

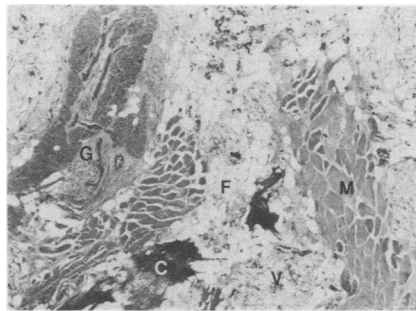
LETTERS TO THE EDITOR

Histomorphometry and immunohistochemistry of beef sausages

There has been recent popular concern that the transmissible agent of bovine spongiform encephalopathy (BSE) might enter the human food chain through the incorporation of brain or spinal cord into beef products.¹ Although the risk to consumers of acquiring spongiform encephalopathy in this way must be miniscule,² central nervous tissue is now banned from such foodstuffs.³ Methods of carcass stripping, however, mean that rigorous exclusion of all nervous tissue from meat intended for products such as sausages might be difficult to achieve (personal communication). I therefore undertook a histomorphometric and immunohistochemical study to determine whether central nervous tissue could be detected in the beef sausage and, if so, to what extent.

Three beef sausages were obtained, one from a local butcher (specimen A) and two from well known supermarket chains (specimens B and C). Three 3 mm blocks of tissue were taken from each sausage, fixed in 10% neutral buffered formalin, and processed in a routine manner as for surgical biopsy material. Paraffin wax sections (5 µm) were cut and stained with haematoxylin and eosin (figure) and with a rabbit anti-cow antibody to glial fibrillary acidic protein (GFAP) using an indirect immunoperoxidase technique (Dakopatts). One thousand points were counted in each stained section in random microscope fields using a ×6.3 objective and an eyepiece graticule. Results are expressed in the table. No GFAP positive cells were seen in any of the tissue sections.

The results are reassuring for those concerned about transmission of the BSE agent



Histology of the beef sausage: M = skeletal muscle; F = fat; V = vegetable material; G = glandular tissue; C = cartilage. (Haematoxylin and eosin.)

through the human food chain. GFAP is a reliable marker of astroglial cells,⁴ working well in a variety of species, including bovine tissue (Dakopatts' data sheet). In my experience results are acceptable, even in necropsy tissue showing some degree of autolysis. Its absence effectively excludes the presence of central nervous tissue.

Of much greater concern for public health is the morphometric demonstration of very high proportions of fat in all these specimens. The high concentration of saturated fat in the British diet is a well known factor in the cause of atherosclerosis. Sausages are clearly an important source of "hidden" fat. It seems, then, that the demented life-long beef sausage consumer is unlikely to be a victim of the BSE agent. One should perhaps, rather suspect the more mundane sequelae of cerebral atherosclerosis.

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- Holt TA, Phillips J. Bovine spongiform encephalopathy. *Br Med J* 1988;296:1581-2.
- Baker HF, Ridley RM. Bovine spongiform encephalopathy. *Br Med J* 1988;297:133.
- Ministry of Agriculture, Fisheries and Food. *Meat products and spreadable fish products regulations*. London: HMSO, 1984.
- Duffy PE. *Astrocytes: normal, reactive and neoplastic*. New York: Raven Press, 1983.

Composition of beef sausages by histomorphometry (percentage section area)

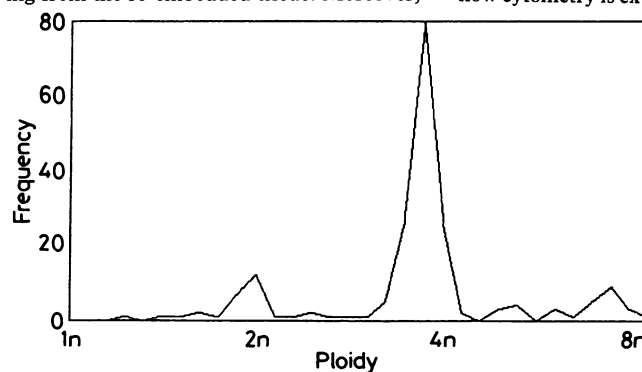
Specimen	Vegetable material	Fat	Skeletal muscle	Fibrous tissue	Cartilage	Other tissue
A	40.5	27.2	13.3	13.0	2.7	3.3
B	54.3	27.7	9.5	8.5	0	0
C	59.3	21.9	3.2	13.8	1.8	0

DNA ploidy in adenocarcinoma in situ of the uterine cervix

Ploidy studies of invasive cervical adenocarcinoma have been reported,¹ but little research has been done in the area of the glandular precursors of adenocarcinoma. We examined the ploidy patterns in three histologically confirmed cases of pure adenocarcinoma in situ (AIS) and report our preliminary observations.

Slides and paraffin wax blocks from each of three pure cases of AIS were examined. In each case one slide was chosen which showed a high concentration of AIS separate from any squamous epithelium or squamous abnormality. By comparing the section with the tissue profile of the block, the focal AIS lesions could be identified and extracted for

re-embedding. Tissue orientation was maintained throughout this procedure. Sections of 50 µm thickness were cut for further processing from the re-embedded tissue. Moreover,



Ploidy/frequency distribution graph. Two cases of AIS showed prominent peaks in the tetraploid region while one case showed a typical diploid distribution.

a 5 µm section stained with haematoxylin and eosin was cut to confirm the presence of pure AIS.

Cell nuclear suspensions were prepared according to the method of Hedley.² Two 50 µm thick sections were dewaxed in xylene and rehydrated through several changes of alcohol of decreasing concentration. Sections were then washed in two changes of distilled water and transferred to 3 ml of a solution of 0.5% pepsin in 0.9% saline (pH 1.5) at 37°C for 30 minutes. At the end of this time the pepsin digestion was stopped by the addition of 10 ml of 0.9% saline. The resultant suspension was passed through a 100 µm mesh and the filtrate was spun down for 10 minutes at 1500 rpm. The supernatant was removed and resuspended in enough 0.9% saline to give a concentration of 1 million cell nuclei/ml. The suspension was centrifuged on to microscope slides using a cytospin microcentrifuge (Shandon).

Cytospin preparations were stained by a Feulgen stain method.³ Measurement of DNA content of the stained cytospin preparations was carried out using an MD-20 image analysis system (Wild Leitz). Corrections were made to take account of the slide background and the inherent glare from the microscope optics. At least 200 diagnostic nuclei were measured from each specimen with selection being restricted to elliptical nuclei typical of AIS. Moreover, 40 lymphocyte nuclei were measured from each specimen as an internal diploid control. The modal integrated optical density (IOD) obtained for the lymphocytes in each specimen was taken as the diploid IOD value for that case. Using this value the raw IOD values for the diagnostic cells were converted to relative DNA content value—that is, relative to the diploid value—and displayed as ploidy/frequency distributions. In two cases (figure) there was a prominent peak in the tetraploid region. The other case showed only a diploid distribution.

Fu *et al* examined the ploidy and nuclear morphometric features of invasive cervical adenocarcinoma and related these to prognosis.¹ This group also looked at ploidy in human papilloma virus infection and in squamous CIN 1-3.⁴ We know that only one third of adenocarcinomas in the endometrium are aneuploid. Thus we were intrigued by the DNA content of cervical AIS. The technique we used offered advantages over conventional flow cytometry. Firstly, it allowed specific areas of interest to be examined. Contaminating squamous abnormalities, which are present in a high proportion of cases of AIS,⁵ could also be excluded. The technique also allowed for the study of whole nuclei, and errors seen when using 5 µm Feulgen stained sections were minimised. Moreover, obtaining material for flow cytometry is extremely difficult as AIS is

a pathological diagnosis and most material has been already fixed and processed. The technique's main disadvantages are that it is labour intensive and the large number of cells examined by a flow cytometer cannot be examined with this method. A near diploid aneuploid peak might not be resolved with this technique either. It will be interesting to examine further cases of AIS to determine if any show definite aneuploidy.

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- 1 Fu YS, Hall TL, Berek JS, Hacker NF, Reagan JW. Prognostic significance of DNA ploidy and morphometric analyses of adenocarcinoma of the uterine cervix. *Anal Quant Cytol Histol* 1987;9:17-24.
- 2 Hedley DW, Friedlander ML, Taylor IW, Rugg CA, Musgrove EA. Method for the analysis of cellular DNA content of paraffin-embedded pathological material using flow cytometry. *J Histochem Cytochem* 1983;31:1333-5.
- 3 Schulte E. Haematoxylin and the Feulgen reagent in nuclear staining. In: Boon ME, Kok LP, eds. *Standardization and quantitation of diagnostic staining of cytology*. Leiden, The Netherlands: Coulomb, 1986:15-26.
- 4 Fu YS, Reagan JW. Benign and malignant epithelial lesions of the uterine cervix. In: Bennington JL, ed. *Pathology of the uterine cervix, vagina and vulva*. Philadelphia: WB Saunders Company, 1989:248.
- 5 Jaworski RC, Pacey NF, Greenberg ML, Osborn RA. The histological diagnosis of adenocarcinoma in situ and related lesions of the cervix uteri. *Adenocarcinoma in situ*. *Cancer* 1988;61:1171-81.

Lymphocytic gastritis and coeliac disease

Lymphocytic gastritis is a recently described histopathological form of gastritis that is characterised by a distinct increase in lymphocytes in the surface and foveolar epithelium.¹ It is commonly associated with a characteristic endoscopic picture featuring erosions and prominent folds (varioliiform gastritis).¹ The incidence of lymphocytic gastritis in gastric biopsy material is about 4%.² A similar increased density of intraepithelial lymphocytes in the small intestinal mucosa is also a typical feature of coeliac disease. The pathogenesis of lymphocytic gastritis is unknown, but it has been suggested that it may represent an immunological response to some local antigen. Dixon *et al* found serological evidence of *Helicobacter pylori* infection, even in cases where bacterium was not detectable in biopsy specimens, and suggested that *H pylori* might be the antigen.² We evaluated the numbers of intraepithelial lymphocytes in biopsy specimens from the gastric mucosa of patients with adult coeliac disease and from controls with no evidence of coeliac disease and studied the presence of *H pylori* in these specimens.

The series comprised all adult patients with coeliac disease diagnosed between January 1 1986 and June 30 1987. There were 30 such patients, from 27 of whom—mean (SD) age 46 (14)—gastric biopsy specimens were taken simultaneously with duodenal biopsy specimens before any treatment. Both the antrum and the corpus were biopsied in 25 cases, while in the remaining two the specimen represented only the antral mucosa.

Intraepithelial lymphocytes/100 epithelial cells in entire groups of coeliac patients and controls and according to *H pylori* state (median; range within parentheses)

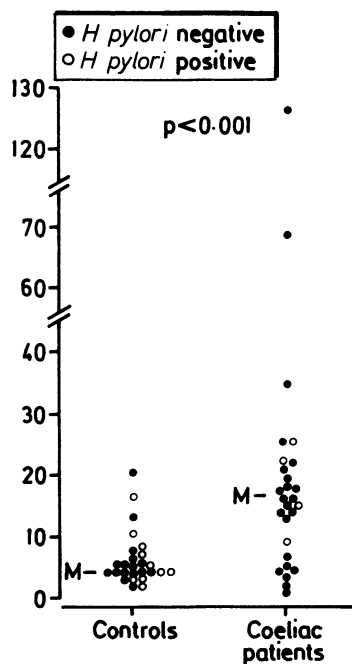
	Patients (n = 27)	Controls (n = 27)	Significance
Antral mucosa:			
All	16.3 (1.6-127.3)	4.3 (2.0-20.6)	p < 0.001
Positive	18.8 (9.1-25.3)	4.3 (2.1-17.3)	
Negative	16.3 (1.6-127.3)	4.7 (2.0-20.6)	
Body mucosa:			
All	12.4 (3.1-53.9)	4.8 (2.0-66.1)	p < 0.001
Positive	16.5 (16.3-17.9)	5.9 (2.0-66.1)	
Negative	9.9 (3.1-53.9)	4.2 (2.0-13.6)	

The groups were compared using the Mann-Whitney U test. There were no significant differences in lymphocyte counts between the *H pylori* positive and negative patients among the coeliac patients and controls.

The control material consisted of 27 consecutive age and sex matched patients without any peptic ulcer, gastric resection, or malignancy and with histologically normal duodenal mucosa, who were examined for upper abdominal complaints. All but one of control patients had had an antral biopsy performed and all but one a corpus biopsy.

Sections stained with haematoxylin and eosin were coded and studied blind in the absence of any information on the state of the duodenal mucosa or the clinical diagnosis. The numbers of lymphocytes and epithelial cells were counted over an uninterrupted length of the surface and foveolar epithelium. One hundred to 200 cells were counted and the results expressed as lymphocytes per 100 epithelial cells.² Modified Giemsa stain² was used to show the presence of *H pylori* in 46 cases and haematoxylin and eosin stain in eight.

The count of intraepithelial lymphocytes was significantly higher in the coeliac patients than in the controls in both the antral and the body mucosa (table, figure). There were four *H pylori* positive patients among the coeliac cases and 10 among the controls, a non-significant difference (χ^2 test). There were no significant differences in the counts of intraepithelial lymphocytes between the *H pylori* positive and negative coeliac patients or the *H pylori* positive and negative controls (table, figure).



Numbers of intraepithelial lymphocytes (IEL)/100 epithelial cells in the antral mucosa of coeliac patients and controls. M = median.

The findings suggest that increased amounts of intraepithelial lymphocytes are present in the gastric mucosa of coeliac patients. It has been suggested that 30 intraepithelial lymphocytes/100 epithelial cells is the minimum figure for the diagnosis of lymphocytic gastritis,¹ and there were four (14.8%) such cases among the coeliac patients and one (3.7%) among the controls. Endoscopic examination showed nodular erosions at the antrum-corporum border in the control patient with a high number of intraepithelial lymphocytes, while none of the cases with coeliac disease showed any evidence of varioliiform gastritis.

No instances of an increase in intraepithelial lymphocytes in the gastric mucosa in association with coeliac disease have been reported previously, except by Wolber *et al*,³ who found high numbers in five out of 10 patients with coeliac disease.

Because the counts of intraepithelial lymphocytes between the coeliac patients and controls overlapped, this increase cannot be regarded as a specific diagnostic feature. On the other hand, it might be used as an indication for a small intestinal biopsy, at least when typical varioliiform gastritis is not present at endoscopy.

An abnormal immunological reaction to gluten is considered to be important in the pathogenesis of coeliac disease, and this may be reflected in the abnormal density and subtype distribution of intraepithelial lymphocytes in the small intestinal mucosa. The change in the stomach may represent the same process. Our results do not support the possibility that *H pylori* may have some role in the pathogenesis of coeliac disease or lymphocytic gastritis, but due to the retrospective nature of this survey, no *H pylori* serology could be performed and we are not able to disprove the suggestion of Dixon *et al*² that lymphocytic gastritis is an abnormal immune response to *H pylori*. An increased incidence of achlorhydria has been reported to occur in coeliac disease⁴ and dermatitis herpetiformis,⁵ but it is unknown whether the present increase in intraepithelial lymphocytes in coeliac disease is associated with any functional abnormality in the gastric mucosa.

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- 1 Haot J, Hamichi L, Wallez L, Mainguet P. Lymphocytic gastritis: a newly described entity: a retrospective/endoscopic and histological study. *Gut* 1988;29:1258-64.
- 2 Dixon MF, Wyatt JI, Burke DA, Rathbone BJ. Lymphocytic gastritis - relationship to *Campylobacter pylori* infection. *J Pathol* 1988;154:125-32.
- 3 Wolber R, Owen MB, DeBuono L, Appelman H. Lymphocytic gastritis in patients with