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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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