Rectal hyperplasia after jejunoileal bypass for morbid obesity

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SUMMARY   Jejunoileal bypass (JIB) has been widely used to treat patients with morbid obesity for the past 20 years. In rats JIB causes adaptive colonic hyperplasia and enhances colorectal neoplasia. In this study crypt cell production rate (CCPR) was measured stathmokinetically in cultured rectal biopsies from nine patients with JIB and seven controls without intestinal operations or disease. Crypt cell production rate in the group with JIB was more than double that of controls (12·80 (2·67) *v* 6·23 (1·49) cells/crypt/h: $p < 0.001$). There were no significant differences in crypt morphometry and histological examination of rectal biopsies was normal. Patients with JIB have a marked and persistent increase in cell proliferation in the large intestine and may be at increased risk of developing colonic cancer.

During the 20 years since jejunoileal bypass (JIB) was introduced for the treatment of morbid obesity,[1] it is estimated that over 100 000 such operations have been carried out in the USA alone.[2] In rats, end-to-side JIB stimulates adaptive growth and enhances chemical carcinogenesis in the colon,[3] although extreme weight loss can limit these effects.[4] Similar phenomena in man would place a large group of young patients at increased risk of developing colorectal carcinoma, even if insufficient time has yet elapsed to reveal the threat. The operation probably reached its peak of popularity in the late 1970s. To date at least two patients have been reported with subsequent colorectal neoplasia: one developed a carcinoma[5] and the other two tubulovillous adenomas.[6]

As experimental hyperplasia precedes and pre-disposes to neoplasia in the large intestine,[7] we have looked for evidence of permanent adaptation in the rectum of patients with previous JIB. The technique has involved measuring crypt cell production in mucosal biopsies maintained in short term organ culture.

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Methods

PATIENTS AND BIOPSIES

Nine patients were studied from among those who had undergone JIB for morbid obesity in Bristol over the last 11 years. The standard operative procedure comprised end-to-side bypass between 35 cm proximal jejunum and 15 cm terminal ileum. Patients were reviewed with particular regard to weight change and current bowel habit. Seven controls who were attending outpatients for the treatment of haemorrhoids, and had no known colonic disease, were also studied. Patients gave informed consent, and approval was obtained from the appropriate health authority. A sigmoidoscopy was carried out and two biopsies were taken from the upper rectum (10–12 cm from the anal verge) using Patterson biopsy forceps (Down Bros). Samples were gently washed before being transported in ice cold Leibowitz L-15 medium (GIBCO).

ORGAN CULTURE

Using an aseptic technique previously described,[8] biopsies were gently orientated and flattened with the mucosal surface uppermost on a Metricell filter. They were then divided into 2–3 mm² explants and trans-ferred onto stainless steel grid platforms placed at

one end of the well of a Lux multiwell culture dish (Flow Labs). The culture medium contained CMRL-1066 (GIBCO) 91·5% v/v, fetal calf serum (GIBCO) 5%, L-glutamine (200 mM) 2%, Hepes buffer (1 M) 1%, and penicillin+streptomycin (10 000 IU/ml) 0·5%. Glucose (20 mg), hydrocortisone 21-hemisuccinate (1 mg), and insulin (1 mg) were added to each 20 ml of medium, which had a final pH of 7·2–7·3. Each well contained 3 ml of medium and was rocked at 3 cycles/min at 37°C in a Bellco controlled atmosphere chamber containing 95% oxygen and 5% carbon dioxide. Explants were established in organ culture within 60–75 minutes of obtaining the biopsy.

CRYPT CELL PRODUCTION RATE (CCPR)
A culture time of 15 h was selected on the basis of a small pilot experiment (see below). The stathmokinetic agent vincristine 0·6 µg/ml (Oncovin, Eli Lilly, Basingstoke) was added to each culture well to bring about metaphase arrest within the intestinal crypts.[9] Explants were removed after one, two, and three hours of culture (between three to five explants/time point). Samples were fixed in Carnoy's fluid for four hours and stored in 70% alcohol. Specimens were later stained by the Feulgen method for DNA, and individual crypts in each sample were separated by microdissection.[10] The number of arrested metaphase figures was counted in 20 crypts/specimen, and the mean value was plotted against time after vincristine administration. The CCPR was calculated by linear regression analysis.[9]

In the pilot experiment rectal biopsies from two controls and one patient with JIB were cultured over 24 hours in order to determine the optimum length of culture before measuring CCPR. Vincristine (0·6 µg/ml) was added to successive wells at three hour intervals from three to 21 hours of culture. Specimens were removed three hours after first exposure to vincristine, and the mean number of metaphase arrests/crypt at each sampling time was determined as previously described.

CRYPT MORPHOMETRY
In four controls and five patients with JIB a modular image analysing system (Kontron MOP/AMO2), consisting of a glass evaluating tablet and a stylus linked to a microcomputer, was used to measure the crypt width and crypt area in 30 crypts per specimen.[11] Camera lucida drawings were made of the microdissected crypts using a Leitz Dialux 20EB light microscope with a drawing tube attachment, and measurements were made on the evaluating tablet.

HISTOLOGY
Five micron sections were cut from the biopsies before and after the period of organ culture. Slides

Table 1 Patients with jejunoileal bypass (JIB)

Age (yr)	Time since JIB (yr)	Wt pre-JIB (kg)	Wt post-JIB (kg)	Daily bowel actions (n)	Rectal CCPR (mean (SE))
49	11	146	77	4	12·0 (1·2)
46	1	146	77	8	16·7 (2·4)
33	0·5	222	185	9	13·1 (2·5)
50	7	141	93	4	12·7 (0·4)
40	2	159	102	3	8·2 (1·0)
43	3	254	146	10	16·7 (2·9)
31	3	124	67	6	10·8 (1·6)
44	0·2	149	117	7	13·4 (0·1)
31	0·5	149	105	6	12·1 (1·4)

CCPR=crypt cell production rate (cells/crypt/hour).

Table 2 Details of control patients

Age (yr)	Wt (kg)	Rectal CCPR (mean (SE))
56	76	7·4 (1·4)
17	140	4·4 (0·6)
38	70	5·7 (0·7)
22	57	5·3 (0·6)
56	73	7·1 (0·1)
40	64	5·4 (0·6)
29	89	8·6 (0·6)

CCPR=crypt cell production rate (cells/crypt/hour).

were stained with haematoxylin and eosin and were examined by routine light microscopy for evidence of inflammation or dysplasia.

STATISTICAL ANALYSIS
Student's t test was used to compare body weight, morphometric indices, and CCPR regression lines.

Results

PATIENTS (Tables 1, 2)
Patients with jejunoileal bypass (median age 43 years; range 31–50) had undergone operations a median two years before biopsy (range two months–11 years). All the patients had lost weight as a result of the operation from a preoperative value of 165 (43) kg (mean (SE)) to 107 (39) kg, a mean decline of 35%. Patients had between three to 10 loose bowel actions/day. Controls were of similar age (median 38 years, range 17–56) but weighed 30% less at the time of biopsy (81 (28) kg, NS).

PILOT EXPERIMENT (Fig. 1)
After an initial 'shock' phase in culture lasting nine hours, the mean number of arrested metaphases/crypt at each three hour period of culture rose to a maximum of 16·25 in controls and 48·5 in JIB when vincristine was added at 15 hours; values then declined to 21 hours. Thus 15 hours was selected as

the optimum length of culture. Values in JIB patients exceeded those of controls at all time points.

CRYPT CELL PRODUCTION RATE (CCPR)
Both groups showed near linear rates for accumulation of arrested metaphases (Fig. 2). Among controls the mean CCPR was 6·23 (1·49) cells/crypt/h (SE). Among patients with JIB the value was 105% higher (12·80 (2·67): $p<0.001$). There was virtually no overlap between the groups (Fig. 3).

CRYPT MORPHOMETRY
After JIB crypts were 18% wider (0·141 (0·048) mm v 0·127 (0·036) mm) and their area was 29% greater (0·079 (0·031) mm^2 v 0·061 (0·020) mm^2). Neither of these differences reached statistical significance.

HISTOLOGY
Examination of histological sections obtained before culture showed no evidence of proctitis nor of dysplasia. After 18 hours culture there was good preservation of the normal mucosal architecture.

Discussion

We have used the technique of organ culture to study adaptation of the human large intestine. Patients who had undergone jejunoileal bypass for morbid obesity had crypt cell production rates that were more than twice as rapid as controls and these rates still remained raised up to 11 years postoperatively. As in the rat, therefore, adaptation of the shortened gut develops rapidly, and persists indefinitely in man.[7]
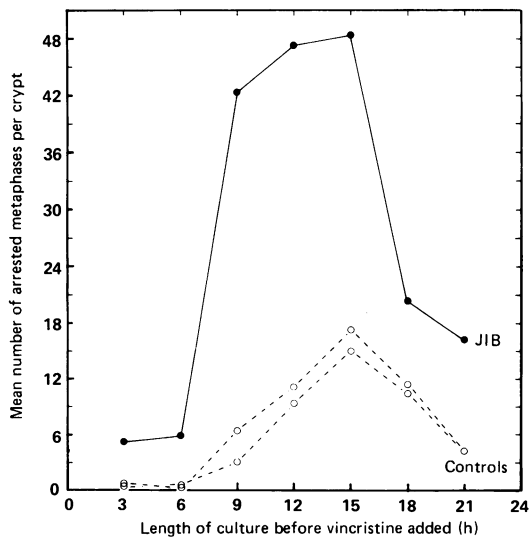

Fig. 1   *Pilot experiment to determine optimum length of culture before measuring crypt cell production rate (CCPR).*
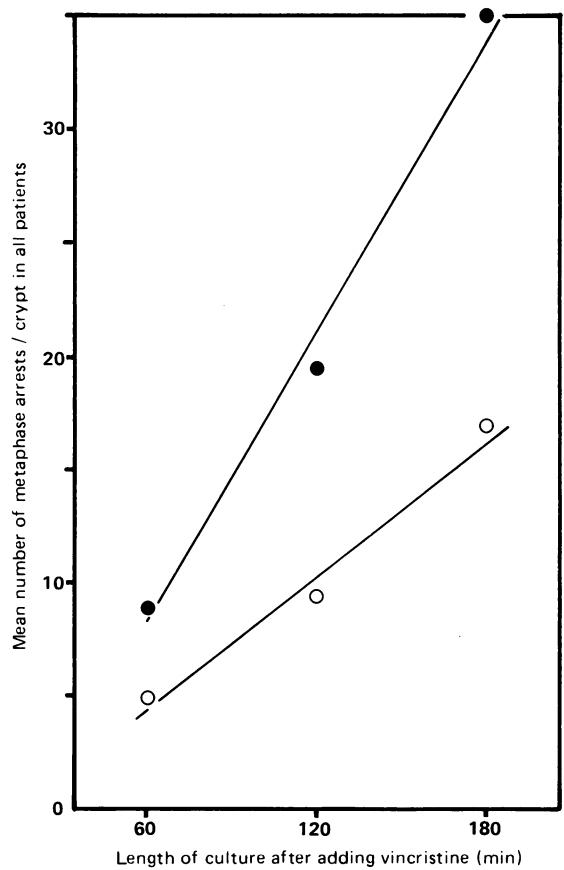

Fig. 2   *Mean crypt cell production rates (CCPR) for the two groups. JIB – ●: controls – ○.*

Crypt cell production rate changes of similar magnitude were seen in the colon of rats killed six months after an equivalent JIB.[3] The extent of the response in man is remarkable, because the rectum (not the colon) was sampled and adaptation is maximal immediately adjacent to the missing segment of bowel.[7] Modest increments in the width and surface area of crypts did not attain statistical significance, probably because of the small numbers sampled in this part of the experiment.

The mechanism of colonic hyperplasia after JIB has not been established. The marked increase in frequency of bowel actions may reflect greater amounts of irritative bile salts reaching the colon because of the short length of small bowel remaining in continuity. Biliary (and pancreatic) secretions are trophic,[12] mitogenic,[13] and carcinogenic[14] to colonic mucosa. Humoral stimuli are a less likely explanation. Enteroglucagon concentrations rise by 1600% after JIB[15] and might contribute to the postoperative
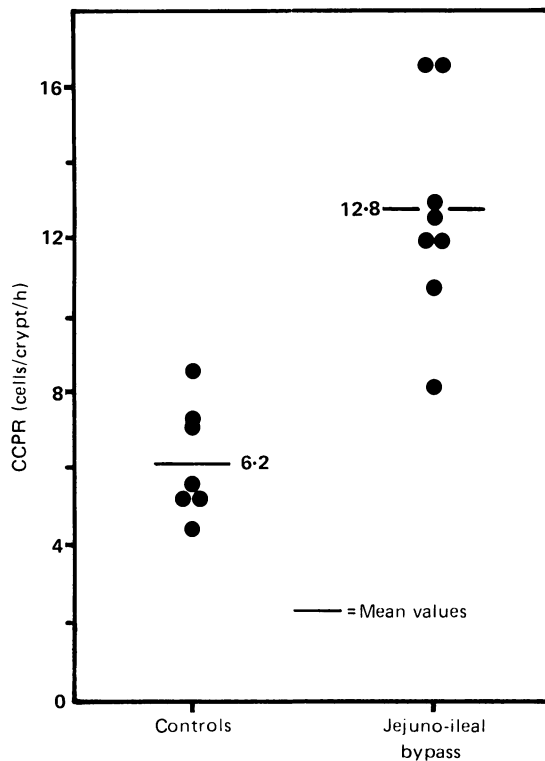
Fig. 3 *Crypt cell production rates (CCPR) in seven controls and nine patients with jejunoileal bypass.*

hyperplasia in the residual functioning small bowel,[16] but enteroglucagon probably does not modulate colonic adaptation.[17] Certainly raised concentrations of circulating enteroglucagon did nothing to overcome the inhibitory effects of colonic defunction on cell kinetics and carcinogenesis in our recent experiment.[18]

In experimental animals the mucosal hyperplasia that follows JIB is a potent promoter of colorectal carcinogenesis.[3] Could the same hold true in man? In support, we have used organ culture to show increased CCPR in rectal biopsies of patients with active ulcerative proctocolitis, a known premalignant condition.[19] Moreover these increments were more modest than those observed after JIB (40% *v* 105%), although they were still detectable in quiescent bowel. Against an increased risk of cancer is the profound weight reduction achieved after a successful JIB. Data from the American Cancer Society indicate that colorectal carcinoma (like many cancers) is more common in the obese and less common in those who are underweight.[20] Patients with a JIB also eat less after the operation,[21] and the reduction in luminal contents would be expected to

cause relative colonic hypoplasia;[22] the type of food ingested might also change substantially. In rats a 99% JIB causes such extreme weight loss that the cocarcinogenic effect of the operation is lost.[4]

In man the stimulatory effect of postoperative hyperplasia on 'spontaneous' carcinogenesis could be balanced by the inhibitory effects of weight loss and altered diet. It is reassuring to note that no evidence of colonic dysplasia could be found in a recent report of 38 patients studied at a mean of 11·5 years after JIB for morbid obesity.[5] Nevertheless we believe that it is premature to discount the longterm risk of colorectal cancer, and patients with JIB should be kept under surveillance indefinitely.

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