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Annotations

Coronaviruses in Balkan nephritis

Viruses found in the kidneys were for a long time considered trivial symbionts, a viral flora. Only recently has it been recognized that these viruses might be subtle pathogens, possibly involved in slow initiation of progressive renal failure.¹ Electron microscopic studies have revealed the presence of virus-like particles in the kidneys of Balkan nephritis (BN) patients,^{2, 3} suggesting the implication of coronaviruses in renal pathology.¹ However, the presence of viruses in tissues does not alone establish causation.⁸ The involved virus could not be isolated as yet, but even the successful recovery of an agent from the kidneys would provide a very low order of evidence for the purpose of proving causality.⁴ In an earlier paper⁷ we described our program of work on the possible viral etiology of BN and presented negative results on attempts at virus isolation and identification of antibody reactivity to selected viral antigens. Because of the suggestion that BN in man might be caused by a slow porcine coronavirus infection,¹ this second progress report of our results is in order.

In the absence of virus isolations, emphasis has been placed on seroepidemiological investigations.⁵ Antibodies have been measured by complement binding, hemagglutination inhibition, and by single radial hemolysis in gel^{10, 11} in serum specimens of 34 BN patients, their relatives sharing their housing and living conditions, and of 67 controls, matched for age and sex.⁵ Sera from 21 pigs kept by patients were also examined. All sera were heat inactivated before testing. Antigens used in this survey were prepared in our laboratory with human, avian, and porcine strains of coronavirus.* The prevalence of antibodies to pig coronavirus was tested with the hemagglutinating encephalomyelitis virus 2063/68 strain. This strain was originally isolated from piglets with vomiting and wasting disease, and it is closely related to, if not identical with, the hemagglutinating encephalomyelitis virus of swine, and also related antigenically to the transmissible gastroenteritis virus of pigs.

None of the pigs from which serum was taken had either vomiting and wasting disease, transmissible gastroenteritis, infantile pyloric stenosis, or encephalitis. Antibodies to the 2063/68 virus strain were found in serum from only one animal, at a titer of 40. Human sera, whether of BN patients or of controls, yielded constantly negative results with this virus. Further investigations with the use of the OC 43 and the 229 E strain of human coronavirus, and of the Beaudette and the Massachusetts 41 strain of avian infectious bronchitis virus revealed so far no statistically significant differences of the incidence of antibodies, the geometrical mean titer, or the frequency of elevated titers, between BN and control sera.

These findings must be interpreted with caution because of

the antigenic diversity of coronaviruses, of the lack of antigens for strains having fastidious growth requirements, and because of the necessity to perform different assay procedures according to the various antigens used. The complement fixation reaction seems rather unsuitable for serosurveys, since complement fixing antibodies are relatively short-lived. Neutralization is effective but expensive, slow and tedious, while the hemagglutination inhibition test is impracticable with non-hemagglutinating coronavirus strains. Moreover, in a chronic viral infection there is always the possibility that antibody production may be masked by complete absorption of circulating antibody by excess antigen. It has to be remembered that the immunofluorescence test actually disclosed in the kidneys of BN patients a granular fluorescence along the basement membrane of the glomeruli,⁶ but the nature of the deposited immune complexes has not been investigated.

Coronaviruses are emerging as important causes of common cold-like illness and are assumed to be involved in non-bacterial gastroenteritis in humans. Lately, their possible role in chronic renal disease has also been suggested.¹ Understanding of the behavior and role of these viruses has been hampered greatly by the difficulties in propagation encountered in the laboratory. If the agent involved in the etiology of BN is an animal RNA virus, then it would account for the predominant rural distribution of the disease.⁶ Our results indicate that the examined BN patients have had no contact with porcine coronaviruses, and they have experienced infections with human and avian coronavirus strains in the same manner, and at a rate similar to that found in the control group. It appears therefore, that the virus-like particles found in renal tubular epithelial cells of BN patients^{1, 3} are of an antigenically unrelated strain, or a different type. The suspected natural focality of BN^{2, 3} (involving for instance some rodent coronavirus) would explain the restricted geographical distribution of this disease, and its dependence on ecological factors.^{4, 6} Since the mouse brain-grown strain OC 43 consistently fixes complement at low levels with antisera hyperimmune to various strains of mouse hepatitis virus, it seems unlikely that this virus would be involved either. Further search for corona- and other RNA viruses of rodents in the endemic area, screening of human sera for antibodies to such strains, and studies to identify their role in BN seem indicated. At the present time the role of coronaviruses in BN remains uncertain.

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REFERENCES

1. Apostolov, K., Spasic, P., and Bojanic, N.: Evidence for viral aetiology in Endemic (Balkan) nephropathy, *Lancet* **2**:127, 1975.
2. Austwick, P. K. C.: Balkan nephropathy, *Proc. R. Soc. Med.* **68**:219, 1975.
3. Diosi, P., Plavoşin, L., and Arcan, P.: Propagation of mouse cytomegalovirus in murine kidney epithelium, *Rev. Roum. Virol.* **25**:91, 1974.
4. Georgescu, L., and Diosi, P.: Balkan nephritis: a synthetic view of 50 studied cases, *Morphol. Embryol. (Buch.)* **22**:13, 1976.
5. Georgescu, L., Diosi, P., Buţiu, I., Plavoşin, L., and Herzog, G.: Porcine coronavirus antibodies in Endemic (Balkan) nephropathy, *Lancet* **1**:163, 1978.
6. Georgescu, L., Diosi, P., Buţiu, I., Plavoşin, L., and Herzog, G.: Viruses and causation of Balkan nephropathy, *Ciba Foundation Symposium on Balkan nephropathy*, London, June, 1978.
7. Georgescu, L., Litvac, B., Diosi, P., Plavoşin, L., and Herzog, G.: Viruses in Endemic (Balkan) nephropathy, *Lancet* **1**:1086, 1976.
8. Georgescu, L., Litvac, B., Diosi, P., Plavoşin, L., and Herzog, G.: Viruses in Balkan nephritis, *AM. HEART J.* **94**:805, 1977.
9. Georgescu, L., Litvac, B., Manescu, N., Petrovici, A., Schwartzkopf, A., and Zosin, C.: Particules virales dans la rein de la néphropathie endémique, *Sem. Hop. Paris* **46**:3526, 1970.
10. Riski, H., Hovi, T., Väänänen, P., and Penttinen, K.: Antibodies to human coronavirus OC 43 measured by radial haemolysis in gel, *Scand. J. Infect. Dis.* (In press).
11. Schild, G. C., Pereira, M. S., and Chakraverty, P.: Single-radial-haemolysis: a new method for the assay of antibody to influenza haemagglutinin. Applications for diagnosis and seroepidemiologic surveillance of influenza, *Bull. WHO* **52**:43, 1975.
12. Tomescu, E.: Constatarea de particule de tip viral într-un caz de nefropatie endemica familiala, *Morfol. Norm. Patol. (Buch.)* **15**:365, 1970.

Cigarette smoking and coronary heart disease: new evidence and old reactions

Although there is little doubt that cigarette smoking is a causal factor in lung cancer and chronic obstructive lung disease, there has been less agreement about the causal nature of the often-found relation between cigarette smoking and coronary heart disease (CHD), for the following reasons. The relation between smoking and CHD has not generally been strong—usually smokers have shown about a twofold excess incidence over non-smokers, in contrast with the tenfold increase for lung cancer. Relatively weak associations are often attributable to some underlying characteristic. Thus, it has been proposed that some constitutional or genetic factors are present in the smoker that both lead him or her to smoke and predispose to CHD. This "counterhypothesis" has had some distinguished support, going back, at least, to the noted statistician, R. A. Fisher, who was primarily concerned with smoking and cancer.¹ Another problem with the evidence concerning smoking and CHD is that the relationship is not found in all study populations. For example, in the seven-country collaborative study of Keys and associates,² U. S. railroad workers showed the smoking-CHD relationship, but using similar data collection methods, men in Finland, the Netherlands, Italy, Greece, Yugoslavia, and Japan did not, and these countries range from low to high CHD incidence. Another troublesome finding is that cigarette smoking tends to be a weaker predictor of CHD in older persons than in young and middle-aged adults. While this is true of other risk factors, too, it does not seem consistent with the generally held notion that smoking acts to induce clinical CHD largely by precipitating acute events such as myocardial infarction and sudden cardiac death.³ Older persons with advanced atherosclerosis should be especially susceptible to this effect.

Then again, the mechanism by which smoking promotes CHD has not been well established, although vascular damage from carbon monoxide, deleterious effects of increased catecholamine release, and promotion of thrombotic tendencies have been proposed. Finally, data from studies of smoking-discordant identical twins where the smokers and nonsmokers are genetically the same have not shown the degree of association between smoking and CHD as has been found in the general population, where there is obviously no such genetic matching.^{4, 5} Nevertheless, because of small numbers of subjects, the twin data to date are too meager to be conclusive in this regard and a number of methodologic questions have been raised about these studies.^{6, 7} Similar considerations apply to the association of smoking with total mortality, of which deaths from CHD (but not from lung cancer or bronchitis) constitute a major component.

Probably the only study design that would convince virtually everyone that cigarette smoking itself does contribute to CHD would be a large-scale controlled experiment in which young healthy persons were randomly assigned to smoke or not and in which they obeyed their assignment and were followed up over most of their lifetimes for CHD development. Such a study would be unethical and impossible to carry out. Therefore, we must do the best we can with observational rather than experimental studies.

We recently published an observational study of the relationship of cigarette smoking to total and CHD mortality, that took advantage of a large body of data that was collected on 4,004 middle-aged cigarette smokers, ex-smokers, and non-smokers who responded to periodic urgings to take multiphasic health checkups.⁸ Because of the recruitment effort,