

Correspondence

Measurement of intra-epithelial lymphocytes

SIR,—In their recent paper, Ferguson and Ziegler¹ conclude that the percentage mitotic index of epithelial lymphocytes is (i) non-specific for coeliac disease and (ii) increases directly with epithelial lymphocyte profile density counts such that it may be discarded in favour of the latter.

The problems that undermine such conclusions stem from the authors' use of the 'differential lymphocyte count per 100 enterocytes' as a valid measure of epithelial lymphocyte population size. This technique provides no such information. It merely gives the percentage density of randomly selected epithelial lymphocyte profiles per 100 enterocyte profiles per specimen.

Profile density counts, apart from actual changes in cell population size, are also affected by two other important variables (i) the size of the cells (as larger cells create more profiles when sectioned) and (ii) their volume of distribution.²⁻⁴ Therefore, in order to have more meaning, profile counts must always be corrected for these additional factors: the rules of morphometry are quite clear on this point.

The spurious 'increases' of epithelial lymphocytes in untreated coeliac disease, so widely acclaimed (and misinterpreted) by many workers who have based their data on crude, uncorrected profile density counts, is a relevant example. These so-called increases are due, not to any significant alteration in total population size at all, but partly to the increased proportion of large, immunoblastoid epithelial lymphocytes in this condition⁵ and to marked reductions in surface epithelial volume.²⁻⁵ Clearly, it is still not widely perceived just how much profile densities are influenced by changes in tissue volumes.

The inverse, non-linear relationship between these variables has been explored with computerised density-volume curves for epithelial lymphocytes.² These revealed rapid and disproportionate rises in profile counts as surface epithelial volumes approximated the 'flat' coeliac disease range, although the absolute number of epithelial lymphocytes in these models remained constant throughout.² Correctly carried-out analyses indicate that absolute epithelial lymphocyte populations in untreated coeliac disease lie within the low-normal range.²⁻⁵⁻⁶

Although appearing to give credence to these principles in their opening sentence, the authors nevertheless persist in using profile density counts irrespective of the errors inherent in the technique.

Thus regarding the results in six coeliac disease, and six dermatitis herpetiformis patients with abnormal mucosae, the fundamental issue is whether their correlations would hold with absolute counts of real lymphocytes: clearly, they would not.

Consider also the 17/40 normal biopsies reported to have high profile density counts. As is pointed out above, a high profile count may arise purely from an increase in cell size, as the larger the object the more profiles created in random sections. Because many of these biopsies came from patients with systemic inflammatory conditions, this possibility should have been excluded, particularly in a critical study of this type. The presence of 'normal' villi, despite statements to the contrary, does not exempt investigators from having to carefully establish mean epithelial lymphocyte sizes: failure to do so may have unexpected consequences.

There are, of course, circumstances in which epithelial lymphocyte populations are truly increased, as in the crypts of untreated coeliac patients,⁴ and in the villi both of treated coeliacs during controlled gluten challenges⁷ and in a large proportion of asymptomatic first degree coeliac relatives.⁸ In these two latter groups, the observation that mitotic activity of epithelial lymphocytes was minimal despite a marked increase in epithelial lymphocyte numbers directly contradicts the authors' main conclusions.

Although mitotic indexes exceeded the proposed arbitrary value of 0.2%⁹⁻¹⁰ in six of 16 specimens reported by these authors, the presence of 'normal' villi excludes coeliac disease, and hence the need for a mitotic index. Some very high mitotic indexes reported in certain specimens, far greater than previously published, raise doubts about technique. Neither is semithin sectioning a critical requirement technically, for with thicker slabs of tissue, the recognition of mitotic figures is aided by 'through focusing'.

While two plus hours is needed by Ferguson and Ziegler to obtain a mitotic index and profile density, in comparison we can (i) determine mitoses per 3000 epithelial lymphocytes in 45 min, and in the remaining 1.5h with a computerised image analysis system, (ii) complete quantitative measurements of surface and crypt epithelial volumes, (iii) obtain a printout of true epithelial lymphocyte diameters corrected for lost profile caps and imperfect sagittal sectioning,² from which (iv) the percentage immunoblasts, (v) flux ratios and (vi) absolute populations of epithelial lymphocytes within both mucosal compartments are easily calculated.

In conclusion, this paper has not established any direct relationship between the population size and mitotic activity of epithelial lymphocytes. On the

contrary, several studies⁴⁻⁸ firmly discredit such relationships. Neither have they disproved the view that, in the presence of a flat mucosa,⁹⁻¹¹ a high mitotic index (>0.2%) is a helpful, prospective index of untreated coeliac disease. The material studied lacked the appropriate disease controls necessary to challenge that assertion.

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Nodular necrobiosis in association with ulcerative colitis

SIR,—In 1982 we reported in this journal a cutaneous manifestation of Crohn's disease which we called nodular necrobiosis.¹ We have now observed an identical lesion in ulcerative colitis.

The patient, a woman aged 53 years, presented with a six month history of diarrhoea and rectal bleeding. Investigation, including colonoscopy, confirmed a diagnosis of ulcerative colitis extending to the mid-descending colon. There were no extra intestinal manifestations of inflammatory bowel disease but after three months she developed a solitary painful lump on the left leg. This gradually became less tender but did not alter in size or colour. On examination there was a 5 cm, purple lesion on the left lower leg which bore a close resemblance to nodular necrobiosis. Biopsy confirmed this diagnosis. The lesion has persisted despite her colitis responding well to therapy with steroid enemas and sulphasalazine.

We therefore conclude that nodular necrobiosis can occur in both Crohn's disease and ulcerative colitis.

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European survey of fertility

SIR,—The authors of the study concerning fertility and pregnancy in women with Crohn's disease¹ should have confined their investigation to the 182 matched pairs who were actually married at the time of the study. Unmarried women in general unequivocally tend to avoid conception. We miss detailed gynaecological data on whether other reasons for infertility have been excluded. The intention to become pregnant, or not, should have been taken into account. Forty patients were advised by their doctor not to conceive; 42 others deliberately refrained from becoming pregnant because of Crohn's disease. Furthermore one should