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Ultrastructural and Immunohistochemical Study of the Human Kidney in Argentine Haemorrhagic Fever

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Summary. In six lethal cases of Argentine Haemorrhagic Fever (AHF) a disease caused by Junin virus, kidney samples were studied by means of immunofluorescent and electron microscopic techniques. — The ultrastructural studies showed that the distal and collecting tubes presented a large number of virus like intracytoplasmic particles. Those particles were present in the lumen of the endoplasmic reticulum cisternae and showed two distinct morphological aspects. Some of them were of high electron density and contained a few granules. The others were larger in size, electron lucid, and contained a variable number of ribosome like granules.

Both types of particles originated from the endoplasmic reticulum wall by a process of budding. The presence of these particles was coincident with a severe cell damage which lead to necrosis and desquamation; and with large quantities of Junin virus antigen as demonstrated by immunofluorescence. — On the basis of these observations it is assumed that in AHF the cell damage is due to direct viral replication within the affected cells.

Epidemic haemorrhagic fevers are characterized because in all of them some degree of kidney involvement exist, and in the lethal cases renal failure is an almost constant component of the disease (Steer, 1966).

Although the morphological modifications of the kidney have been described at the light microscope level (Child *et al.*, 1967; Edington and White, 1972; Elsner *et al.*, 1973; Gallardo, 1970; Hullinghorst and Steer, 1953; Lahdevirta, 1971; Lukes, 1954; Oliver and McDowel, 1957; Oliver and McDowel, 1958; Powel, 1954; Rivero *et al.*, 1959; Steer, 1955; Steer, 1966), to our knowledge no studies exist describing the fine structure of the kidney in these diseases and no immunofluorescent studies have been reported with the object of searching for etiological agents in the affected tissues.

On the other hand, the pathogenesis of the renal damage remains unknown at the present time, although several hypothesis have been put forward in order to explain the mechanisms which lead to the tissue damage, such as the presence of immune complexes or the direct endothelial damage by viral replication with consequent interstitial haemorrhage and anoxia (Steer, 1966).

In the present report the ultrastructural and immunohistochemical findings in the kidney of 6 lethal cases of argentine haemorrhagic fever (AHF) are described.

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Material and Methods

1. Tissue Specimens. Kidneys' samples were obtained from 6 human beings who died from AHF. The material was obtained between 10 min and 2 hours postmortem.

The clinical diagnosis of AHF was based on signs and symptoms previously described (Schwarz *et al.*, 1970; Sabattini y Maiztegui, 1970).

The clinical and virological diagnosis of the 6 cases of AHF included in this study was done according to criteria described elsewhere (Sabattini and Maiztegui, 1970). Junin virus was isolated from the blood in 5 out of the 6 cases included in this report.

2. Immunofluorescent Procedure. Two cryostat sections of one piece of the kidney samples were treated with a) goat antiserum anti human gamma globulin, labelled with fluorescein; the antiserum was prepared and labelled as previously reported (Cossio *et al.*, 1974); b) goat antiserum anti human B_1 C-A globulin, labelled with fluorescein (Hyland Lab., Los Angeles, California); diluted 1:6; c) Hyperimmune mouse ascitic fluid (anti Junin virus), labelled with fluorescein, as described by Nairn (1968). The final labelled globulin fraction was used diluted 1:25, and the fluorescein protein ratio was 1:4. "Blocking" experiments were performed applying the same anti virus Junin antibody, but unlabelled, previously to apply the labelled one; d) normal mouse ascitic fluid (induced by sarcome 180) labelled with fluorescein in the same way as the anti Junin one.

The same procedures were performed on two normal human kidneys and on renal samples of 3 patients with systemic lupus erythematosus (SLE).

3. Histological Procedures. Small kidney samples were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, for 4–24 hours, post-fixed in 1% osmium tetroxide and embedded in Araldite. Thin sections were stained with uranium acetate and lead citrate and examined with a Philips 200 Electron Microscope. In all cases thick (1 μ) sections were mounted on slides and stained with alkaline toluidin blue in order to perform a light microscope study. The remainder of the kidneys was fixed in 10% buffered formaldehyde and processed for routine histological study.

Results

Immunofluorescence Studies. Bound human gamma globulin or B-1 CA globulins were not observed in the 6 cases. Fluorescein labelled mouse anti Junin antibodies gave strong positive reactions in the cytoplasm of the distal and collecting tubes epithelial cells (Fig. 1). In the proximal tubes positive stain with the anti virus antibody was observed as small roundish brightly fluorescent granules located in the nuclei (Fig. 2). Blocking experiments abolished the reaction. Normal ascitic fluid also gave negative results.

The hyperimmune anti Junin virus labelled antibody gave negative reaction with the two normal kidneys as well as with the SLE kidneys.

Light Microscope Studies. The most conspicuous alterations were located at the medullary level. In that place the collecting tubes showed an increase of the cell size and presented invaginations of the epithelium towards the tubular lumen, which were apparently provoked by detachment of the cell from the basement membrane.

This alteration was also present in the distal tubes. The glomeruli, proximal tubes, interstitium and blood vessels did not show evident damage, with the only exception of the proximal tubes, where the cells presented moderated cytoplasmic swelling.

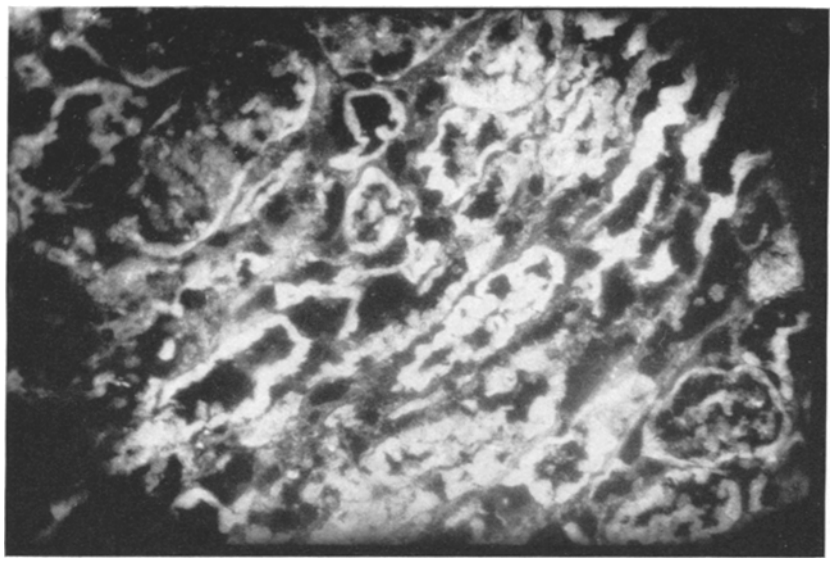

Fig. 1. Renal medullary zone treated with fluorescein labelled anti Junin virus antibodies. The epithelial cells of the distal and collecting tubes show an intense reaction. × 400

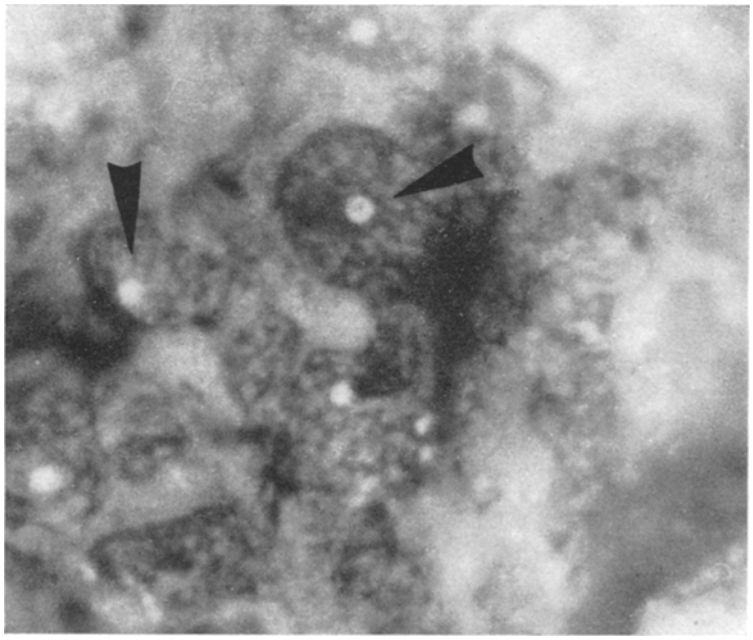

Fig. 2. High-power view of epithelial cells of the proximal tubes stained with fluorescein labelled anti Junin virus antibodies. The arrows point to brightly stained intranuclear granules. × 1200

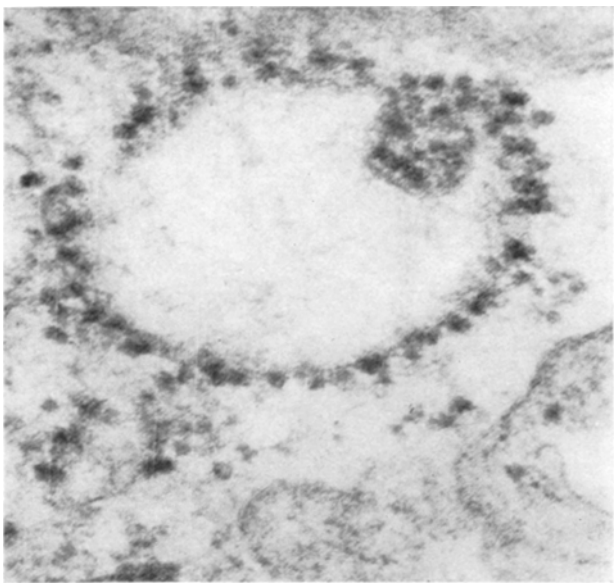

Fig. 3. High power view of a virus-like particle. It appears roundish in shape, with an electron dense matrix containing electron dense granules resembling ribosomes. × 65000

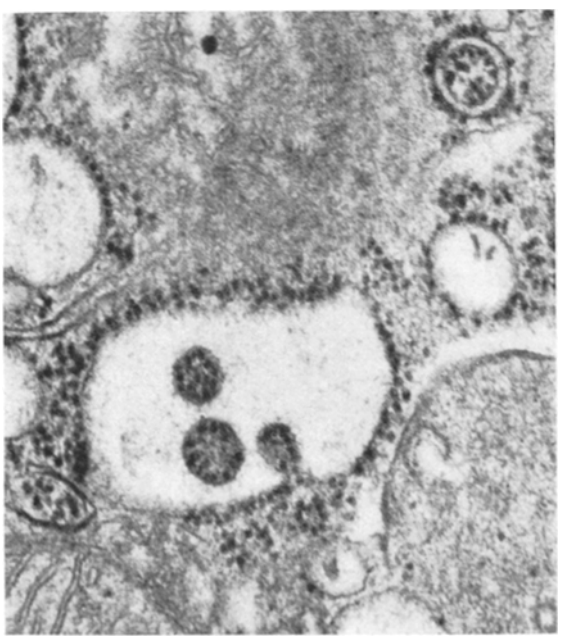

Fig. 4. Three virus-like particles within the lumen of a rough-surfaced endoplasmic reticulum cisternae. One of the particles appears budding from the endoplasmic reticulum wall. × 29000

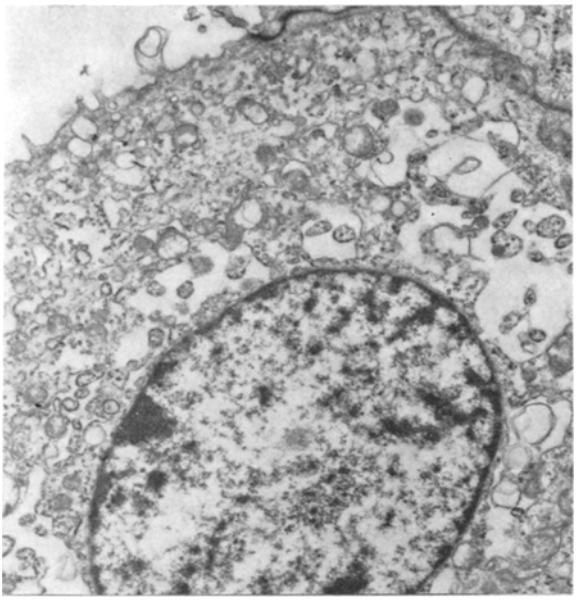

Fig. 5. Low-power view of a collecting tube epithelial cell. The endoplasmic reticulum cisternae appear dilated and contain several roundish bodies of variable size. × 11000

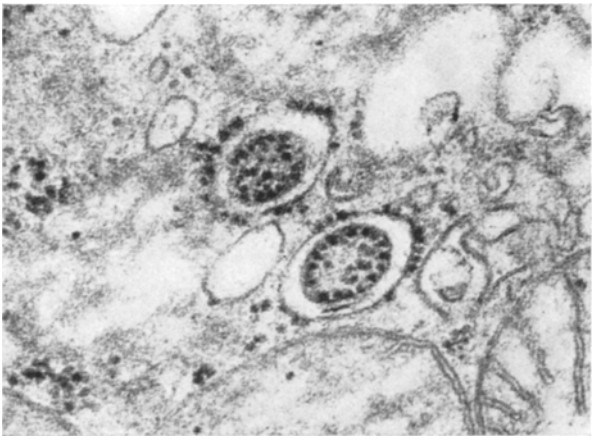

Fig. 6. Two virus-like particles located within endoplasmic reticulum cisternae. × 38000

Electron Microscope Studies. The cells of the distal and collecting tubes showed modifications heralded by the appearance of numerous particles of two distinct morphological aspects within the endoplasmic reticulum cisternae. Some of these particles were round in shape, measuring 70–90 nm in mean diameter. Uniform in size, they showed an outer envelope consisting of a single membrane and an electron dense matrix containing a dense core which at times could be

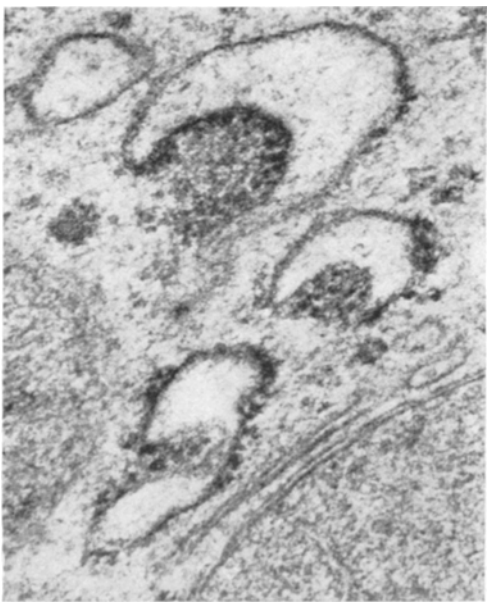

Fig. 7. Electron micrograph showing buddings which protrude towards the lumen of three endoplasmic reticulum cisternae. The buddings contain ribosomes-like granules. × 70000

resolved into a few tiny granules (Fig. 3). Apparently these particles originated by budding from the wall of the smooth endoplasmic reticulum. Intermediate images ranging from the accumulation of an electron dense material inmediately beneath the endoplasmic reticulum to the presence of intracisternal particles connected with the endoplasmic reticulum wall by means of a thin pedicle, were frequently to be seen (Fig. 4). In some cells a different type of particle could be seen in the lumen of the rough-surfaced endoplasmic reticulum cisternae. Roundish in shape, they measured 80–200 nm in mean diameter. Generally they presented an envelope and an electron lucid matrix containing variable number of granules resembling ribosomes (Figs. 5 and 6).

Apparently they formed by a process of budding from the endoplasmic reticulum wall (Fig. 7).

This last type of particles resembled morphologically those described in tissue cell cultures or rodents experimentally infected with other arenaviruses (Lascano and Berria, 1969; Murphy *et al.*, 1969, 1970, 1973; Speir *et al.*, 1973; Mannweiler and Lehman Grube, 1973). However, they showed an exclusive intracytoplasmic localization, in contrast to that reported for all arenaviruses, in which they appear in an extracellular localization or budding from the plasma membrane. On the basis of the uniformity in size and shape, of the origin from budding of the endoplasmic reticulum wall, and of the presence of large quantities of Junin virus antigen as demonstrated by immunofluorescence procedures in the same cells where those particles were especially numerous, it was assumed that they could be the morphological representation of Junin virus.

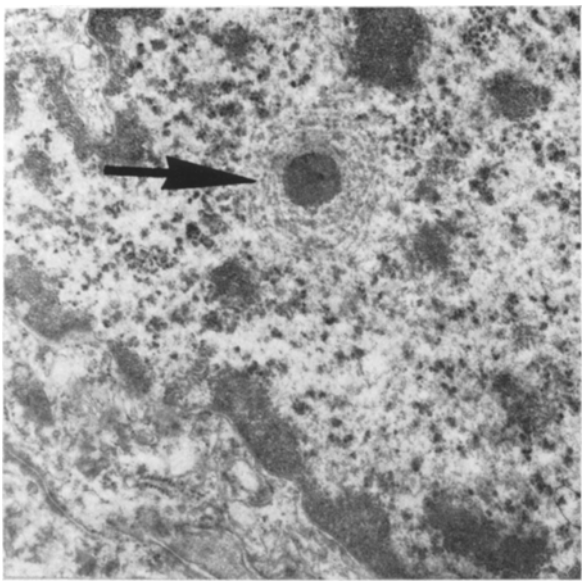

Fig. 8. Nucleus of a proximal tube epithelial cell. The arrow points to a body consisting of an electron dense core surrounded by several layers of thin fibrils. × 14000

The presence of these particles in the tubular cells was coincident with a severe damage with lead to cytoplasmic disorganization. The damaged cells desquamated towards the tubular lumen. Tubular remnants consisting of naked basement membrane with no epithelial cells were frequently observed. Intermediate steps of desquamation could at times be seen in neighbouring tubules.

The proximal tubes and glomeruli showed minor modifications. In the former the epithelial cells showed intranuclear bodies of characteristic aspect consisting of a concentric array of fibrils (Fig. 8).

Very few virus like particles could be seen in the cytoplasm. The cell organelles were abnormal and presented mitochondrial, endoplasmic reticulum and matricial swelling. However, no morphological evidence of necrosis could be seen.

In the glomeruli the mesangium, endothelium and basement membrane showed no modifications. The epithelial cells were hypertrophic and occasionally some virus like particles were present in their cytoplasm.

Discussion

In the present report, by means of immunofluorescence techniques it was possible to show antigenic determinants of the Junin virus in the cytoplasm of the cells of the distal and collecting tubes of 6 patients who died of AHF These cells also contained *virus-like particles* within the endoplasmic reticulum.

With the present evidence it is hard to say if the particles observed in the affected cells represent true virions. Both types of particles present some morphological similarities with those described for other arenaviruses, especially with

those recently reported by Mannweiler and Lehman Grube (1973), who described two kinds of particles in cells infected with lymphocytic choriomeningitis virus, but the exclusive intracellular localization of these particles differs with all the other description of arena virus infected cells. However, most of the ultrastructural studies have been made on cells or animals in which the arena virus infection produces a mild cytopathic effect but no severe cell damage or necrosis, as is the case for human beings infected with Junin virus. On the basis of this fact, it is tempting to speculate that the site of replication of the virus and the formation of particles, as shown by immunofluorescence and ultrastructural methods, is in some way related with the pathogenic action of the virus.

In *the proximal tubes,* antigenic determinants of the Junin virus were observed by immunofluorescence methods mainly as small inclusions in the nuclei. By conventional electron microscopy methods, those cells presented nuclear bodies; this observation suggests that the latter could represent some step in the virus cycle. The presence of numerous particles in the lumen of the reticulum cisternae was coincident with an intense cellular damage.

The epithelial cells showed nuclear and cytoplasmic disorganization, which lead to cell necrosis and desquamation. The cell damage was more evident at the distal segments of the nephron and the collecting tubes. This finding is in agreement with those reported in other haemorrhagic fevers where the alterations were more marked at the corticomedullary junction and medullary zone (Steer, 1966), suggesting that in the different haemorrhagic fevers there would exist similar mechanisms of renal damage.

The presence of large quantities of particles seen with the electron microscope and of the viral antigen as demonstrated with immunofluorescent methods in the damaged components of the kidney, would indicate that the cell damage is due in some way to viral replication within the infected cells. If this is the case, this would be the first observation of a human renal disease in which the cell damage is due to the direct action of a virus.

Although in other viral diseases kidney involvement has been described (for review see Smith and Aquino, 1971) no direct pathogenetic action of the viral agents have been found. Even in cytomegalic virus infection, which is associated with the viral replication in the tubular cells, no severe functional alterations of the kidney have been found (Smith and Aquino, 1971), as is the case for AHF. In this way, our observations suggest that the kidney damage in AHF is produced directly by the viral replication in the infected cells.

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