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## EVIDENCE OF A VIRAL ÆTIOLGY IN ENDEMIC (BALKAN) NEPHROPATHY

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**Summary** Segmental and focal pathological changes were found in the glomeruli and tubules of postoperative renal-biopsy specimens from seven cases of clinically confirmed endemic (Balkan) nephropathy. In the glomeruli, there was mesangial reaction and segmental thickening of the basement membrane with subendothelial and membranous depositions. In the tubules there was spongiform degeneration and fusion of cells. In all the cells of the nephron numerous cytoplasmic vesicles containing free and budding particles (80–200 nm) were found. These particles had the characteristics of a coronavirus. Balkan nephropathy occurs almost exclusively in people who have been in close contact with pigs. Coronaviruses have been isolated from pigs, and it is suggested that a slow coronavirus infection causes endemic nephropathy in man.

### Introduction

ENDEMIC nephropathy (E.N.), also known as Balkan nephropathy, was first described as a clinical entity in the late 1950s.<sup>1 2</sup> However, despite intensive investigation its ætiology is still unknown.<sup>3</sup> Post-mortem studies suggested that the changes were confined to the tubules, and most of the research was directed towards a search for a possible toxin. These efforts were unsuccessful, as were attempts to find an infective agent.<sup>3</sup> However, various epidemiological features have come to light. The disease is found only in adjacent areas of three Balkan countries, Yugoslavia, Bulgaria, and Rumania. It is estimated that there are now 20 000 cases in these three countries.<sup>4 5</sup> The disease is endemic in certain villages.<sup>6</sup>

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The patients are country dwellers who have been born in, or have spent some years in, a focus of E.N.<sup>6-9</sup> There is a tendency to familial clustering of cases.<sup>1 6 10</sup> Clinically, the outstanding features of the disease are its insidious onset, the mild proteinuria, absence of œdema, low urinary specific gravity, and an accompanying normochromic or hypochromic anæmia.<sup>11</sup> There is bilateral, symmetrical, progressive reduction in the size of the kidneys. Uræmia develops slowly.<sup>12</sup> Hypertension, perhaps surprisingly, appears later.<sup>12</sup> Tumours are common in the proximal regions of the urinary tract.<sup>13 14</sup>

### Materials and Methods

Postoperative kidney-biopsy material was obtained from seven cases of clinically confirmed E.N.<sup>15</sup> The material was fixed in formalin and stained with hæmatoxylin-eosin and periodic-acid/Schiff (P.A.S.). For electron microscopy small pieces of tissue were double fixed in glutaraldehyde and osmic acid and embedded in methacrylate. The sections were double stained with uranyl acetate and lead citrate, and examined in a Philips 300 electron microscope.

### Results

**Histology.**—Pathological changes were found in the whole of the nephron as well as in the small blood-vessels. These changes seemed to be at different stages of development in any one area. Patent hypertrophied glomeruli and occluded hyalinised ones were found next to each other, with others showing various degrees of pathological change in between. There was a conspicuous lack of inflammatory cellular infiltration. The regular changes included an increase in the numbers of mesangial and endothelial cells, diminution or obliteration of the lumen of the capillary loops, and an uneven thickening of the P.A.S.-positive basement membranes. The epithelial cells were swollen and the urinary spaces narrowed. Dilated urinary spaces were found only when the glomeruli were collapsed. The membrane of the Bowman's capsule was unevenly thickened and P.A.S.-positive. These changes were found in various degrees and combinations in different glomeruli, but mostly in the subcapsular cortical region. Pathological changes were also found in the tubules, especially in the cells of the proximal tubules. The cells were in various stages of vacuolar degeneration. The cell boundaries were often lost, and granular material was present in the lumina of the tubules. The basement membranes of the tubular cells were unevenly thickened and P.A.S.-positive. In addition, there were also changes in the extra-glomerular blood-vessels, consisting of uneven thickening of the wall and deposition of P.A.S.-positive material in the tunica media.

**Ultrastructural changes.**—These confirmed and complemented the histopathological findings. There was a mesangial reaction in the glomerulus. The basement membrane was convoluted and grossly thickened, sometimes as much as ten times (fig. 1). Deposits of amorphous, fibrillar, or granular appearance were present, mostly subendothelially and in the membrane (fig. 1). Membrane-bound particles were seen embedded in the basement membrane. It was striking that in some glomeruli, despite pronounced changes in the endothelial cells and the membrane, the foot processes of the epithelial cells (podocytes) looked normal. The Bowman's capsule was also unevenly thickened and contained deposits similar to those of the glomerular membrane. The cells

of the tubules showed various degrees of spongiform degeneration (figs. 2 and 3). One important finding was that most of the cells of the tubules were fused with each other (figs. 2 and 3).

*Subcellular ultrastructural changes.*—The affected cells of the whole of the nephron showed increased numbers of cytoplasmic vesicles, degenerate mitochondria, and general proliferation of membranes, giving the cytoplasm a spongy appearance (figs. 1-3). Rough endoplasmic reticulum was seen only occasionally. A large number of the vesicles lined with a single membrane contained pleomorphic particles varying in size from 80 to 200 nm (figs. 1 and 3). The particles were membrane

bound and some had peripheral projections. Many of these particles were seen in the process of budding into the cytoplasmic vesicles (fig. 3). Such particles were seen in endothelial mesangial and epithelial cells of the glomerulus, in the basement membrane, and in large numbers in the cells of the tubules (fig. 1-3). They contained threadlike internal components about 10 nm in diameter (fig. 3 inset). Dense granular masses of varying size (300-500 nm) without limiting membranes were also seen in the cytoplasm of the affected cells. A characteristic structure associated with these darkly stained masses is seen in fig. 4. Such structures are found

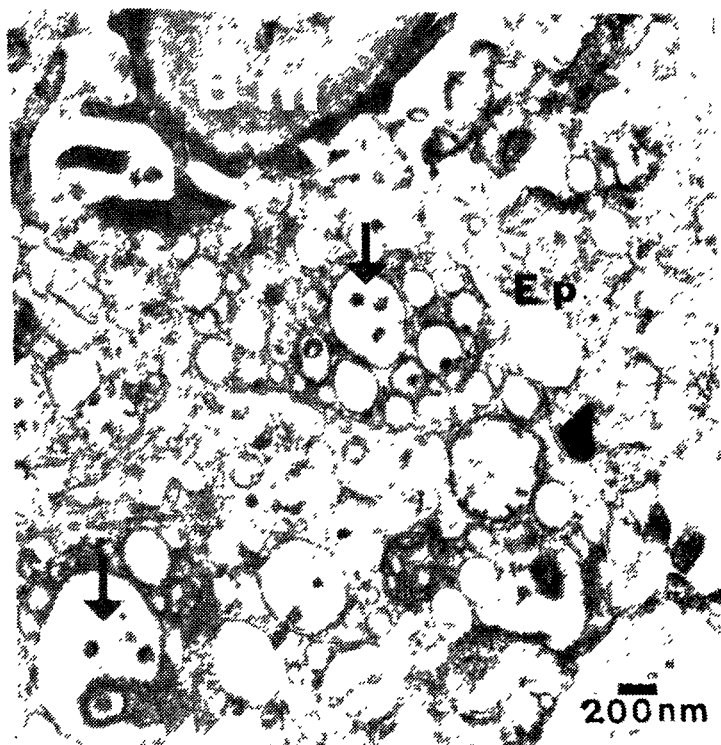


Fig. 1—Basement membranes (Bm) from two capillary loops, with fused epithelial cells in between (Ep).

Spongiform degeneration of the cells with numerous particles in cytoplasmic vesicles (arrows). ( $\times 19\ 000$ ).

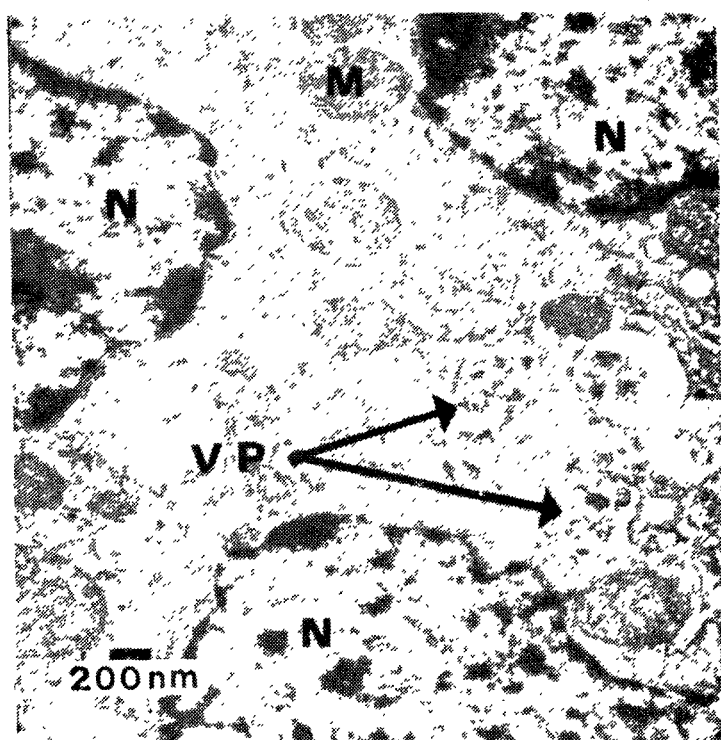


Fig. 2—Three nuclei (N) of fused cells from proximal tubules. Particles in cytoplasmic vesicles (arrows, VP). ( $\times 22\ 000$ ).

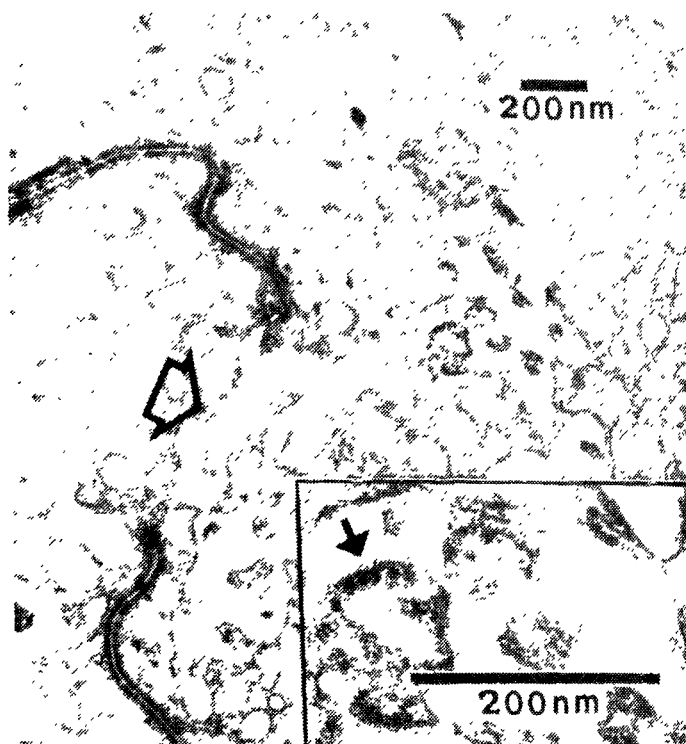


Fig. 3—Cytoplasm of two tubular cells with particles budding from vesicles.

Area of fusion, breaking the continuity of cell membranes, shown by open arrow. ( $\times 37\ 500$ ). Inset: budding particle with internal component (9-10 nm) cut across (arrow). ( $\times 142\ 500$ ).

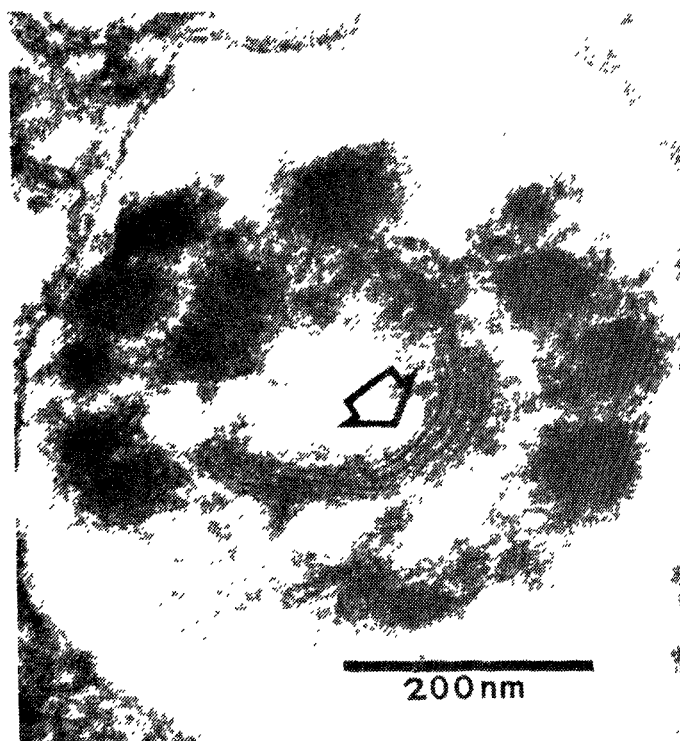


Fig. 4—Electron-dense material in a cytoplasmic vesicle.

Note onion-like arrangement with a periodicity of 10 nm ( $\times 140\ 000$ ).

usually in cells infected with foamy viruses<sup>16</sup> and infectious bronchitis virus of fowls.<sup>17</sup>

### Discussion

The histological and ultrastructural pathology of E.N. presented in this paper accords with most reports on biopsy material from patients with confirmed E.N. and from those with suspected E.N.<sup>12 18-21</sup> Similar changes were also found in biopsy specimens from children and adolescents who showed no clinical evidence of E.N. but came from families with cases of proven E.N.<sup>19 22</sup> Taking into account the progressive character of the disease and the asynchronous histopathological changes, we think that a more appropriate term for this condition would be chronic progressive pan-nephritis.

IgG deposits were found by immunofluorescence in the basement membrane of all seven cases, C3 deposits were also found in the mesangium of the glomeruli.<sup>15</sup> The immunofluorescent and subcellular findings strongly indicate that a biological agent is involved in the aetiology of E.N. and we suggest that E.N. is produced by a slow infection of the kidney by a virus. Some chronic diseases of the nervous system have been shown to be caused by viruses, and are classed as slow (persistent) virus diseases.<sup>23</sup> Subacute sclerosing panencephalitis, which is caused by measles, bears close comparison with E.N.<sup>24</sup> Both conditions are characterised by an insidious onset and a long latent period, measured in decades rather than years. Once the clinical signs appear there is a subacute or chronic progressive course leading to complete loss of organ function in both conditions within a few years.

The subcellular findings, found in all the cells of the nephron but seen most prominently in the proximal tubules, strongly suggest that a coronavirus is involved. The coronaviruses are known to have an affinity for the kidneys. Mouse hepatitis virus,<sup>25</sup> infectious bronchitis virus (I.B.V.) of chickens,<sup>26</sup> and transmissible gastroenteritis virus of pigs<sup>27</sup> are all coronaviruses, and they have all been isolated from the kidneys of infected animals. I.B.V. is also known to be the cause of "nephrosonephritis" in chickens.<sup>28</sup> Coronaviruses have also been isolated from people, with respiratory symptoms.<sup>29</sup> The main criteria for their classification are the morphology of the virion and the changes seen in infected cells.<sup>30 31</sup> The ultrastructural subcellular findings presented in this paper are similar to findings in cells infected with known coronaviruses. The most striking features are the maturation of the virus by budding into cytoplasmic vesicles, the absence of budding from the cell surface, and the capacity of the virus for fusing cells.<sup>32</sup> The epidemiological studies also strongly support the concept of an infective aetiology in E.N. The findings in favour are its endemic focal character,<sup>6</sup> the familial incidence,<sup>5 6</sup> and the fact that the patients are predominantly peasants living in close association with animals<sup>5 6 11</sup> and that immigrants into the endemic areas contract the disease,<sup>6</sup> as well as emigrants from the foci, after a latent period of some years.<sup>7 8</sup> One outstanding and interesting finding is that in one village in which E.N. was endemic the population of 2400 was 50% Muslim and 50% Christian, only 1 out of 54 cases of E.N. was Muslim.<sup>10</sup> Muslims avoid pigs, and there is reason to believe that they are strict in their observance of this prohibition. This suggests that a pig coronavirus may be the causal agent.

Pig husbandry on a domestic scale is common in all the endemic regions so far described, with pigs roaming free in the farmyards of Christians. In villages where no pigs are kept there are no cases of E.N., and vice versa.<sup>4</sup> The peasants could be infected from the excreta of the animal and the initial illness pass as a trivial respiratory infection. During a viræmic phase, the virus, which has an affinity for the kidneys, and is of a low cytolytic capacity, would establish itself in the endothelial cells of the capillary loops, then, via the basement membrane, infect the epithelial cells of the glomerulus, and finally the proximal tubules. The cell-to-cell spread of the virus could be by cell fusion, which can occur even in the presence of a high titre of antibody. The persistence of the infection would be favoured by the fact that coronaviruses are known not to produce specific changes in the cytoplasmic membrane, so that the T lymphocytes are not attracted to the infected cells, even though they may be sensitised to the virus. This could explain the lack of the usual inflammatory cell infiltration.

The pathological findings in biopsy material in children and adolescents from families with E.N., who themselves had no symptoms of the disease, accorded with the suggested progressive character of the disease.<sup>19 22</sup> The rapidly accelerating course of the disease, once the clinical symptoms appear, fits in with the concept of centrifugal cell-to-cell spread of the infection by fusion.

We thank Prof. A. P. Waterson for discussions and help in preparing the manuscript, and Mr. P. A. Lister for technical help.

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