since 1965² has undoubtedly stemmed an increase in deaths from drug overdosage, and there has not been a compensatory increase in suicides by other means.³ The number of barbiturate deaths per million population, however, did not decrease as rapidly after 1965 as might have been expected from the decreases in the corresponding rates for hospital admission and for barbiturate prescribing. One factor that probably militated against such a decrease was the concomitant increase in the quantity of barbiturate dispensed with each prescription, partly because more dose units were given each time and partly because the proportion of hypnotic-strength doses increased in relation to sedative doses. In addition, patients who continued to take barbiturates may have had a greater-than-average risk of dying from an overdose because of their age or because their social circumstances made it unlikely that they would be found and admitted to hospital before they died.⁴ These conclusions are supported by the increasing death rate from barbiturate poisoning per million prescriptions and by the observations that most deaths involved barbiturates of hypnotic rather than sedative strength and occurred without the patients being admitted to hospital.

Compared with barbiturate hypnotics the benzodiazepines nitrazepam and flurazepam are at least as effective in the short term (a few days) and are more effective in the long term,^{5,6} less apt to produce dependence, and much less toxic in overdosage.^{7,8} They are, however, more expensive. Most patients in general practice can be transferred from barbiturate to benzo-

diazepine hypnotics provided that it is done gradually (over two to four weeks), and many can cope without hypnotics altogether if they are withdrawn gradually.⁹

Improvements in the hospital care of patients will not reduce the death rate from self-poisoning with drugs as much as would further limitation on the prescribing of barbiturates—that is, on the number of prescriptions and number of dose units, particularly those of hypnotic strength.

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DNA synthesis by jejunal mucosa in responsive and non-responsive coeliac disease

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Summary

DNA synthesis by jejunal biopsy specimens from patients with coeliac disease and from controls was measured by an organ culture technique. The rate of synthesis in the mucosa of patients with untreated coeliac disease was almost eight times that in normal mucosa. Patients whose jejunal mucosa remained flat despite prolonged gluten withdrawal showed a rate of DNA synthesis significantly lower than that of the untreated patients, while those whose jejunal mucosa had responded to gluten withdrawal showed a rate similar to that of normal subjects. Impaired enterocyte production in non-responsive coeliac disease may be responsible for the failure to regenerate villi after gluten withdrawal.

Introduction

The jejunal mucosa of patients with adult coeliac disease usually improves morphologically, with villous regeneration, after gluten

is withdrawn from the diet. In about 15% of cases, however, the jejunal mucosa remains flat despite gluten withdrawal. These patients often require additional treatment with corticosteroids, parenteral nutrition, or elemental diets, but even then their condition may progressively deteriorate.¹ This failure to regenerate villi might be due to impaired enterocyte production. We therefore used an organ culture technique to determine the enterocyte production rate in patients with coeliac disease.

Patients and methods

Jejunal mucosa was studied in four groups of patients: (a) 13 controls with histologically normal mucosa who had previously been suspected of having malabsorption; (b) six patients with untreated coeliac disease who had flat mucosa (subtotal villous atrophy) and malabsorption of at least two dietary components including folate; (c) seven patients whose jejunal mucosa remained flat despite prolonged documented gluten withdrawal; and (d) nine patients with coeliac disease in whom gluten withdrawal had improved jejunal morphology; when studied these patients had either normal villi or only partial villous atrophy.

Jejunal biopsy specimens were collected with the Watson-Crosby capsule as part of the clinical management of the patients. The specimens were divided into representative pieces for routine histological examination, enzyme analysis,² or organ culture.³ The investigations reported here were approved by the local ethical committee.

DNA synthesis was measured by culturing the mucosa for three hours with 135 µg ³H-thymidine (specific activity 74 Ci/g; Radiochemical Centre, Amersham). The tissue was homogenised and DNA selectivity precipitated with cadmium chloride.⁴ The radioactivity was counted in triton-toluene scintillant with a Beckman LS 250 scintillation counter. For each patient DNA synthesis was measured

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in at least three pieces of mucosa, which were cultured separately. Total DNA in tissue homogenate and culture medium was assayed fluorimetrically by the method of Le Pecq and Paoletti.⁵ Results were expressed as cpm incorporated/3 hours/ μ g total (tissue and medium) DNA. Results were analysed by the Wilcoxon test for two samples.

Results

The figure shows the rates of incorporation of tritiated thymidine into DNA by jejunal mucosa in the four groups. Patients with untreated coeliac disease had a significantly higher rate of DNA synthesis (mean ($\pm$ SE of mean) 92 ± 16 cpm/ μ g DNA) than the controls (13 ± 0.7 cpm/ μ g DNA; $P < 0.01$). The patients whose jejunal mucosa remained flat despite gluten withdrawal showed a rate of synthesis (34 ± 1.1 cpm/ μ g DNA) significantly lower than that of the untreated coeliac group ($P < 0.05$).

The treated patients who had responded morphologically to a gluten-free diet also showed a rate (27 cpm/ μ g DNA, SEM 2.6) significantly lower than that of the untreated coeliac group ($P < 0.05$); their rate was not significantly different from that of the controls. The three patients who showed a partial response—that is, their biopsy specimens showed partial villous atrophy—had higher rates of DNA synthesis than the patients who responded more completely and achieved morphologically normal villi.

Incorporation of ^3H -thymidine into DNA by mucosa from four groups of patients. Rate of synthesis for mucosa from treated responsive group was not statistically different from that of control group.

Discussion

Our findings indicate that biochemically there are two forms of adult coeliac disease. The more usual form is characterised by subtotal villous atrophy and an enhanced rate of DNA synthesis and, presumably, an enhanced rate of enterocyte production. The other form is also characterised by subtotal villous atrophy but the rate of DNA synthesis is not enhanced to the same extent.

Before correlating these results with previous findings we must consider the possible reasons for the differences in the rate of ^3H -thymidine incorporation into DNA. It is unlikely that the increased numbers of lamina propria cells contribute appreciably to the enhanced DNA synthesis, as autoradiograms show that nearly all the cells incorporating radioisotopes into their nuclei are found in the crypt cells of the biopsy; very few interstitial cells are labelled.³ Possibly differences in in-vitro DNA synthesis might reflect a lack of tissue folate in the coeliac mucosa. Nevertheless, we used a complex medium containing folic acid,³ and in-vivo and in-vitro supplementation with folic acid did not affect the rate of DNA synthesis (P E Jones and T J Peters, unpublished result).

The observation that typical patients with coeliac disease show enhanced enterocyte production rates agrees with the findings of morphological studies showing crypt cell hyperplasia⁶ and with intestinal perfusion studies, in which the rate of DNA loss into the lumen—the enterocyte shedding rate—was measured. Both Pink *et al*⁷ and Barry and Read⁸ have shown that DNA loss into the small bowel lumen in patients with untreated coeliac disease may be increased compared with that in control subjects. The preliminary results of the Bristol group's perfusion studies indicated that there are two subgroups of patients with coeliac disease.⁸ One group has a low rate of DNA loss, suggesting relative crypt hypoplasia, and this group probably corresponds to our group of patients with non-responsive coeliac disease.

The reason for the non-responsive patients' failure to show such an enhanced rate of DNA synthesis remains unknown, but if the jejunal mucosa is to regain normal architecture the rate of enterocyte production must, at least temporarily, exceed the rate of enterocyte loss into the lumen. The reduced rate of DNA synthesis suggests that the rate of enterocyte production is reduced, and this abnormality may be responsible for the failure to regenerate villi.

At present non-responsive coeliac disease can be diagnosed only when the mucosa has failed to improve after gluten withdrawal. This means that several months may have passed before the failure to respond can be shown and more effective treatment applied. Possibly measurements of DNA synthesis may be used to predict response to gluten withdrawal. Prospective studies are in progress to answer this question.

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