels were measured before and five hours after ingestion of vitamin A (I.C.N.N.D., 1964).

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For the lactose-tolerance test an oral dose of lactose (1.5 g./kg. body weight) was administered. The glucose-tolerance test was performed with an oral dose of 0.75 g. of glucose/kg. body weight, an amount equimolar to the glucose content of the lactose in the lactose-tolerance test. Blood glucose was estimated (Technicon AutoAnalyzer method, 1937) before and 15, 30, 45, 60, 90, and 120 minutes after each sugar was administered.

Stools were collected for three to six days and were analysed for fat by the method of van de Kamer *et al.* (1949). Because fat intake in the average Thai diet is low (I.C.N.N.D., 1962), a daily supplement of 75 g. of butter was given during the collection period. Carmine red dye was given orally to mark the beginning and end of the collection periods.

Fluoroscope examination of the upper gastrointestinal tract, with small-bowel follow-through studies, was also made. (We are indebted to Major Chitti Palavatana for carrying out the x-ray studies.)

Small-bowel biopsy specimens were taken from the jejunum with a Crosby biopsy capsule (Crosby and Kugler, 1957) after x-ray verification of the position of the capsule. The specimens were orientated with the mucosal side up on filter paper and were fixed in 10% buffered formalin. Haematoxylin and eosin, periodic acid-Schiff, and iron stains were done on all material. In order to minimize artifacts resulting from tangential cutting, care was taken to ensure that the plane of sectioning was parallel to the long axis of the villi and perpendicular to the luminal surface (Rubin and Dobbins, 1965). Measurements of overall mucosal thickness and villus/crypt ratio were made in eight patients by methods similar to those of Swanson and Thomassen (1965).

### Results

Laboratory data are shown in the Table. Urinary excretion of D-xylose after a 25-g. dose was  $5.84 \pm 1.54$  (S.D.) g. in 36 subjects. The mean two-hour serum level was  $39.8 \pm 7.5$  (S.D.) mg./100 ml. The percentage of xylose excreted by the three subjects given the smaller dose (0.5 g./kg. body weight) was comparable to that after a 25-g. dose. Mild diarrhoea consisting of two to four watery stools occurred after ingestion of the 25-g. dose in three subjects. Five-hour urine volumes were above 225 ml. in all subjects tested.

Little or no increase in blood glucose occurred after oral lactose administration in the 39 subjects tested (Fig. 1), the mean maximum rise being  $2.97 \pm 3.07$  mg./100 ml. Diarrhoea, consisting of up to 10 loose stools, or abdominal cramps, or both, occurred in 28 of the 39 subjects.

In contrast with the flat lactose-tolerance test, glucose absorption was usually normal. The mean maximum rise in blood sugar after ingestion of 0.75 g./kg. body weight of glucose was  $42.5 \pm 20.5$  (S.D.) mg./100 ml. in 33 subjects (Fig. 1). Only four subjects showed a rise of less than 20 mg./100 ml. (7, 15, 16, and 19 mg./100 ml.). In four other subjects given 100 g. of glucose the mean maximum rise in blood sugar was 61.5 mg./100 ml.

Vitamin-B<sub>12</sub> absorption was normal in 33 out of 34 subjects tested (Fig. 2). One subject with a haemoglobin of 12.4 g. excreted 4% of the dose.

Stool fat excretion averaged 2.31 ± 1.37 g./day in 35 subjects tested. The values were below 5 g. in all except two, in whom the fat excretion was 6.3 and 6.8 g./day (Fig. 2).

Vitamin-A tolerance in 24 out of 27 subjects showed a rise of more than 125 µg./100 ml. above the control value (Gardner and Perez-Santiago, 1956).

The mean serum total cholesterol was 177.5 ± 31 mg./100 ml. in 25 subjects tested. There was no correlation between xylose excretion and serum cholesterol (Robins *et al.*, 1967).

The following mean levels (± S.D.) were obtained (the number of subjects tested is in parentheses): serum calcium, 9.31 ± 0.49 mg./100 ml. (29); serum beta-carotene, 133.1 ± 65.0 µg./100

ml. (34); serum albumin, 4.18 ± 0.56 g./100 ml. (32); haemoglobin, males 13.8 ± 1.9 g./100 ml. (18), females 12.1 ± 1.2 g./100 ml. (22).

Fifteen subjects were found to harbour the following small-intestinal parasites: *Ascaris* alone in 11, *Ascaris* and hookworm in one, hookworm alone in one, hookworm and *Strongyloides* in one, and *Giardia lamblia* in one. No difference in absorption tests was noted between these subjects and the 24 whose stools were negative for parasites, with one exception. Subject 8, whose stools contained *G. lamblia*, excreted the lowest xylose recorded—2.99 g.

Laboratory Investigations

| Subject No. | Age | Sex | Urine Xylose Excretion After 25-g Dose (g) | Schilling Test with Intrinsic Factor (%) | Vitamin A Tolerance* Max. Rise (µg./100 ml.) | Lactose Tolerance† Max. Rise Blood Sugar (mg./100 ml.) | Glucose Tolerance‡ Max. Rise Blood Sugar (mg./100 ml.) | Stool Fat (g./day) |
|-------------|-----|-----|--|--|--|--|--|--------------------|
| 1           | 31  | F   | 4.05§                                      | 15.8                                     | 444  | 0  | 42   | 3.79               |
| 2           | 24  | F   | 6.98                                       | 17.3                                     | 190  | 5  | 51   | 3.30               |
| 3           | 27  | F   | 4.73                                       | —  | 307  | 1  | 61   | 2.4                |
| 4           | 22  | F   | 5.06                                       | 7.8                                      | 455  | 3  | 56   | 2.08               |
| 5           | 46  | F   | 3.59                                       | 29.8                                     | 180  | 0  | 67   | 1.88               |
| 6           | 22  | F   | 5.70                                       | —  | —  | 0  | —  | —                  |
| 7           | 46  | F   | 6.77                                       | 4.0                                      | 156  | 8  | 57   | 2.07               |
| 8           | 43  | F   | 2.99                                       | —  | —  | 0  | 57¶  | —                  |
| 9           | 31  | F   | 4.05                                       | —  | —  | —  | —  | —                  |
| 10          | 17  | F   | 9.39                                       | 10.3                                     | 252  | 0  | 19   | 1.82               |
| 11          | 72  | F   | 3.22                                       | 9.5                                      | 343  | 8  | 84¶  | —                  |
| 12          | 25  | F   | 4.43                                       | 11.3                                     | 276  | 8  | 79   | 0.6                |
| 13          | 29  | F   | 4.85                                       | 15.2                                     | 242  | 5  | 98   | 2.7                |
| 14          | 16  | F   | 6.30                                       | —  | 421  | 3  | 33   | 1.00               |
| 15          | 32  | F   | 4.30                                       | —  | 190  | 0  | —  | 1.40               |
| 16          | 16  | F   | 7.00§                                      | 31.3                                     | 345  | 0  | 34   | 0.93               |
| 17          | 40  | F   | 7.77                                       | 16.8                                     | 227  | 0  | 21   | 2.46               |
| 18          | 18  | F   | 7.80                                       | 19.9                                     | —  | 3  | 66   | 1.94               |
| 19          | 25  | F   | 6.66                                       | 8.4*                                     | —  | 2  | 33   | 1.60               |
| 20          | 35  | F   | 5.97                                       | 12.3*                                    | —  | 5  | 27   | 1.84               |
| 21          | 27  | F   | —  | 14.9                                     | 49   | 0  | 28   | 2.14               |
| 22          | 19  | F   | 4.73                                       | 17.6                                     | —  | 0  | 66   | 1.70               |
| 23          | 15  | M   | 7.40                                       | 25.7                                     | 225  | 0  | 51   | 2.61               |
| 24          | 16  | M   | 6.50                                       | 29.0                                     | 1,266  | 5  | 60   | 1.40               |
| 25          | 16  | M   | 4.70                                       | 28.0                                     | 262  | 0  | 56   | 2.80               |
| 26          | 17  | M   | 5.55                                       | 23.0                                     | 95   | 0  | 15   | 1.14               |
| 27          | 16  | M   | 5.50                                       | 10.7                                     | 429  | 0  | 23   | 1.71               |
| 28          | 18  | M   | 8.59                                       | 28.0                                     | —  | 5  | 62¶  | 6.30               |
| 29          | 17  | M   | 6.87                                       | 15.0                                     | —  | 2  | 43¶  | 3.00               |
| 30          | 17  | M   | 7.27                                       | 26.5                                     | 280  | 6  | 58   | 3.80               |
| 31          | 13  | M   | 4.41§                                      | 10.0                                     | 235  | 8  | 30   | 0.44               |
| 32          | 17  | M   | 7.39                                       | 24.0                                     | 205  | 6  | 28   | 2.00               |
| 33          | 45  | M   | 7.20                                       | 25.7                                     | —  | 2  | 32   | 1.65               |
| 34          | 40  | M   | 4.16                                       | 20.8                                     | 217  | 6  | 7  | 4.26               |
| 35          | 42  | M   | 4.48                                       | 17.0                                     | 112  | 0  | 44   | 6.81               |
| 36          | 15  | M   | 6.16                                       | 23.6                                     | 145  | 4  | 52   | —                  |
| 37          | 17  | M   | 6.04                                       | 15.8                                     | 355  | 10   | 44   | 1.58               |
| 38          | 17  | M   | 4.16                                       | 21.7                                     | —  | 3  | 42   | 2.63               |
| 39          | 19  | M   | 5.94                                       | 25.7                                     | —  | 1  | 23   | 1.45               |
| 40          | 17  | M   | 7.01                                       | 22.8                                     | —  | 7  | 34   | 1.62               |

\* Test dose 260,000 i.u. † Test dose 1.5 g./kg. body weight. ‡ Test dose 0.75 g./kg. body weight. § Test dose 0.5 g./kg. body weight. ¶ No intrinsic factor given. ¶ Test dose 100 g.

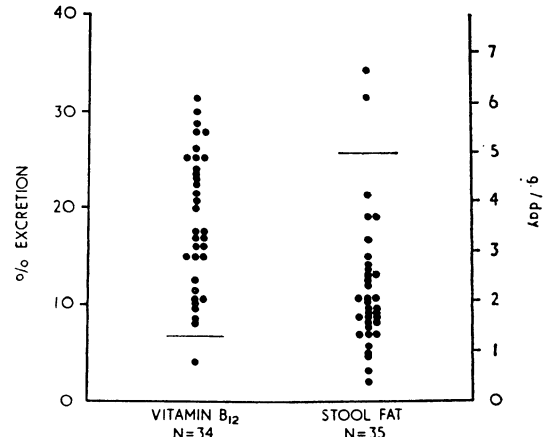


FIG. 2.—Individual values for cobalt-57 vitamin B<sub>12</sub> urinary excretion (Schilling test) and for stool fat excretion in Thai subjects. Horizontal lines indicate lower and upper limits of normal, respectively, by Western standards.

The small-bowel pattern by x-ray examination was normal in the 29 subjects studied, except for mild mucosal irregularity in the ileum of one. In six subjects wormlike objects, probably ascarids, were seen in the mid-ileum.

A jejunal biopsy was obtained in each of the 40 subjects. The specimens were reasonably uniform in appearance, with the same mild histological abnormalities as noted in a previous report from Thailand (Sprinz *et al.*, 1962). Under the dissecting-microscope the villi were irregular in height and thickness

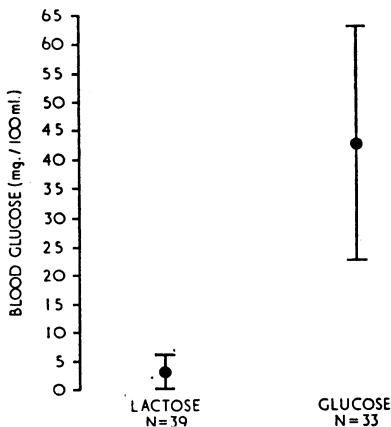


FIG. 1.—Comparison of mean maximal rise in blood glucose over fasting value in Thai subjects after lactose- and glucose-tolerance tests ± 1 S.D.

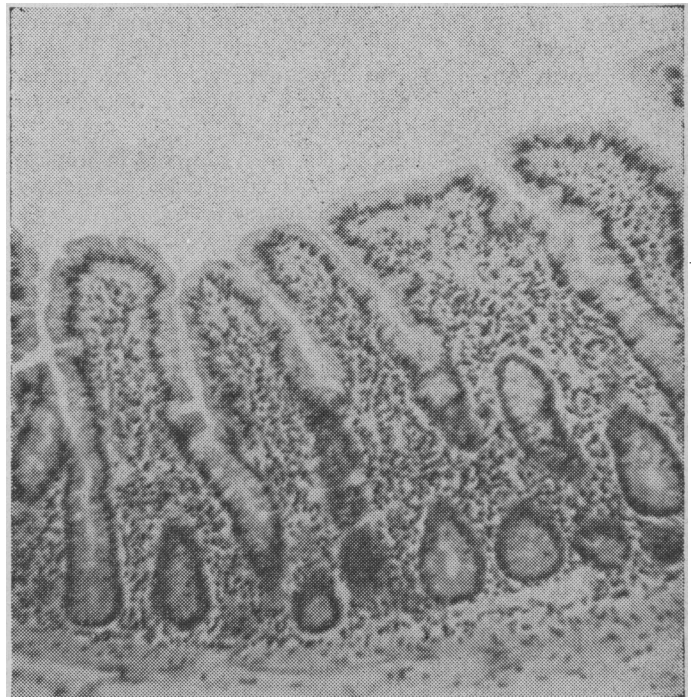


FIG. 3.—Biopsy showing villus shortening, decreased villus/crypt ratio, and increased cellularity of the lamina propria. (Haematoxylin and eosin. × 125.)

and usually leaflike or tonguelike in overall shape. In no specimen did fingerlike forms predominate. A flat or convoluted mucosal pattern was not seen in any patient. Under the light microscope the most impressive changes were villus-shortening with a decrease in the villus/crypt ratio, and uniform infiltration of the lamina propria with plasma cells and lymphocytes (Fig. 3). In the eight biopsies measured the average total villus/crypt thickness was 520  $\mu$  (range 420–630  $\mu$ ) and the average villus/crypt ratio was 2.3:1 (range 1.9:1–3.1:1). Periodic-acid-Schiff and iron stains were unremarkable. No correlation was noted between the degree of microscopic or gross abnormality of the biopsies and any of the laboratory findings.

### Discussion

In this population of asymptomatic Thai subjects the results of fat, vitamin-B<sub>12</sub>, vitamin-A, and glucose-absorption tests were comparable to the results obtained in North American subjects (Sheehy and Floch, 1964). Xylose and lactose absorption and the histological appearance of the jejunal mucosa were different, however. Changes of similar degree have been found by others in apparently healthy subjects and interpreted as indications of subclinical intestinal disease (Baker *et al.*, 1962; Russell *et al.*, 1966; Lindenbaum *et al.*, 1966b). Is there any evidence to support this contention?

Urinary xylose excretion after a 25-g. dose in this population (5.84±1.54 g.) is essentially the same as Gardner and Perez-Santiago (1956) (5.60±0.60 g.) and Butterworth *et al.* (1959) (5.70±1.40 g.) have reported from Puerto Rico, and Lindenbaum *et al.* (1966b) from East Pakistan (5.38±1.57 g.). The Thai results are significantly lower than those of a group of North Americans studied in our laboratory (Keusch *et al.*, unpublished data). A similar difference was found by Lindenbaum *et al.* (1966b) between "protected" Westerners living in Pakistan and Pakistanis. While the reasons for this mild reduction in xylose excretion are not apparent, it is important to point out that these results are clearly higher than values found in classical tropical sprue (Sheehy and Floch, 1964).

The xylose excretion in the present study and in Pakistanis (Lindenbaum *et al.*, 1966a) has a bell-shaped distribution. Therefore it is not valid to use a criterion of normality—for example, greater than 5 g. excretion—derived from a different group (North Americans), as is customary in many clinics. Arbitrarily applying such a criterion would divide these homogeneous populations studied in the tropics.

Though lactose malabsorption has been observed in tropical sprue (Bayless *et al.*, 1964), it has also been described in a variety of other gastrointestinal diseases (Haemmerli *et al.*, 1965), as a possible genetically controlled enzyme defect (Cook and Kajubi, 1966), and in a surprisingly high percentage of normal adults (Friedland, 1965). The cause in the Thai is not clear. However, in view of what is apparently a universal occurrence of lactose malabsorption in Thais, the test is of no value in this population as a screening test for tropical sprue (Sheehy and Anderson, 1965).

Interpretation of the very mild non-specific jejunal abnormality is also difficult. Similar histological changes have been reported in kwashiorkor (Burman, 1965), hookworm disease (Tandon *et al.*, 1966), viral illnesses (Sheehy *et al.*, 1964), rosacea (Watson *et al.*, 1965), acute diarrhoea (Lindenbaum, 1965), and after triparanol administration (McPherson and Summerskill, 1963). None of these conditions was present in our subjects. What, then, is the relation between this non-specific histological abnormality and tropical sprue? It has been suggested that there is a spectrum of small-bowel disease in the tropics which ranges from mild and asymptomatic malabsorption to overt sprue (Klipstein *et al.*, 1966). Furthermore, this coexistence of tropical sprue and milder forms of

jejunal abnormality in the same tropical population has been cited as evidence for an association between the two processes (Klipstein, 1967). However, our results fail to support this contention, since intestinal function in Thai subjects with non-specific jejunal abnormalities is different from that in overt tropical sprue. Furthermore, Pakistanis with similar histological changes failed to show improvement in absorption tests after treatment with tetracycline or folic acid, which are usually effective in tropical sprue (Lindenbaum *et al.*, 1966a).

### Summary

Forty asymptomatic Thai subjects were studied for evidence of tropical sprue by means of the following tests: xylose excretion, <sup>57</sup>Co vitamin-B<sub>12</sub> and vitamin-A absorption, faecal fat excretion, lactose and glucose tolerance, small-intestine radiographs, and jejunal biopsy. Xylose excretion was lower than in North American subjects but above the range expected in tropical sprue. Lactose malabsorption and mild non-specific histological abnormalities of the jejunal mucosa were present in all. The other studies showed nothing abnormal. These findings were considered insufficient to support a diagnosis of subclinical tropical sprue, and thus we have no evidence for sprue as a widespread disease in Thailand.

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