

form filling with better clinical information coming from nurses than from their medical colleagues.

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Measurement of morphokinetic status in experiments on intestinal adaptation

EDITOR,—The protracted debate concerning appropriate measurements of morphokinetic status in experiments on intestinal adaptation, seems to have passed Reilly *et al* (*Gut* 1995; 37: 81–6). There are several points of major concern in their paper.

(1) 'Crypt cell proliferation rate (is determined) by bromodeoxyuridine incorporation'. How? The references quoted are to BrdU in different systems and cannot be used to assess the methodology of Reilly *et al*. The measurement, called 'crypt cell proliferation (%)', is not one we recognise: could it be the percentage of BrdU labelled cells? But then, percentage of what? It is surely no longer acceptable in such studies to be allowed to give such measurements without giving readers an indication of the target interphase nuclear population and counting statistics; of course, BrdU does not measure 'crypt cell proliferation', but only one indirect component of it – the S phase fraction. The changes in crypt depth (Table II) show that the several groups have different sized crypt populations: therefore the putative increases in labelling index in SCFA/control, saline/gastrin receptor blockade, and SCFA/gastrin receptor blockade may in fact be modified because of changes in the interphase nuclear population. Such problems are simply avoided by using the whole crypt as a denominator, as is now widely used.¹

(2) The fact that both groups receiving the gastrin blocker (saline/gastrin receptor blockade and SCFA/gastrin receptor blockade) show a significant increase in labelling index compared with saline control, and that the SCFA/gastrin receptor blockade group is significantly larger than the SCFA control receives no mention in the discussion; these differences are also reflected in the crypt depth and villous height measurements (Table II). What is going on here: does the gastric receptor antagonist itself stimulate cell proliferation?

Some light may be cast on this by the weight data: both gastrin receptor blockade groups gained significantly more weight than the control group. Food intake in the various groups is an important modulator of intestinal proliferation and was evidently not monitored: it is certainly not mentioned. Consequently, a possible interpretation is that gastrin receptor blockade increases food intake and hence intestinal cell proliferation: the addition of SCFA in this model fails to further increase cell proliferation. And to argue that other measures such as DNA and protein, support the interpretation is neither here nor there: it is difficult to support such measurements for critical work.² What is meant by 'jejunum'? Clarke pointed out many years ago that rats do not have jejunums and ileums, so what does jejunal weight mean? We assume that 'crypt death' measurements were a figment of inadequate proof reading.

It is difficult, if not impossible, to make any conclusions from this study: it will no doubt have to be repeated, monitoring food intake

or feeding rats parenterally, with more critical measurements.

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- 1 Goodlad RA. Microdissection-based techniques for the determination of cell proliferation in gastrointestinal epithelium: application to animal and human studies. In: Celis JE. *Cell biology: a laboratory handbook*. San Diego: Academic Press, 1994.
- 2 Wright NA, Alison MR. *The biology of epithelial cell populations*. Vol 2. Oxford: Oxford University Press, 1984.

Reply

EDITOR,—George Bernard Shaw once said 'England and America are two countries divided by a common language'.¹ The letter by Wright and Goodlad personifies this divisiveness. Citing their own studies of the crypt cell production rate (CCPR) as the purported 'gold standard' for measurement of intestinal proliferation, Wright and Goodlad question our measure with bromodeoxyuridine (BrdU) and wonder why we did not use their technique. Interestingly, we are not the first 'non-conformists' to incur the vent of their partisan wrath, as expressed in another vituperative letter by Goodlad 'Defective denominators or will people never learn?' (*Gastroenterology* 1995; 108: 1963). On this side of the pond, experienced investigators use tritiated-thymidine incorporation to quantify 'S' phase cells, proliferating cell nuclear antigen (PCNA), and BrdU to measure proliferating cells, or enzyme markers such as thymidine kinase or ornithine decarboxylase as indicators of cell proliferation. Flow cytometry is also used in combination with some of the staining techniques. We believe all of these methods are valid comparisons within a single experiment. There is clearly not a general agreement on a 'gold standard' for this measure. BrdU immunohistochemistry, performed in our study, is used by many to yield semi-quantitative information that directly correlates with cell proliferation in many *in vitro* and *in vivo* experimental systems. The accuracy of BrdU labelling indices to study *in vivo* proliferation in the digestive tract in rats has been clearly shown.²

We agree that BrdU immunohistochemistry is an imperfect measure of crypt cell proliferation because (similar to tritiated-thymidine) it only measures the number of cells cycling through the 'S' phase of the cell cycle. Readers of *Gut* and most gastrointestinal scientists are well aware of this characteristic. The reality remains that although imperfect, BrdU labelling indices (as well as labelling indices obtained by tritiated-thymidine or PCNA) are to date widely accepted as accurate and reliable indices of intestinal proliferation, which can be used for internal comparisons within a single experiment.

It is well accepted that labelling indices can be expressed as the number of labelled cells, divided by the total cells (labelled plus unlabelled) in the entire crypt. Many investigators multiply this ratio by 100, such as performed in our study, to express the index as a percentage value rather than a fraction.^{3–8} One of the references included in our paper clearly explains this simple calculation³ (Table I, page 700), information seemingly overlooked by Wright and Goodlad. Perhaps the confusion stems, in part, from the unintended omission of the number of crypts

counted per rat. As indicated in our report for the measurements of villous height and crypt depth, 20 well oriented crypts per animal were randomly selected for assessment of BrdU labelled and unlabelled cells. Labelling index was then calculated by counting the number of BrdU labelled cells and expressing the results as percentage of the total cells (labelled plus unlabelled) counted in the crypts assayed in each rat.

Many scientists^{4–8} choose to study intestinal proliferation by BrdU incorporation assessed and expressed as labelling index percentage such as described in our study. This sample of references is woefully incomplete but serves to strengthen our point. These publications include *in vitro* and *in vivo* studies all of which use BrdU labelling indices as a measure of intestinal proliferation in the same manner as reported in our paper. While Wright and Goodlad continue to express their bias for the CCPR technique, a search of Current Contents Article Records using the key words 'proliferation' and 'intestine' yielded 89 publications in 1995, only two of which reported use of the CCPR. While the majority may not rule in science, it is clear that the majority have not embraced CCPR.

Wright and Goodlad correctly state that 'food intake is an important modulator of intestinal proliferation.' We agree that controlled dietary intake, such as provided by total parental nutrition, gastrostomy feeding, or pair feeding is important in nutritionally oriented studies. Moreover, we have used these methods extensively in other investigations.^{9–10} These methods were not used in this study based upon pilot data (unpublished) showing no significant differences in dietary intake between the gastrin receptor blocker and control groups. Despite the results of our unpublished pilot investigations, the gastrin receptor blockade groups collectively gained more weight than the control groups in the published study. There was no observed increase in dietary intake in the gastrin receptor blockade groups, however, actual food intake was not measured and it may have been increased in the gastrin receptor blockade groups. Nevertheless, we intend to repeat these studies with controlled dietary intake.

Wright and Goodlad quibble about our use of the terms 'jejunum' and 'ileum' to describe the anatomy of the small intestine in the rat. We would like to point out that they have extensively and correctly used the terms jejunum and ileum in many of their previous publications.^{11–14} Thus a review of their own work, in addition to a standard textbook on the anatomy of the laboratory rat¹⁵ is strongly recommended.

In summary, an objective review of the literature clearly shows that there are widespread fundamental differences as to whether there is a single best measurement of cell proliferation and what that measure should be. The weight gain in our gastrin receptor blockade groups may have influenced some of the morphometric measures, and further studies with controlled dietary intake are planned. Wright and Goodlad's comments that 'rats do not have jejunums and ileums' is incorrect. To return to Shaw, 'No man fully capable of his own language ever masters another'.¹⁶

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