The cell cycle time in the flat (avillous) mucosa of the human small intestine

NICHOLAS WRIGHT, ALEXANDER WATSON, ADRIAN MORLEY,
DAVID APPLETON, JANET MARKS, AND ADRIAN DOUGLAS

From the Departments of Pathology, Medical Statistics, Dermatology, and Medicine, University of Newcastle upon Tyne

SUMMARY A hyperproductive mucosal state in gluten-sensitive enteropathy has been proposed on the basis of an elevated mitotic index, but this parameter is dependent on the mitotic duration when used as an index of proliferative status. The mitotic duration was therefore measured in two control patients with normal villous mucosae and in two patients with the flat avillous mucosa of untreated gluten-sensitive enteropathy, using two different stathmokinetic techniques with vincristine. No significant difference in mitotic duration was found but values obtained for cell cycle time showed a halving in the flat mucosae. An increased rate of cell production in the small bowel mucosa of untreated gluten-sensitive enteropathy is thus confirmed.

In a further article (Wright, Watson, Morley, Appleton, and Marks, 1973) we report an increased incidence of dividing crypt epithelial cells in the flat avillous mucosa of untreated gluten-sensitive enteropathy, so confirming earlier reports (Padykula, Strauss, Ladman, and Gardner, 1961; Yardley, Bayless, Norton, and Hendrix, 1962). On the basis of such findings the crypt cells in gluten-sensitive enteropathy have usually been supposed to have a shortened intermitotic or cell cycle time. But Winawer and Lipkin (1971) have recently cautioned that an increased mitotic incidence in gluten-sensitive enteropathy connotes a decreased cell cycle time only if the duration of mitosis remains constant. Under steady state conditions, the mitotic index (I_m) is related to the mitotic duration (t_m) and the cell cycle time (T_c), thus

$$I_m = \frac{t_m}{T_c} \quad \dots (1)$$

An increase in mitotic index could therefore be caused by an increase in mitotic duration as well as by a reduction in cell cycle time. It is clearly necessary to make direct measurements of t_m and T_c . In this paper we report measurements of these parameters in two patients with morphologically normal small bowel mucosa, and in two patients with the flat mucosa of untreated gluten-sensitive enteropathy.

Materials and Methods

The full experimental procedure was explained in detail to the four subjects before informed consent was obtained. Two procedures, both employing the metaphase-arresting properties of vincristine (Oncovin, Lilly), were used to measure T_c and t_m in these four patients.

Patient A was a male aged 61, with a clinical diagnosis of rosacea and leg ulceration resulting from stasis.

Patient B was a male aged 37 who had suffered from dermatitis herpetiformis for over 10 years, and who had recently presented with a megaloblastic anaemia due to folic acid deficiency. He had been treated with Dapsone and had been eating a normal diet.

On day 1 at 13.50 hr a peroral biopsy was taken from the duodenojejunal region of each patient using a modified Crosby capsule. On day 2 the capsule was again passed to the same position under radiological monitoring. Then at 11.00 hr vincristine sulphate was administered by rapid intravenous infusion in a dose of 0.045 mg per kg body weight. At 13.50 hr the capsule was fired and a mucosal biopsy obtained.

Patient C was a male aged 61, who was being investigated for diarrhoea.

Patient D was a female aged 55 with a clinical diagnosis of untreated coeliac disease.

In these two patients a different procedure was used. The capsule of a Quinton hydraulic multiple suction biopsy machine was passed into the jejunum and a base-line biopsy obtained at 1100 hours. Vincristine sulphate was immediately given in the same dose as before by rapid intravenous injection. Serial mucosal biopsies were then obtained at approximately 15-min intervals.

All biopsies were orientated under the stereomicroscope and spread on glass, fixed in 10% neutral buffered formalin, and postfixed in Carnoy's solution. The tissue was wax embedded, and serial sections were cut at a thickness of 3 μ m and stained with Harris's haematoxylin. The counting techniques used for analysis of the biopsies from patients A and B are described in a further paper (Wright *et al.*, 1973). In patients C and D, at least 3000 crypt nuclei were counted in each biopsy. Only optimally sectioned crypts, ie, those with the base, middle, and mouth present in the plane of section, were analysed.

Results and Interpretation

In patient A both biopsies showed only finger- and narrow leaf-shaped villi, while histological examination showed no notable abnormality. Patient B had a 'flat' mucosa with a cobblestone appearance in both samples and the histological appearances were characteristic of untreated gluten-sensitive enteropathy.

The cytokinetic data are summarized in the table. The native or resting mitotic index ($I_{m(o)}$), obtained on day 1 in each case, was 2.36% in patient A and 5.09% in patient B ($P < 0.001$). In these biopsies all phases of mitosis were counted. In the biopsies after vincristine taken after 2.5 hr in each case, these values were raised to 7.75% and 14.81% respectively. In these samples no anaphases or telophases were seen and metaphase arrest was concluded to be complete. Only prophase and metaphases were therefore counted. Degeneration of arrested metaphases was not encountered (Aherne and Camplejohn, 1972), probably as a consequence of the short arrest period.

The mitotic duration t_m can be calculated from the

		Total Cells Counted	Cells in Mitosis	Mitotic Index (%) (I_m)
Patient A				
Biopsy 1	$I_{m(o)}$	6781	160	2.36
Biopsy 2	$I_{m(t)}$	9048	701	7.75
Patient B				
Biopsy 1	$I_{m(o)}$	8421	429	5.09
Biopsy 2	$I_{m(t)}$	15 288	2264	14.81

Table Summary of cytokinetic data in patients A and B

following expression (Lala, 1971), assuming steady state conditions.

$$t_m = \frac{I_{m(o)} \cdot t}{(I_{m(t)} - I_{m(o)})} \quad \dots (2)$$

where t is the period of metaphase or stathmokinetic arrest, in each case 2.5 hours. From this equation the mitotic duration in patient A was 1.09 hr and in patient B, 1.31 hr ($P > 0.05$). It cannot be stated therefore that there is a real increase in t_m .

The cell cycle time can now be calculated from equation 1. In this calculation it is necessary to incorporate Tannock's constant (Tannock, 1967), a factor by which I_m must be multiplied to compensate for the spatial distribution of mitoses and interphase small intestinal mucosal cells; this is because mitotic cells move towards the crypt axis, and would therefore be counted preferentially in sections of the crypt. Measured by the method of Tannock (1967) the constant was 0.71 in patient A and 0.72 in patient B.

Now the apparent cell cycle time ($T_{c(a)}$) is the cell cycle time if all the crypt cells are proliferating, ie, if the growth fraction or proliferating population is equal to 1. $T_{c(a)}$ was accordingly found to be 64 hr in patient A and 36 hr in patient B ($P < 0.001$). The growth fraction can be approximately determined from mitotic index distribution curves (Wright *et al.*, 1973) and was found to be 0.83 in patient A and 0.61 in patient B. As noted elsewhere (Wright *et al.*, 1973) these are maximum values for the growth fraction. Such estimates of the proliferating population can be used to modify the mitotic index, so that it deals only with the crypt cells actually dividing, by substituting in the expression

$$I_m = \frac{N_m}{(N_p/N_t)N_t} \quad \dots (3)$$

where I_m is the corrected mitotic index, N_m the number of cells in mitosis, N_p the number of proliferating cells, and N_t the total number of cells (Gavasto and Pileri, 1971). The cell cycle times then become 54 hr in patient A and 22 hr in patient B.

The use of equation 2 to calculate t_m assumes not only that vincristine exerts its stathmokinetic effect immediately upon injection, but also that linear accumulation of metaphases occurs thereafter. These assumptions were investigated in patients C and D using the multiple biopsy technique.

In patient C 11 serial biopsies were obtained over a period of 150 minutes. Stereomicroscopy showed in all specimens a predominance of finger- and narrow leaf-shaped villi, with a small minority of broader leaves. Histological examination showed no abnormality. In patient D nine serial biopsies were obtained over a period of 105 minutes. All specimens showed a flat mucosa on stereomicroscopy, and the appear-

Fig 1a

Fig 1b

Fig. Metaphase accumulation lines for (a) patient C, and (b) patient D. In each case vincristine was administered at time 0, and the slopes of the regression lines represent the rate of accumulation of arrested metaphases in the intestinal mucosa. The dotted lines represent 95% confidence limits for the line.

ances of untreated gluten-sensitive enteropathy on histological examination.

The metaphase accumulation graphs for patients C and D (see fig) show that there is no apparent lag in the onset of vincristine metaphase arrest, and that a good degree of linearity is present over the experimental period. The slopes of the lines did not differ significantly ($0.05 < P < 0.06$). $T_{c(a)}$ was 58 hr in patient C and 39 hr in patient D. The corresponding values for T_c (corrected for growth fraction) were 42 hr and 21 hr respectively. In patient C t_m was 1.10 hr and in patient D 1.55 hr ($P > 0.05$).

Discussion

It is concluded that the cell cycle time in the crypt

cells of the avillous mucosa in untreated gluten-sensitive enteropathy is about half that in control villous mucosa. In patients A and B the difference between the $T_{c(a)}$ values was found to be statistically significant; presumably the difference between the T_c values is also real. It was not possible to prove statistical significance between the $T_{c(a)}$ values of patients C and D. However, when the growth fraction is incorporated into the calculation, it can be seen that again there is a halving of the cell cycle time in the flat mucosa. In each case t_m is calculated from a quotient of mitotic indices; consequently because of the larger standard errors involved it is not possible to say whether a real difference exists in t_m values between the villous control and avillous flat mucosae.

The nature of the metaphase accumulation lines suggest that the assumptions made in the first experiment were probably justified. In experimental animals Wright, Morley, and Appleton (1972) have shown that the values for mitotic duration obtained by the use of equation 2 were in close agreement with an accurate technique involving measurement from a metaphase collection graph. All the equations in the present report assume steady state and they are easily modified to allow for exponential conditions; however, there is as yet no evidence that in the hyper-productive mucosal state found in flat mucosae steady state conditions do not apply.

The values given for cell cycle time in the control mucosae are in reasonable agreement with the values obtained by Lipkin, Bell, and Sherlock (1963) using labelling techniques with tritiated thymidine. Values for cell cycle time in flat mucosae have not been reported hitherto and all measurements of the proliferative state have been indirect (Wright *et al*, 1973) and largely dependent upon mitotic indices only. The T_c values given in this paper are direct measurements and have been corrected for changes in proliferating population, and consequently are free from the several criticisms which can be levelled against other measurements of proliferative status.

The implications of this study for the mucosal dynamic state in flat mucosae are several. The decrease in cell cycle time of crypt cells indicates that even if there were no increase in the proliferative population, cell production rate would be at least doubled. In a further paper (Wright *et al*, 1973) we shall demonstrate a threefold increase in the number of proliferating cells per crypt in gluten-sensitive enteropathy; this is despite the lowered growth fraction, and is a consequence of the three-dimensional increase in crypt size. Independent evidence is available which supports these observations; Croft, Loehry, and Creamer (1968) have demonstrated an increased cell loss (in terms of

nuclear DNA) in patients with the coeliac syndrome in relapse. Trier and Browning (1970), employing an elegant *in-vitro* technique using tritiated thymidine, reported increased labelling indices and cell migration rates in flat mucosa compared with control villous mucosae. Combined with the present observation of a markedly decreased cell cycle time, the existence of a hyperproductive mucosal state in untreated gluten-sensitive enteropathy (Booth, 1970) can be regarded as proven.

We are grateful to Professor A. G. Heppleston for continued encouragement and advice. Mrs Glynis Stones rendered skilled technical assistance and the work was supported by a grant from the North of England branch of the Cancer Campaign.

References

- Aherne, W. A., and Camplejohn, R. S. (1972). On correcting the error due to metaphase degeneration in stathmokinetic studies. *Exp. Cell Res.*, **74**, 496-502.
- Booth, C. C. (1970). Enterocyte in coeliac disease. *Brit. med. J.*, **3**, 725-731.
- Croft, D. N., Loehry, C. A., and Creamer, B. (1968). Small-bowel cell-loss and weight-loss in the coeliac syndrome. *Lancet*, **2**, 68-70.
- Gavast, F., and Pileri, A. (1971). Cell cycle of cancer cells in man. In *The Cell Cycle and Cancer*, edited by R. Baserga. Dekker, New York.
- Lipkin, M., Bell, B., and Sherlock, P. (1963). Cell proliferation kinetics in the gastrointestinal tract of man. 1. Cell renewal in colon and rectum. *J. clin. Invest.*, **42**, 767-776.
- Padykula, H. A., Strauss, E. W., Ladman, A. J., and Gardner, F. H. (1961). A morphologic and histochemical analysis of the human jejunal epithelium in nontropical sprue. *Gastroenterology*, **40**, 735-765.
- Tannock, I. F. (1967). A comparison of the relative efficiencies of various metaphase arrest agents. *Exp. Cell Res.*, **47**, 345-356.
- Trier, J. S., and Browning, T. H. (1970). Epithelial-cell renewal in cultured duodenal biopsies in coeliac sprue. *New Engl. J. Med.*, **283**, 1245-1250.
- Winawer, S. J., and Lipkin, M. (1970). Proliferative abnormalities in the gastro-intestinal tract. In *Modern Trends in Gastroenterology*, vol. 4, pp. 60-72, edited by W. I. Card and B. Creamer. Butterworths, London.
- Wright, N. A., Morley, A. R., and Appleton, D. R. (1972). Variation in mitotic duration in the crypts of Lieberkuhn of the rat: a cytokinetic study using vincristine. *Cell Tiss. Kinet.*, **5**, 351-364.
- Wright, N. A., Watson, A. J., Morley, A. R., Appleton, D., and Marks, J. (1973). Cell kinetics in flat (avillous) mucosa of the human small intestine. *Gut*, **14**.
- Yardley, J. H., Bayless, T. M., Norton, J. H., and Hendrix, T. R. (1962). Celiac disease: a study of the jejunal epithelium before and after a gluten-free diet. *New Engl. J. Med.*, **267**, 1173-1179.