Section of Comparative Medicine

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Comparative Aspects of Renal Disease

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Balkan Nephropathy

Occurrence and Nature

The chronic, atrophying and fatal kidney disease of man known as endemic or Balkan nephropathy occurs in some ten restricted areas of Yugoslavia, Romania and Bulgaria in terrain ranging from flood plain to low montane valley always within the Danube Basin (Wolstenholme & Knight 1967). There are villages in which over 12% of the population and some 20% of the households are affected, and cases may even be restricted to one side of a street. In Bulgaria alone between 1961 and 1970 1546 cases were diagnosed and 663 deaths reported (Puchlev 1971). The main group affected is the farming population but people in other occupations including the law and the church have suffered from the disease. In most areas the ratio of men to women affected is 1:2, whilst the main age group is between 35 and 55 with cases known from ages 20 to 70. Reported survival times from diagnosis have ranged from a few months to ten years or more but generally 50% of the patients die within two years (Čeović 1971). The chief diagnostic criteria are listed in order of importance in Table 1, but it must be understood that there are regional differences of opinion on all of these points – a situation which underlines the present urgent need for common standards in diagnosis. Recent investigations have shown that there is a 30–40% incidence of benign and malignant tumours of the urinary tract in certain areas. Hall & Vasiljević (1973) have shown that an increasing concentration of β_2-microglobulin in the urine is an important diagnostic feature, no doubt associated with initial tubular damage. The pathological changes seen at autopsy are of an interstitial fibrosis with destruction of tubules and hyalinization of glomeruli. Glomerular changes are late and sometimes minimal. Recent electron microscope findings in biopsy material show a pronounced thickening of the capillary basement membrane at an early stage (Doitchinov, 1974 personal communication).

Table 1

Diagnostic criteria for Balkan nephropathy

Present	*Absent*
Familial association	Hypertension
Anæmia	Œdema
Low proteinuria	Signs of cardiovascular disease
Xanthodermia	Eye fundus changes
'Coppery' sheen to skin	
Smooth, atrophied kidneys	
Kidney weight 20–30 g	

Etiological Investigations

For over 25 years intensive etiological investigations have been made embracing many fields including genetics, infection, immunology, allergy and mineral and organic toxicology. Almost all seem to indicate exposure to a common etiologic agent but the findings also present a situation remarkable in modern medicine in which contagion and infection have been neither proved nor disproved. Familial connexion is established but it is clear that genetic inheritance cannot play a role, for the risk of contracting the disease seems to be as high in spouses as it is in the indigenous occupants of a house, provided they have lived with an affected family for 15 or more years.

Endemic nephropathy seems unrelated to the occurrence and distribution of leptospiral, *Brucella*, coliform and streptococcus infections

and no good evidence has yet come of general viral involvement, despite valid observations by Georgescu *et al.* (1970) of virus particles in the tubular epithelium of cases. Epidemic hæmorrhagic fever has been a suspected cause but the acute kidney lesions in this disease apparently resolve without complications (Trencséni & Keleti 1970). Limited investigations of renal disease in the domestic and wild animal populations of the endemic areas have revealed some nephropathy of unknown origin, but adequate comparative studies have not been carried out. Malaria was eradicated in the region in 1945 and no other protozoan, helminth or fungal pathogen has been implicated. The immunological aspects of the disease have been neglected until recently and the finding of autoimmune antibodies (Craciun & Roscolescu 1970) may be an important advance, although possibly to be expected in the presence of such extensive kidney damage (Macanović 1973).

The Toxin Theory

Much of the epidemiological evidence points to a toxicological origin for the disease and extensive investigations have been made in this field, so far without success in revealing a significant single toxic agent. Some 30 known toxic macro- and micro-elements have been analysed in affected tissues, in diets and in the environment, but despite seemingly strong indications that for example lead (Danilović 1958) or silicon (Marković & Arambašić 1971) are involved, there is no general agreement as to the role of minerals. Likewise natural environmental radioactivity has been studied inconclusively (Karamikhailova *et al.* 1965). The widespread use of herbal medicines in the Balkans provides some grounds for suspecting that nephrotoxins of plant origin might be involved and at least one product, namely aristolochic acid, might be significant, but the source, *Aristolochia clematitis* L. (birthwort), is unevenly distributed in the region and it is difficult to envisage how the toxin could become incorporated in foodstuffs except by design (Dammin 1972). Another aspect of the natural occurrence of potential nephrotoxins of presumably plant origin is the finding of up to two hundred times the admissible limit of phenolic compounds in the well waters used by patients with the disease (Mustață *et al.* 1971).

The theory that fungal toxins might be causally involved seems to hold greater promise than most since it was first mooted by Dimitrov (1960) and reiterated by Barnes (1967). Preliminary survey of 200 samples of 54 different types of food from the endemic areas has shown that there are certainly toxin-producing fungi in the environment. Twenty-six species of fungi were isolated and are currently being tested for toxin production. So far ochratoxin has been found in a small number of wheat samples and zearalenone in maize (Krogh & Peberdy 1972, personal communication).

Ochratoxin is of great potential significance because it is the cause of a comparable nephrotoxicosis of pigs in Denmark, which develops after feeding with barley invaded by *Penicillium viridicatum* Westling (Krogh *et al.* 1974). Zearalenone (also known as F_2) is an œstrogenic compound produced by *Fusarium* spp. which invade maize grains on the cob around harvest time. Samples collected in endemic villages from the 1970 harvest have shown high levels of this substance, which is responsible for outbreaks of vulvovaginitis in pigs and is thought to be associated with other reproductive disorders (Mirocha *et al.* 1967). Reproductive problems of this type have been reported from the region by Ošegović (1970). Parallel with the known carcinogenic action of other œstrogenic substances, it is conceivable that there might be a connexion with the high incidence of urinary tract carcinoma in Balkan nephropathy (Schoental 1974).

Correlation with Weather

The possible role of fungal metabolites and the intimate connexion between the growth of fungi and the weather during crop growth and harvesting, led to an investigation of the morbidity and mortality from endemic nephropathy in relation to climatic conditions. A pilot study on Yugoslavian data showed highly significant correlations ($r=0.80$) between the amount of late summer and autumnal rain and the number of people who die during the succeeding two years (Austwick & Smith 1975). Further analysis showed that the most significant weather factor was the excess of rainfall over evaporation summed over the four months from August to November (Smith & Austwick, unpublished). This gives some numerical indication of the length of time that a surface is likely to remain wet or damp and hence suitable for fungal growth. The existence of morbidity data enabled an estimation to be made of the number of people in the affected locality at maximum risk and this together with the weather factor (excess rain) provided an accurate forecast of the deaths occurring during the following calendar year.

A similar weather factor could be calculated for the one area in Bulgaria for which mortality

but not morbidity data were available. The excess rain for each of two successive years was therefore used on the assumption that this would partially replace the lack of morbidity data, and it was then found that a clear relationship existed with the number of deaths in the succeeding two years. No satisfactory estimate of evaporation in Romania could be made, so that the total rainfall for September, October and November was used as a parameter. August rainfall was omitted because no heavy rain fell in this month during the years of the sample. The totals of each of two successive years were again used to allow for the absence of morbidity data but a significant difference was found from the results of the Bulgarian analysis. The deaths appeared to be occurring earlier and the rainfalls of years 1 and 2 were most closely linked statistically to the deaths occurring in years 2 and 3 plus half the number in year 4. Some corroboration of the nature of this difference emerged from the general observation that in one location in Romania the interval between diagnosis and death was much shorter than the Bulgarian average. Although the precise nature of the disease-weather relationship seems to vary in detail from country to country, the dominant weather factor of a damp autumn was common to each of the three numerical investigations. Furthermore the regression equations were verified successfully by the use of independent data for succeeding years. Unless, therefore, a most unusual succession of weather-disease coincidences has occurred, it is reasonable to deduce that some biological event associated with damp autumnal conditions, either in the field or in storage of food, or in both, is an etiological factor in the occurrence of the disease. It is also interesting to note that the geographical siting of the affected villages is such that long periods of high humidity may be expected.

Discussion

Speculation that an unknown toxin of fungal origin could be the cause of Balkan nephropathy would explain several epidemiological anomalies of the disease. If an intake of the toxin leads to a permanent loss of nephrons and this loss is proportional to the amount of the substance ingested, then in the year following a wet harvest the increased level of toxin would be likely to exert its influence on a greater proportion of the population at risk, i.e. those who are approaching the fatal threshold of a loss of 80% of their nephrons. It cannot be disputed that the disease can kill people who have long since moved away from the affected areas, but exposure to a toxic agent in quantities governed by the year-to-year harvest weather might account for the close correlation between mortality from Balkan nephropathy and the late summer and autumn rainfall in all three countries.

At the present time it seems prudent to keep an open mind on the etiology of Balkan nephropathy and perhaps concentrate on the comparison of the disease in the different areas in an effort to find a common underlying set of environmental features differing from those in so-called non-endemic areas in the region. Standardization of methods and a greater exchange of data, material and views would be major contributions to the final solution of this tragic disease problem.

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