THE ROUTE OF ENTRY OF LEPTOSPIRES INTO THE KIDNEY TUBULE

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PLATES X and XI

It has been known for many years that pathogenic leptospires multiply in the blood stream of an infected animal, invade many organs of the body, and finally become localised in the proximal convoluted tubules of the kidney. Most observations of infected kidneys have been made in the later stages of natural and experimental infections (Hartley, 1952; Brito *et al.*, 1965; Brito *et al.*, 1967; Sitprija and Evans, 1970; Marshall, 1974). It is not clear how leptospires reach the tubular lumina from the bloodstream, or how much damage, if any, is caused by their migration. From a study of so-called pre-nephritic kidneys of pigs, Michna and Campbell (1969) postulated that the invading leptospires travel to the tubules by way of interstitial spaces and through the tubular epithelial cells or between adjacent cells. Miller and Wilson (1962) noted the formation of lysosomal vacuoles in tubular epithelial cells of infected hamsters, and Michna and Campbell (1969) inferred that organisms migrate from the interstitial space to the tubular lumen inside such vacuoles.

This study was undertaken to make further observations on the early stages of renal infection and on the time taken for leptospiral migration within the kidney. Mice were used because of the need to kill sequentially a considerable number of infected animals.

MATERIALS AND METHODS

Animals. White mice aged 2 or 3 weeks were derived from a conventional colony that showed no evidence of leptospiral infection on serological and cultural examination.

Inocula. Two different isolates of *Leptospira interrogans* serotype *pomona* from sheep were used. Mice were infected intraperitoneally (i.p.) with 0.1-ml doses derived from primary cultures in Fletcher's medium incubated at 30°C for 3 weeks. The inocula were uncontaminated and the organisms had not been subcultured.

Bacterial counts. These were carried out by means of a Petroff-Hausser counting chamber (C. A. Hausser & Son, Philadelphia, USA) and were subject to a greater degree of error than usual because of the uneven distribution of organisms in the semi-solid medium.

Perfusion technique. Mice anaesthetised with ether were perfused with a mixture of 3% glutaraldehyde and 2% formaldehyde in 0.1M phosphate buffer, pH 7.2; c. 0.5 ml of this fixative was injected i.p. to bathe the kidneys *in situ*. A further 0.2–0.3 ml was injected by the intracardiac route until death occurred. The right kidney was then removed as quickly

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as possible and cut into 1-mm cubed fragments. These were immersed in 10 ml of cold glutaraldehyde-formaldehyde fixative and held at 4°C.

Electron microscopy. Primary fixation with glutaraldehyde-formaldehyde fixative was carried out overnight and the tissues were subsequently washed twice in cold phosphate buffer. Secondary fixation with osmium tetroxide was carried out for 2 h. To ensure complete removal of the osmium tetroxide the fragments were again washed twice in phosphate buffer. Dehydration was effected by ethyl alcohol in a graded series of ascending strengths. The dehydrated tissues were then infiltrated with Durcupan (Fluka A.G. Chemische Fabrik Buchs, S.H.G.) and the final embedding was in Durcupan to which had been added 2% of accelerator, 10% of hardener and di-n-butyl phthalate as a plasticiser. Sections were cut on an ultramicrotome (L.K.B. Instruments Ltd), mounted on formvar-coated grids and stained with uranyl acetate and lead nitrate.

Experimental design. The experiment was carried out in two parts. In the first part, 24 3-week-old mice were given i.p. inoculations of 4×10^6 organisms that had been cultured from the urine of an infected ewe. Twelve control mice were given sterile Fletcher's medium i.p. At weekly intervals, six infected and three control mice were killed for examination.

In the second part of the experiment, 12 two-week-old mice were given i.p. inoculations of 7×10^6 organisms that had been cultured from the kidney of a naturally-infected lamb and six control mice received sterile medium. Two infected mice and one control were killed for examination every 2 days for a period of 12 days.

RESULTS

Two days after inoculation, organisms were seen within the lumen of capillaries and within capillary endothelial cells. From the 4th to the 8th day, the organisms were mainly in the interstitial tissue of the kidney (fig. 1). In many of the sections taken during this period the leptospire were surrounded by a clear area interpreted as oedema fluid. From the 2nd day after infection, the interstitial tissues showed oedema that became progressively more severe and the basement membrane structure around the cells of the proximal convoluted tubules became thicker and less electron-dense. No change was observed in the uriniferous tubules or glomeruli at this stage. In a few sections of kidney from mice that had been infected for 8 days, an infiltration of neutrophils and lymphocytes into the interstitium had taken place. Ten days after inoculation, leptospire were seen between adjoining epithelial cells of the proximal convoluted tubules (fig. 2). Approximately 45 sections were examined from various levels of each embedded block of tissue yet this was the only site at which leptospire were observed apparently *en route* from interstitial tissue to tubular lumina. At no time were intact organisms seen invading or within epithelial cells. Where organisms were found close to tight junctions they were contained within small pockets and surrounded by electron-translucent material (fig. 2). In some sections, the organisms were seen within the infoldings at the base of the proximal convoluted tubules (fig. 3). By the 14th day leptospire were located within the tubular lumen (fig. 4), but some organisms could still be found between epithelial cells and in the interstitial spaces. In some sections of kidneys at the 4th and 8th day after infection, vacuoles apparently containing portions of leptospire could be identified within epithelial cells of the proximal convoluted tubules. On no occasion did these organisms appear intact or viable.

DISCUSSION

One of the earliest changes seen in the mouse kidney was an interstitial oedema. This was present on the 2nd day and may have resulted from endothelial damage by the leptospires or have been due to an inflammatory response provoked by leptospiral toxins. This oedema probably aided the passage of the organism through the tissues to the base of the proximal convoluted tubules. The basement membrane structure around the cells of the proximal convoluted tubules became thicker and less electron-dense; this may indicate that its effectiveness as a barrier to leptospires was reduced. Having reached the base of the cells of the proximal convoluted tubules, the organism entered the space between adjoining tubular epithelial cells and created small pockets by parting the otherwise tightly opposed cell membranes. The 10th day was the earliest at which leptospires were seen in this intercellular site. At no stage were the organisms seen penetrating between cells in other parts of the tubule. The forces attracting these organisms towards the lumen of the proximal convoluted tubules are not known. Although the passing of the organism through the tight junction was not observed, it must be assumed that these junctions relax sufficiently to allow the organism to pass to the tubular lumen. In only three electron micrographs of tissues taken on the 4th to the 8th day after infection could structures resembling leptospiral fragments be seen within cells of the proximal tubules. These fragments were inside structures resembling lysosomes, suggesting that the organism might on occasion have actively penetrated the cells but become digested by the lysosomal enzymes. An alternative explanation might be that a few organisms died between adjoining cells and then became engulfed. However, it must be emphasised that these structures were not sufficiently well delineated to make identification conclusive.

It would be reasonable to assume that any passage of organisms through the glomerulus would result in visible ultrastructural change. The absence of glomerular lesions in the mice at any stage up to and including the 14th day of infection probably excludes the glomerulus as a route by which the leptospires gain entry to the proximal convoluted tubules.

SUMMARY

To study the migration of *Leptospira interrogans* serotype *pomona* through the kidney, conventionally-reared mice aged 2 or 3 weeks were infected intraperitoneally with this organism. Within the first 4 days, the organisms migrated from the capillary lumina to the interstitial tissue and provoked an interstitial oedema. By the 10th day they were seen between the epithelial cells of the proximal convoluted tubules and by the 14th day many were located within tubular lumina. There was no evidence of viable leptospires within the cells of the proximal tubules, though occasionally structures resembling leptospiral fragments inside lysosomes were observed. At no stage during the study were glomerular lesions seen.

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REFERENCES

- BRITO, T. DE, FREYMÜLLER, E., PENNA, D. O., SANTOS, H. S., SOARES DE ALMEIDA, S., AYROZA GALVÃO, P. A. AND PEREIRA, V. G. 1965. Electron microscopy of the biopsied kidney in human leptospirosis. *Am. J. trop. Med. Hyg.*, **14**, 397.
- BRITO, T. DE, PENNA, D. O., PEREIRA, V. G. AND HOSHINO, S. 1967. Kidney biopsies in human leptospirosis: a biochemical and electron microscopy study. *Virchows Arch. path. Anat. Physiol.*, **343**, 124.
- HARTLEY, W. J. 1952. Ovine leptospirosis. *Aust. vet. J.*, **28**, 169.
- MARSHALL, R. B. 1974. Ultrastructural changes in renal tubules of sheep following experimental infection with *Leptospira interrogans* serotype *pomona*. *J. med. Microbiol.*, **7**, 505.
- MICHNA, S. W. AND CAMPBELL, R. S. F. 1969. Leptospirosis in pigs: epidemiology, microbiology and pathology. *Vet. Rec.*, **84**, 135.
- MILLER, N. G. AND WILSON, R. B. 1962. *In vivo* and *in vitro* observations of *Leptospira pomona* by electron microscopy. *J. Bact.*, **84**, 569.
- SITPRIJA, V. AND EVANS, H. 1970. The kidney in human leptospirosis. *Am. J. Med.*, **49**, 780.

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FIG. 1.—Leptospires (arrows) surrounded by oedema fluid (Oe) in the interstitium of the mouse kidney 2 days after infection, having migrated there from the capillary lumen (CL). EM. $\times 35\ 000$.

FIG. 2.—A leptospire (arrow) migrating between adjoining convoluted tubular epithelial cells of the mouse kidney 10 days after infection. The organism is contained within a pocket of electron-lucent material and is lying close to a tight junction (TJ) and the brush border (BB). EM. $\times 47\ 600$.

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FIG. 3.—A leptospire (arrow) within an infolding at the base of a proximal convoluted tubular cell. The mouse had been infected 10 days previously. EM. $\times 27\,700$.

FIG. 4.—Mouse kidney 14 days after infection. Organisms (arrow) are well established within the tubular lumen and are closely associated with the brush border (BB) which appears normal. EM. $\times 40\,000$.