ULTRASTRUCTURAL CHANGES IN RENAL TUBULES OF SHEEP FOLLOWING EXPERIMENTAL INFECTION WITH LEPTOSPIRA INTERROGANS SEROTYPE POMONA

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Infections of domestic livestock with Leptospira interrogans serotype pomona are responsible for considerable economic loss in New Zealand. In addition they create a potential public health hazard for those engaged in animal production and related industries.

Serotype pomona, like many other mammalian leptospires, becomes localised in renal tubules, frequently establishing a carrier state. Lesions associated with leptospiral infections are common in sheep kidneys and their appearance has been described both macroscopically and by light microscopy (Hartley, 1952). Ultrastructural studies of such lesions have not apparently been recorded.

Ultrastructural studies have been made on biopsy material taken from the kidneys of human beings with leptospirosis (Brito et al., 1965; Brito et al., 1967; Sitprija and Evans, 1970) but none of the infections studied was due to pomona. Renal ultrastructural changes in guinea-pigs experimentally infected with L. interrogans serotype icterohaemorrhagiae have been described (Brito et al., 1966). A similar study of a naturally occurring leptospiral infection of unknown serotype of the rat kidney (Martino et al., 1969) revealed that organisms were localised in the epithelial cells of the proximal tubules. Miller and Wilson (1966) recorded similar findings in hamsters with experimentally-induced pomona infections.

The aim of the present study was to determine by electronmicroscopy the degree and nature of the changes occurring in the convoluted tubules of the sheep kidney after an experimental infection with serotype pomona.

MATERIALS AND METHODS

Animals. Eight 5-year-old Romney ewes with their 1-month-old lambs at foot were infected with serotype pomona. A further four ewes with lambs were kept in a pen isolated from the infected animals and used as controls. The sheep were killed and examined 34 days after inoculation.

Inoculum. The experimental sheep were given intraperitoneal injections of approximately $8 \times 10^7$ leptospires in 2 ml of Stuart's medium. The infecting organisms were derived from a subculture of serotype pomona, inoculated 7 days earlier with a primary culture of infected porcine kidney in Fletcher's semisolid medium.

Collection and preparation of specimens. Immediately after the sheep were killed by exsanguination, the right kidney was removed and selected portions from both cortex and
medulla were placed in cold 3% glutaraldehyde with 2% formaldehyde in 0.1M phosphate buffer, pH 7.2, and cut into cube-shaped fragments approximately 1 mm in width. All specimens were post-fixed in 1% phosphate-buffered osmium tetroxide (pH 7.2) for 2 hours. The tissue specimens were dehydrated in ascending strengths of ethyl alcohol, embedded in Durcapan (ACM Fluka) and cut on an LKB ultramicrotome. The sections were mounted on formvar-coated grids and stained with uranyl-acetate and lead nitrate.

RESULTS

In each experimentally-infected sheep, lesions were visible on the cortical surface of the kidney. They were roughly circular, often ill-defined white spots varying from 1-3 mm in diameter. The lesions were invariably present in both kidneys in approximately equal size and numbers and distributed evenly throughout the cortex. When examined by light microscopy, the lesions were seen to consist of foci of interstitial infiltration by lymphocytes and plasma cells. Adjacent to or within the inflammatory foci there was usually some evidence of tubular damage. When these areas of tubular damage were examined by electron microscopy, leptospires were seen concentrated around the periphery of the proximal tubules and intermingled with the brush border (fig. 1). These organisms were concentrated at the apical plasma membrane close to the tight junction and in some cases even appeared to penetrate into the intercellular space. Other material which at times filled the tubular lumen was probably of a proteinaceous nature, and presented a balloon-like appearance. Many of the infected tubules had a complete or almost complete absence of brush border, whilst in others the brush border was intact in spite of the presence of large numbers of organisms. An outstanding feature in many areas was the bulbous ends of the microvilli (fig. 1). These bulbous ends reached a size of 0.5 μm in diameter while still remaining attached by the basal stalk to the apex of the epithelial cell. Some even larger-sized bulbous structures were observed, but without preparing serial sections it was not clear whether these were free in the lumen of the tubules or remained attached to the microvilli (fig. 1). In some sections, approximately two-thirds of the microvilli showed some degree of bulbous swelling of their ends. A few of the microvilli had increased in diameter more or less evenly along their whole length. Swellings of the whole apical region of individual cells could be seen (fig. 2) and these protruded well into the lumen of the tubule. These apical protrusions were all devoid of microvilli. Some of the tubular epithelial cells showed an increased number of cytosomes and some had an occasional dense inclusion (fig. 2). Individual cells in an advanced stage of necrosis were seen in a few sections and these were usually free of organisms, although the remains of one necrotic cell found free in the lumen had leptospires both within the cytoplasmic membrane and within the nuclear membrane (fig. 3). The nuclei of epithelial cells of infected tubules were normal in appearance.

Mitochondria, lysosomes, fragments of endoplasmic reticulum and remnants of brush border, together with much unrecognisable cellular debris were occasionally present in the lumen (fig. 4). The leptospires were easily recognisable; they could be seen to be helical
Fig. 1.—Numerous leptospires within the tubular lumen and in association with the brush border. The bulbous swellings (arrow) at the ends of the microvilli are a feature of these infected tubules. EM. × 12,600.
Fig. 2.—The apical region of one tubular epithelial cell (A) is seen protruding into the lumen. Within a tubular epithelial cell is a dense inclusion (Ic). In the tubular lumen is an accumulation of cell debris (D) and free organelles. EM. × 12,600.
Fig. 3.—A necrotic cell lying within a tubular lumen. Organisms can be seen apparently within the cell membrane and the nucleus (arrows) of this necrotic cell. EM. $\times 12,600$. 
Fig. 4.—A tubular lumen containing mitochondria, lysosomes and pieces of brush border, together with unrecognisable cellular debris. EM. ×12,600.
and the axostyle was often visible when viewed at high magnifications. Also visible at high magnifications was the variable density of the cytoplasm of the organism which contained an occasional granule of approximately 15 nm diameter.

**DISCUSSION**

Although immersion-fixation is not considered as effective as perfusion techniques, none of the tubular changes described, with the exception of the proteinaceous "balloons", was seen in the control animals. These epithelial changes were seen only in kidney tubules that were colonised by leptospires; they are therefore unlikely to have been artifacts.

The majority of tubular changes seen were related to degeneration and necrosis of the epithelial cells. Of particular interest was the association between the degeneration of the brush border and the close proximity of the leptospires.

Various hypotheses can be put forward to explain the loss of the microvilli. A general swelling of the microvilli might result in their obliteration; alternatively, the replacement of degenerate cells by less well differentiated cells without a brush border might occur (Chatelanat and Simon, 1969). A further possibility is that of detachment or destruction of the microvilli with rejoicing of the plasma membrane at their bases. The changes in the shape of the microvilli described in this study might form part of a sequence of events leading up to their detachment.

Necrotic debris and large "balloons" of proteinaceous material occurred in the lumina of many tubules. Although some "balloons" were seen in the tubular lumina of normal kidneys, they were probably the result of poor fixation and may not have been present had perfusion techniques been employed. The greater size and frequency of occurrence of these structures in the pathological material make them a significant finding.

At no time were leptospires seen to have actively invaded an otherwise normal cell. Martino et al. (1969) noted that they were found only in the cytoplasm of cells in which the brush border showed severe degeneration. In the present study they were found only in the cytoplasm of severely necrotic tubular cells of the type shown in figs. 2 and 3, and in the nucleus of the necrotic cells lying free in the tubular lumen (fig. 3). It was assumed that invasion of the cytoplasm and certainly of the nucleus occurred after the death and detachment of the cells. In support of this assumption, obviously necrotic cells were seen in position in the tubular epithelium, and these showed no evidence of leptospiral invasion in spite of the presence of large numbers of organisms in the adjacent lumen.

From the evidence considered above, it is difficult to substantiate one of the theories proposed by Harrington and Sleight (1966) that mechanical disruption plays an important part in cellular damage by *pomona*. It appears more likely that such damage is due to the elaboration of a substance or substances toxic to the epithelial cells. Electron micrographs of infected kidneys illustrate the large number of organisms and their close proximity to tubular-epithelial cells.
Any toxins elaborated by the organisms would probably be at high local concentrations under such circumstances.

SUMMARY

Sheep were experimentally infected with *Leptospira interrogans* serotype *pomona* and after 34 days the kidneys were examined by electron microscopy. Colonisation of the kidneys was well established at this time and changes in the kidney tubules included a concentration of leptospires around the periphery of the proximal tubular lumen. The microvilli making up the brush border were in some instances greatly reduced in numbers and in others the microvilli had developed bulbous ends. Necrotic epithelial cells within the tubules were observed and these were sometimes found free in the tubular lumen. The possible sequence of events leading to these changes is discussed.

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REFERENCES


