WINTON *ET AL.* REPLY— O'Sullivan *et al.* challenge our estimate[2] of the rate of spontaneous mutation at the *Dlb-1* locus in intestinal epithelium. We suspect that the apparent discrepancy between their results and our own may be due to the scoring of two different types of mutant clone. O'Sullivan *et al.* score the large descendant clones induced by mutation during embryonic development, whereas we are scoring the smaller clones arising from mutation of individual crypt stem cells in adult mice. Other of their criticisms seem to arise from misunderstanding. Our view has consistently been that the best way to resolve the disagreement would be for us to compare experimental material. Unfortunately, O'Sullivan *et al.* have not agreed to this course of action.

Taking their criticisms in turn: (1) Our finding of more mutations is said to be due to poor methodology: tissue damage, incomplete staining and sampling error. We refute this. In all our experiments, heterozygous mice and control B6/B6 ($Dlb\text{-}1^b/Dlb\text{-}1^b$) homozygous mice are scored blind. We have never found mutations in the homozygous controls, which argues strongly against our having scored artefacts as mutations. In addition, we obtain a linear accumulation of mutations with age in untreated mice, and consistent dose–response curves for several different mutagens: unlikely if we were scoring artefacts. We have preferred to sample a large number (12 mice) at each time point. If we were scoring descendant clones like O'Sullivan *et al.*[1], we would need to score the entire intestine because these clones are so much less frequent (three or fewer per mouse in their material). In fact, the physiological and anatomical differences between different regions of the small intestine suggest that, in mutation experiments, these regions may be best treated separately[6].

The standard deviations of our measured rates are due partly to Poisson variation associated with counting, and partly due to variation between animals. The contribution of Poisson variation to the rates at later ages is quite small, since these are based upon relatively large counts. Of course, at younger ages, where mutations are less frequent, Poisson variation is a greater source of variability and there would be something to be said for sampling a larger part of the gut. However, O'Sullivan *et al.* are wrong in suggesting that such variation is excluded by counting the whole gut. The precision of their method, like ours, is limited by the number of counted events upon which the estimates are based. They give us no information about this. Their 'minuscule' standard deviations may or may not be impressive; it must be remembered that these apply to much smaller rates. A fairer comparison would be provided by the coefficient of variation.

(2) O'Sullivan *et al.* suggest that we are scoring the components of descendant clones as separate adult mutational events. This may occasionally be true; but most descendant clones are easily recognized by the clustering of mutant villi in a small area. O'Sullivan *et al.*'s own data (Table 1 in ref. 1) show that these clones are in any case very infrequent, and therefore will seldom fall within our sampling area. More important, counting of descendant clones cannot explain our observation of the linear increase in mutations with age, which is the main point at issue.

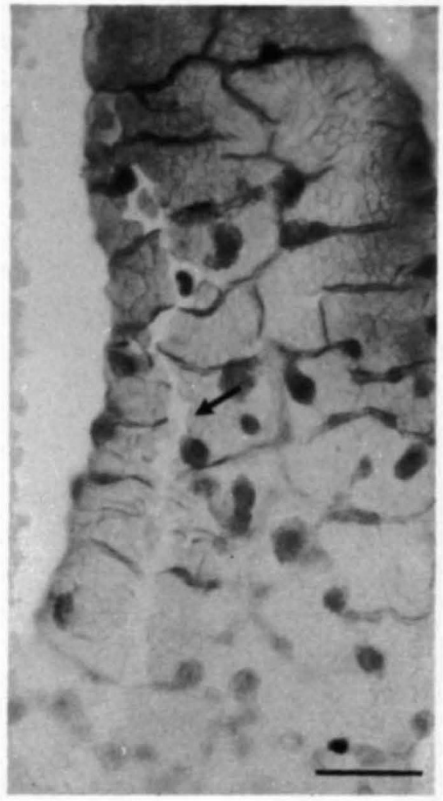

FIG. 3 A jejunal villus dissected from a small intestinal whole mount made from an adult B6 × SWR F1 mouse treated two weeks previously with ENU (50 mg kg^{-1}). The whole mount was stained with a peroxidase conjugate of *Dolichos biflorus*. Note the unstained ribbon of epithelial cells (arrow) which arises by mutation of a single stem cell and which is between one and two cells wide. Scale bar, 100 μm.

(3) O'Sullivan *et al.* suggest that our published results show large inconsistencies. The comment that our ethylnitrosourea (ENU) experiments show discrepancies of 50-fold in mutation rates shows a misunderstanding of the data. The cells in the base of the crypt turn over slowly, and it takes 12 weeks to see mutated crypts[3] as opposed to the two weeks to see mutated villus ribbons. The data are therefore not inconsistent, but as one might expect from the generally accepted model of crypt organization. There is one, much smaller, real difference between the mutation rates scored on villi and in crypts. There are two *Dlb-1* mutant phenotypes: one which results in complete loss of DBA-peroxidase binding (and thus of staining) in crypt and villus cells, and one which is expressed as loss of staining on the villi only. These occur in different ratios following treatment with different mutagens in B6 × SWR F1 mice. When these phenotypes are allowed for, there is no discrepancy between mutations scored in crypts or on villi.

The comparison with the results of Griffiths *et al.* may not be relevant because they were scoring a different epithelium in different mice with a different marker, mutation of which might well confer a selective disadvantage. Other studies in human colonic epithelium, using a mucin marker more comparable to *Dlb*, have suggested a significant accumulation of mutations[7].

In conclusion, O'Sullivan *et al.* would like to develop the *Dlb-1* assay to detect mutation of embryonic progenitor cells and the resulting descendant clones. But they have not shown that they can detect the much smaller clones which arise by mutation of adult renewing stem cells in fully developed small intestine (Fig. 3). In respect of the latter we have demonstrated dose responses in the induction of *Dlb-1* clones for ENU[2], irradiation[8] and several other mutagens including ENNG, MNNG and MNU (D.J.W. and B.P., unpublished observations). Until O'Sullivan *et al.* demonstrate that they can detect mutations occurring in adult small intestine, we suggest that the discrepancy in the estimate of the spontaneous mutation rate in adult mice between our two groups may result from the low resolution of their scoring method.

We find it surprising that O'Sullivan *et al.* challenge our data when they present so few of their own. Their published data are confined to the description of a few descendant clones, and the data they present here in relation to mutations in mice over 12 weeks of age come from three mice at one timepoint in one experiment. Even so, we take their comments seriously and are anxious to know if there is really a discrepancy due to some factor or factors which we have not identified. Unfortunately, the inadequate description of their scoring method and their reluctance to compare experimental material make it likely that the problem, if there is one, will remain unresolved.

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