Vindaloo and you

Ingvar Bjarnason, Sassoon Levi, Paul Smethurst, Ian S Menzies, A Jonathan Levi

Recent developments have led to the introduction of various test substances to assess intestinal permeability, some of which test the barrier function of the small intestinal mucosa.[1-4] Most of the tests are simple, comprising ingestion of a solution containing a poorly metabolised test substance and subsequent collection of urine over 5-24 hours for analysis of the test markers. Such tests have shown abnormal intestinal permeability in several gastrointestinal conditions,[1-4] and because of their non-invasive nature they can be applied to diagnostic screening and research.[5-7] Furthermore, close attention to apparent anomalies of intestinal permeability has led to the description of a new enteropathy caused by non-steroidal anti-inflammatory drugs that is not evident using conventional techniques.[8-10]

Permeability tests have not, however, been met with universal success as diagnostic screening tests. In particular, the indigenous "normal" populations of east and west Africa,[11 12] the Middle East,[13] Indonesia, Papua New Guinea, India, and Thailand (I S Menzies, unpublished observations) have such high permeability values that the tests do not discriminate between subjects with disease and normal subjects. This is in sharp contrast with the low normal values and sensitivity of the tests in whites in England,[1-4] the Continent,[14-19] America,[20-22] and Australia.[23] The reasons for these differences are unknown, but as ethnic Asian (from India or Pakistan) residents in the United Kingdom[24] and white residents in tropical countries mentioned (I S Menzies, unpublished observations) have pronounced intestinal hyperpermeability it has been suggested that dietary factors may be important in disrupting intestinal integrity. We assessed whether ingesting three Indian meals on consecutive evenings altered intestinal function by a combined absorption and permeability test.

TABLE I—*Indian dishes eaten by volunteers*

	No of subjects
Weak:	
Massala	3
Biriani	4
Malai	1
Medium:	
Meat or chicken curry	4
Dopiaza	1
Rogan Josh	1
Sag Gosth	1
Bhuna	1
Hot:	
Madras	3
Patia	1
Vindaloo	8
Pal	2

Section of Gastroenterology, MRC Clinical Research Centre, Harrow, Middlesex HA1 3UJ
Ingvar Bjarnason, MD, *honorary senior medical registrar*
Paul Smethurst, MIBIOL, *senior technician*

Northwick Park Hospital, Harrow
Sassoon Levi, MRCP, *registrar*
A Jonathan Levi, FRCP, *consultant gastroenterologist*

Department of Chemical Pathology and Metabolic Disorders, United Medical and Dental Schools of Guy's Hospital, London
Ian S Menzies, FRCPATH, *consultant chemical pathologist*

Correspondence and reprints to: Dr Bjarnason.

Subjects and methods

We studied 10 healthy white volunteers, which is the same number of subjects that has given clear cut results in previous studies of permeability.[25] Six were men and four were women, mean age 30 (range 22-39), and all were staff at the Medical Research Council's clinical research centre. All abstained from drinking alcohol[26] for at least one week before the study and none had taken aspirin or non-steroidal anti-inflammatory drugs in the preceding three weeks.[8 20] They were all taking a stable English diet and did not, on average, consume hot or spicy food more than fortnightly.

PROCEDURE

After fasting overnight the subjects drank a test solution (100 ml) at 8 am and remained fasting for a further two hours, when normal food and fluids were permitted. The test solution (105 mmol/l) contained: 3-*O*-methyl-D-glucose 0·2 g to assess active carrier mediated transport[1]; D-xylose 0·5 g to assess passive carrier mediated transport[1]; L-rhamnose 1·0 g to assess transcellular permeation (through aqueous pores)[1]; and edetic acid labelled with chromium-51, 3·7 MBq, to assess intracellular permeation.[1 4] We assessed intestinal permeation by measuring the fraction of the ingested dose of these poorly metabolised test substances excreted in urine collected for a total of five hours from 8 am to 1 pm. The urine was collected into sample bottles containing 1 ml 1% (wt/vol) thiomersal as a preservative. Aliquots of urine were stored at 4°C and analysed within six weeks after collection.

Intestinal permeation of the four test substances was assessed 12 days before and the day after the last test meal. The food was bought and eaten in selected Indian restaurants in Harrow. Each subject had a full portion of an Indian dish on three consecutive evenings between 8 and 10 pm; table I shows the dishes. The overall strategy was to increase the "hotness" of the meals each evening, the subjects starting at a strength that suited them and finishing with the last meal being hotter than each had been accustomed to. Onion salad, raita, nan, pappadams, bhajee, tarka pal, chana mossalia, and water were permitted ad libitum.

The study was approved by the Harrow Health Authority Ethical Committee.

ANALYSIS OF TEST SUBSTANCES

A modified thin layer chromatographic technique was used for estimating monosaccharides, which involved scanning densitometry with an arabinose internal standard to overcome errors of application.[27] Separation of sugars in the test samples and sugar standards was achieved by multiple development on half plates (10×20 cm) of F1500, plastic backed silica gel (Schleider, Schill, Dassel, West Germany) after three consecutive ascending runs of 8·5 cm with ethyl acetate:pyridine:acetic acid:water (75:15:10:10 by volume). The plates were dried for a minimum of 30 minutes between each run and then overnight to remove the pyridine before performing a 4-aminobenzoic acid:phosphoric acid colour reaction at 120-130°C. After location of the monosaccharides the chromatograms were kept at 4°C in polyethylene envelopes, and exposure to light was minimised during scanning. The peak heights were measured and corrected to a standard peak height with an internal standard. The concentrations of the test sugars were then obtained by interpolation from standard curves for each sugar, which had been derived from the same chromatograms. The technique is accurate and sensitive, recovery being above 90% with a minimum level of detection <0·1 mmol/l. The precision coefficient of variation without replication is 3-8%.

Radiolabelled edetic acid was determined by counting 5 ml aliquots of urine from the volunteers with an LKB Wallac 1280 gammacounter for five minutes, together with 5 ml of a one in 500 dilution of the original test solution, and the percentage labelled edetic acid excreted in the five hour urine samples

was calculated. The minimum detectable activity is <0·03% of the original dose/l of urine.[26]

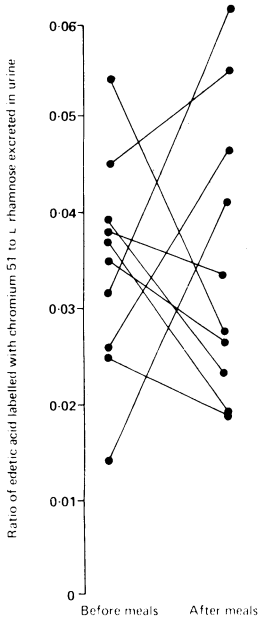
STATISTICS

Statistical analysis was assessed by the paired Student's *t* test.

Results

Table II shows that the percentage urinary excretion of each of the four test substances increased significantly after the Indian meals. The figure shows that the ratio of excreted labelled edetic acid to L-rhamnose, which specifically reflects alterations in intestinal permeability, did not differ significantly between the two tests (mean 0·034 (SE 0·004) *v* mean 0·036 (SE 0·005). Likewise, the ratios of labelled edetic acid to 3-*O*-methyl-D-glucose and D-xylose and the various saccharides to one another did not differ significantly between the tests.

No subject had serious side effects from the ingested food. Perspiration was a consistent finding, all felt bloated after the last meal, and six had mild diarrhoea the following morning.



Ratios of edetic acid labelled with chromium-51 to L-*rhamnose before and after eating Indian meals on three consecutive evenings. Mean ratios 0·034 v 0·036; mean (SE) difference 0·001 (0·0004); 95% confidence interval for difference 0·0004 to 0·0022, p>0·5*

Discussion

3-*O*-methyl-D-glucose, D-xylose, L-rhamnose, and edetic acid labelled with chromium-51 were selected as test substances because their mode of permeation across the intestinal mucosa is well defined and fairly specific.[1] Accordingly, their permeation across the mucosa is predominantly determined by an active and a passive carrier mediated transport system and transcellular and intercellular permeability, respectively.

To a large extent they fulfil the requirements of an ideal test substance for assessing intestinal permeation.[1 28] They are non-toxic, readily assayed, and rapidly excreted and quantitatively recovered in urine with minimal metabolic loss after intravenous administration, so that a five hour urinary excretion value after ingestion corresponds closely to the total amounts absorbed. Table III shows the main factors that govern the permeation of the test substances and shows the advantage of giving them simultaneously. Thus changes in the urinary excretion of a single test substance may be due to alterations in mucosal, premucosal, and postmucosal factors. Giving the markers together reduces the possible mechanisms as changes in blood flow and the premucosal determinants of absorption affect the markers identically (table III) so their urinary excretion ratios are unchanged. Altered mucosal function is, however, associated with a selective change in the excretion of a single test substance or of two in opposition to each other.[1-3]

The effect of the Indian meals was to increase the urinary excretion of all four test substances equally, as was evident from the unchanged urinary excretion ratios. This suggests that the food or its ingredients were affecting premucosal factors or intestinal blood flow. The precise mechanism is unknown, but a number of possibilities exist. Most people who regularly eat curry report immediate postprandial fullness that commonly continues to the next day. The discomfort is not unlike that experienced by patients with mild forms of the irritable bowel syndrome, which in turn may be a dysmotility phenomenon.[29] Our results are certainly consistent with the hypothesis that the food prolongs the transit time through the small intestine. Furthermore, such an effect would be potentiated by dehydration, which accompanied the diarrhoea. Alternatively, and not mutually exclusively, the spices used in the food may increase intestinal blood flow as a consequence of mucosal hyperaemia. That the spices are indeed irritant is perhaps best shown by the well known phenomenon of perianal discomfort ("ring sting") that commonly occurs during and immediately after defecation 10-12 hours after a particularly hot curry.

The original hypothesis of damage to the small intestinal mucosa is not substantiated by this study. Further effort is needed to identify possible environmental factors capable of increasing intestinal permeability. Their identification may not be a trivial matter. Indeed, the morphological counterpart of increased intestinal permeability is likely to be partial villus atrophy (tropical enteropathy), which is common in normal subjects in the tropics.[30-35] Tropical enteropathy in turn is associated with mild to moderate malabsorption, which may be of major importance in regions where the nutritional adequacy of the diet is borderline. Furthermore, the agent(s) responsible for increased intestinal permeability in normal subjects in Ireland[36] may be the key to understanding the high prevalence of coeliac disease there.[6 37]

TABLE II—*Five hour urinary excretion of the four poorly metabolised test substances before and after eating three Indian meals of increasing strength on consecutive evenings. Values are percentages*

	Mean value before meals	Mean value after meals	Mean difference	Standard error of mean difference	95% Confidence interval	p Values
3-*O*-methyl-D-glucose	49·1	63·4	14·3	3·2	7·1-21·5	<0·05
D-Xylose	30·9	36·9	6·0	2·4	0·6-11·4	<0·05
L-Rhamnose	15·2	22·4	7·2	2·1	2·5-12·0	<0·01
Edetic acid labelled with chromium-51	0·50	0·83	0·33	0·19	0·02-0·64	<0·05

TABLE III—*Factors affecting urinary excretion of ingested test substances*

	3-*O*-methyl-D-glucose	D-Xylose	L-Rhamnose	Edetic acid labelled with chromium-51
Premucosal:				
Gastric emptying	=	=	=	=
Gastric dilution	=	=	=	=
Intestinal transit	=	=	=	=
Intestinal dilution	=	=	=	=
Bacterial degradation	(+)*	+	+	NA
Mucosal:				
Mode of permeation	≠	≠	≠	≠
Blood flow	=	=	=	=
Postmucosal:				
Metabolism	NA	(+)†	NA	NA
Tissue distribution‡	≠	≠	≠	≠
Renal handling§	=	=	=	=

=Test substances do not differ.
≠Test substances differ.
+ (+) Present or occurs.
*More resistant than D-xylose and L-rhamnose.
†Small quantities metabolised by liver.
‡Radiolabelled edetic acid is a classic extracellular volume marker, other markers equilibrate with the cellular compartment and at different rates.
§Mechanism may be the same, but rate of urine excretion varies because of tissue distribution.

1 Menzies IS. Transmucosal passage of inert molecules in health and disease. In: Skadhauge E, Heintze K, eds. *Intestinal absorption and secretion.* Lancaster: MTP,1984:527-43 (Falk symposium 36.)
2 Hamilton I. Small intestinal permeability. In: Pounder RE, ed. *Recent advances in gastroenterology.* Vol 6. Edinburgh: Churchill Livingstone, 1966:73-91.
3 Cooper BT. The small intestinal permeability barrier. In: Losowski MS, Heatley RV, eds. *Gut defences in clinical practice.* Edinburgh: Churchill Livingstone, 1986:117-32.
4 Bjarnason I, Peters TJ, Levi AJ. Intestinal permeability; clinical correlates. *Dig Dis* 1986;**4**:83-92.
5 Walker WA, Isselbacher KJ. Uptake and transport of macromolecules by the intestine. Possible role in clinical disorders. *Gastroenterology* 1974;**57**: 531-50.
6 Peters TJ, Bjarnason I. Coeliac syndrome: biochemical mechanisms and the missing peptidase hypothesis revisited. *Gut* 1984;**25**:913-8.
7 Bjarnason I, Peters TJ. Helping the mucosa make sense of macromolecules. *Gut* 1987;**28**:1057-61.
8 Bjarnason I, Williams P, So A, *et al.* Intestinal permeability and inflammation in rheumatoid arthritis; effects of non-steroidal anti-inflammatory drugs. *Lancet* 1984;ii:1171-4.
9 Bjarnason I, Zanelli G, Prouse P, *et al.* Blood and protein loss via small intestinal inflammation induced by non-steroidal anti-inflammatory drugs. *Lancet* 1987;ii:711-4.
10 Bjarnason I, Price A, Zanelli G, *et al.* Clinical pathological features of non

steroidal anti-inflammatory drug-induced small intestinal strictures. *Gastroenterology* 1988;**94**:1070-4.
11 Ukabam SO, Homeida MA, Cooper BT. Small intestinal permeability in Sudanese subjects: evidence of tropical enteropathy. *Trans R Soc Trop Med Hyg* 1986;**80**:204-7.
12 Behrens R, Northrop C, Lunn P, Neale G, Hanlon P. Factors affecting the integrity of intestinal mucosa in Gambian children. *Proc Nutr Soc* 1986; **45**:67a.
13 Peled Y, Watz C, Gilat T. Measurement of intestinal permeability using ^{51}CrEDTA. *Am J Gastroenterol* 1985;**80**:770-3.
14 Casellas F, Aguadé S, Soriano B, Accarino A, Molero J, Guarner L. Intestinal permeability to ^{99m}Tc diethylene-tetraaminopentaacetic acid in inflammatory bowel disease. *Am J Gastroenterol* 1986;**81**:767-70.
15 Auer IO, Habscheid W, Hiller S, Gerhards W, Eilles C. Nicht-steroidale antiphlogistika Erhöhen die Darmpermeabilität. *Dtsch Med Wochenschr* 1987;**112**:1032-7.
16 Ruppin H, Hotze A, Düring A, *et al.* Reversible Funktions-Sjörungen des Intestinaltraktes durch abdominelle Strahlentherapie. *Z Gastroenterol* 1987; **25**:261-9.
17 Leclercq-Foucart J, Forget P, Sodoyez-Goffaux F, Zappitelli A. Intestinal permeability to ^{51}CrEDTA in children with cystic fibrosis. *J Pediat Gastroenterol Nutr* 1986;**5**:384-7.
18 Leclercq-Foucart J, Forget P, Van Cutsem JL. Lactulose-rhamnose intestinal permeability in children with cystic fibrosis. *J Pediat Gastroenterol Nutr* 1987;**6**:66-70.
19 Turck D, Ythier H, Maquet E, *et al.* Intestinal permeability to ^{51}CrEDTA in children with Crohn's disease and coeliac disease. *J Pediatr Gastroent Nutr* 1987;**6**:535-7.
20 Jenkins RT, Rooney PJ, Jones DB, Bienenstock J, Goodacre RL. Increased intestinal permeability in patients with rheumatoid arthritis: a side effect of oral non-steroidal anti-inflammatory drug therapy. *Br J Rheumatol* 1987;**26**:103-7.
21 Jenkins RT, Jones DB, Goodacre RL, *et al.* Reversibility of increased intestinal permeability to ^{51}CrEDTA in patients with gastrointestinal inflammatory bowel disease. *Am J Gastroenterol* 1987;**82**:1159-64.
22 O'Morain CA, Abelow AC, Chervli LR, Fleischner GM, Das KM. ^{51}CrEDTA test: a useful test in the assessment of inflammatory bowel disease. *J Lab Clin Med* 1986;**108**:430-5.
23 Hetzel DAS, Labrody JT, Bellon M, *et al.* The ^{51}CrEDTA absorption test in coeliac patients treated with a gluten free diet. *International Research Communication Journal of Medical Science* 1986;**14**:1183-4.
24 Bjarnason I, Peters TJ, Veall N. ^{51}CrEDTA test for intestinal permeability. *Lancet* 1984;ii:523.
25 Bjarnason I, Williams P, Smethurst P, Peters TJ, Levi AJ. Effect of non-steroidal anti-inflammatory drugs and prostaglandins on permeability of the human small intestine. *Gut* 1986;**27**:1292-7.
26 Bjarnason I, Ward K, Peters TJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. *Lancet* 1984;i:179-82.
27 Menzies IS, Mount JN, Wheeler MJ. Quantitative estimation of clinically important monosaccharides in plasma by rapid thin-layer chromatography. *Ann Clin Biochem* 1978;**15**:65-76.
28 Chadwick VS, Phillips SF, Hofmann AF. Measurements of intestinal permeability using low molecular weight polyethyleneglycols (PEG 400). II. Application to normals and abnormal permeability states in man and animals. *Gastroenterology* 1977;**73**:247-51.
29 Kumar D, Wingate DL. The irritable bowel syndrome; a paroxysmal motor disorder. *Lancet* 1985;ii:973-7.
30 Baker SJ. Geographical variations in the morphology of the small intestinal mucosa in apparently healthy individuals. *Pathologia et Microbiologia* 1973;**39**:222-37.
31 Baker SJ, Mathan VI. Syndrome of tropical sprue in south India. *Am J Clin Nutr* 1968;**21**:984-93.
32 Brunser O, Eidelman S, Kupstein FA. Intestinal morphology of rural Haitians. A comparison between overt tropical sprue and asymptomatic subjects. *Gastroenterology* 1970;**58**:655-68.
33 Schenk EA, Klipstein FA, Tomasini JT. Morphological characteristics of jejunal biopsies from asymptomatic Haitians and Puerto Ricans. *Am J Clin Nutr* 1972;**25**:1080-3.
34 Cooke WJ, Holmes GKT. The jejunal mucosa. In: Cook WJ, Holmes GKT, eds. *Coeliac disease*. Edinburgh: Churchill Livingstone, 1984:23-66.
35 Lindenbaum J, Harmon JW, Gerson CD. Subclinical malabsorption in developing countries. *Am J Clin Nutr* 1972;**25**:1056-106.
36 O'Mahoney CP, Stevens FM, Bourke M, McCarthy CF, Weir DB, Feighery CF. ^{51}CrEDTA test for coeliac disease. *Lancet* 1984;i:1354-5.
37 Mylotte M, Egan-Mitchell B, McCarthy CF, McNicholl B. Incidence of coeliac disease in the west of Ireland. *Br Med J* 1973;i:703-5.

A place in the sun?

Izhar Ben-Shlomo, Geoffrey Goodman

The number of original medical articles is ever increasing, and evaluation of trends may contribute to understanding the nature of medical progress. An increase in the number of authors per article is well recognised, as is the increasing number of references. We report a statistical analysis in which we compared certain aspects of articles, such as length of title and number of authors, in three medical journals in 1955 and 1985. We also carried out a longitudinal study of a representative number of articles published during January in the *British Medical Journal* during 1955-85.

Method and results

We chose three journals, the *Lancet*, *Circulation*, and *Fertility and Sterility*, representing disparate aspects of medical literature, and selected from each between 43 and 57 consecutive articles from 1955 and 1985. Only 43 articles were published in *Fertility and Sterility* in 1955, and, accordingly, we chose articles from the middle of the year in 1985. Articles in the *Lancet* (original contributions and preliminary communications) and *Circulation* were taken from the beginning of the two years. The variables analysed were the number of words in the title and the number of authors, references, and pages. Adjustment was made for the change in format of *Fertility and Sterility*; the other two journals maintained their format. The results were analysed with the Mann-Whitney U test, regression analysis, and rank correlation.

To check for the continuity of trends identified the first 10 full articles that appeared every year in the *BMJ* were similarly analysed, and the median values were calculated.

In all three journals the titles of the articles were longer and the numbers of authors and references were larger in 1985 than in 1955 (table). The numbers of words in the titles were significantly correlated with the numbers of authors ($p<0.05$) but not with the other variables. The change in the number of pages was more limited, with a small but significant reduction in that of *Circulation* in 1985 ($p<0.01$).

In the *BMJ* there was a highly significant and consistent yearly increase in the number of words per title (Spearman's correlation coefficient=0·59 (95% confidence interval 0·30 to 0·78; $p<0.001$)) and in the number of authors accredited per article (0·82 (0·65 to 0·91; $p<0.001$)), confirming the trends identified previously.

Comment

One reason why titles have become longer may be the increasing complexity of medicine. On this basis,

Department of Obstetrics and Gynaecology, Rebecca Sieff Government Hospital, Safed, Israel 13100
Izhar Ben-Shlomo, MD, *senior registrar*

Amrad (Research and Development), Amiad, Hevel Korazim, Israel 12335
Geoffrey Goodman, PHD, *scientific director*

Correspondence to: Dr I Ben-Shlomo, Amiad, Hevel Korazim, Israel 12335.

Survey of articles published in three medical journals in 1955 and 1985. Values are medians (95% confidence intervals)

	Lancet		*Circulation*		*Fertility and Sterility*	
	1955 (n=43)	1985 (n=57)	1955 (n=48)	1985 (n=51)	1955 (n=47)	1985 (n=48)
No of words in title	7 (6 to 8)	10 (9 to 12)*	12 (10 to 13)	15 (14 to 18)†	9 (6 to 10)	13 (11 to 15)*
No of authors	2 (1 to 2)	4·5 (3 to 5)*	3 (3 to 3)	5 (5 to 6)*	1 (1 to 2)	4 (4 to 5)*
No of references	8 (5 to 11)	19 (16 to 21)*	16·5 (13 to 21)	24 (22 to 30)*	9 (6 to 12)	18 (16 to 21)*
No of pages	2·3 (1·4 to 3·2)	2·3 (2·1 to 2·5)	8·0 (7·0 to 10·0)†	7·0 (5·7 to 7·5)	4·3 (3·3 to 5·3)‡	4·5 (4·0 to 4·5)

*$p<0.001$. †$p<0.01$. ‡Adjusted for change in format of journal.