In prion diseases, an abnormal isoform of the prion protein (PrPSc), which is generated by a posttranslational modification of the cellular protein (PrPC), accumulates in the brains of affected animals. The detection of PrPSc is essential for the diagnosis of prion diseases, and immunohistochemistry (IHC) and Western blotting (WB) are conventionally used for this purpose. IHC can be used for topographic examination of PrP Sc in brain tissues, but it is unable to distinguish between PrP C and PrP Sc. WB however can be used for subquantitative analysis of PrP C and PrP Sc, but it cannot be used to topographically examine both forms of the prion protein.

Recently, a histoblot technique was used for the in situ distinctive detection of PrP C and PrP Sc in brain tissues of mice and hamsters experimentally infected with scrapie. In that study, the PrP C signal was diminished in certain regions, specifically regions where the PrP Sc signal was detected.

In the present study, the histoblot technique was applied to detect and distinguish between prion proteins in brains of cattle affected with bovine spongiform encephalopathy (BSE) and sheep affected with scrapie to evaluate the technique for use in animals with naturally acquired disease.

The brains were removed from 5 cattle clinically suspected of having BSE and from 3 sheep clinically suspected of having scrapie. All of these animals had been raised in the United Kingdom. For normal controls, brains were collected from 2 healthy cattle and 2 healthy sheep that had been raised in Japan. At necropsy, a block of medulla oblongata at the level of the obex or the spinomedullary transition was collected from each brain. The blocks were cut into cranial and caudal halves. The cranial halves were immediately frozen in liquid nitrogen and stored at $-80^\circ$C until application of the histoblot technique. The technique enabled the in situ distinctive detection of the cellular (PrP C) and abnormal (PrP Sc) isoforms of the prion protein. In BSE- or scrapie-affected brains, the PrP C signal decreased, especially in those areas where the PrP Sc signal was detected.
Figure 1. Medulla oblongata at the level of the spinomedullary transition from a cow affected with bovine spongiform encephalopathy. a, spongiform change of neuropil was observed in the substantia gelatinosa. HE. Bar = 50 μm. b, granular deposition of PrP was observed in a spongiform lesion. Immunohistochemistry with anti-PrP antibody. Bar = 50 μm.

cattle (Fig. 2, left). The signal for BSE-affected cattle, however, was much weaker than that of normal cattle, especially in the gray matter. When the membranes were treated with both proteinase K and hydrated autoclaving, the signal was detected in tissue of gray matter only from BSE-affected cattle, not from normal cattle (Fig. 2, right). The signal was strongest in the substantia gelatinosa. When the membrane was treated with proteinase K only, no signal was seen from either BSE-affected or normal cattle (Fig. 2, center). These results indicate that signals on the membrane without either treatment preferentially represent PrPc, as previously described using brains of mice experimentally infected with scrapie. However, PrPSc can be exclusively detected on the membrane when both proteinase K and hydrated autoclave treatments are used. Furthermore, the distribution of PrPSc in the histoblot technique completely coincided with histopathologic changes. The PrPc signal diminished in BSE-affected samples, and the PrPSc signal was clearest in certain areas where the PrPc signal became weakest.

There were similar findings in membranes on which tissues of scrapie-affected and normal sheep were blotted (Fig. 3). The PrPSc signal in sheep samples, however, was stronger than that in BSE-affected cattle samples, despite the fact that the sheep samples had been treated with stronger proteinase K.

Histoblotting was used for the in situ detection of both PrPc and PrPSc in brains of BSE-affected cattle and scrapie-affected sheep. The results might have been predicted from the previous study in which the histoblot technique was used to locate PrPc and PrPSc in brains of hamsters experimentally infected with scrapie. WB is not applicable for such in situ detection of both forms of the protein. Although a few investigators reported detection of PrPc in tissues of normal animals using a sensitive IHC technique,1,2 that method cannot be used to distinguish PrPc from PrPSc without antibodies highly specific to each form of the protein. The histoblot technique also has other merits. The histoblot can be used for tissue damaged by autolysis, and it is easy and quick (<24 hours) to perform. Thus, the histoblot can be used not only for diagnosis but also for analysis of PrPc and PrPSc kinetics in tissues of animals affected with prion diseases.

PrPc decreased in brains of hamsters inoculated with a scrapie agent as the illness progressed, and this phenomenon coincided with PrPSc accumulation.6 The functional loss of PrPc has been suggested to underlie the pathogenesis of prion diseases.6 Although sequential observation of the kinetics of PrPc and PrPSc was not possible in the present study, the PrPc signal decreased in certain areas, i.e., those where the PrPSc signal was detected, in BSE- and scrapie-affected brains. These observa-
Figure 3. Histoblot analysis of medulla oblongata at the level of the spinomedullary transition from normal sheep and sheep affected with scrapie. With neither proteinase K nor autoclave treatment, the PrPc signal was diffuse. The signal for scrapie-affected sheep, however, was much weaker than that for normal sheep, especially in the gray matter (left). When the membranes were treated with proteinase K, no signal was seen in either scrapie-affected or normal sheep (center). When the membranes were treated with proteinase K followed by hydrated autoclaving, the PrP Sc signal was detected in gray matter tissues from scrapie-affected sheep but not in those from normal sheep (right). Bar = 1 cm.

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Sources and manufacturers
a. Dr. C. Birkett, Institute for Animal Health, Compton Laboratories, Compton, Newbury, Berkshire, UK.
b. Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA.
c. Amersham, LittleChalfont, Buckinghamshire, UK.

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