1. Howard, J. G., Brit. J. Exp. Path., 1961, v42, 72.
2. Schooley, J. C., Kelly, L. S., Dobson, E. L., Finney, C. R., Havens, V. W., Cantor, L. N., Res., 1965, v2, 396.
3. Miller, J. F. A. P., Howard, J. G., ibid., 1964, v1, 369.
4. Troup, G. M., Walford, R. L., Transplantation, 1967, v5, 43.
5. Sjodin, K., Dalmasso, A. P., Smith, J. M., Martinez, C., ibid., 1963, v1, 521.
6. Parrott, D. M. V., East, J., Nature, 1962, v195, 347.
7. Miller, J. F. A. P., Proc. Roy. Soc. Biol., 1962, v156, 415.
8. Brumfitt, W., Glynn, A. A., Brit. J. Exp. Path., 1961, v42, 408.
9. Barnes, J., ibid., 1940, v21, 264.
10. Suu, V. T., Congdon, C. C., Kretchmar, A. L., Proc. Soc. Exp. Biol. & Med., 1964, v115, 825.
11. Congdon, C. C., Kretchmar, A. L., Exp. Mol. Path., 1963, v2, 277.
12. Perri, G. C., Faulk, M., Shapiro, E., Money, W. L., Proc. Soc. Exp. Biol. & Med., 1964, v115, 189.
13. Bealmear, P. M., Wilson, R., Exp. Hematol., 1966, v12, 37.
14. Zinbar, S. N., Svet-Moldavsky, G. J., Nature, 1967, v214, 295.
15. de Vries, M. J., van Putten, L. M., Balner, M., van Bekkum, D. W., Rev. franc. études clin. biol., 1964, v9, 38.
16. Brézin, C., Cannat, A., Sekiguchi, M., ibid., 1965, v10, 839.

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Coxsackie B_4 Viral Nephritis in Mice and Its Autoimmune-Like Phenomena.* (32595)

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During a recent investigation on viral valvulitis in mice produced by Coxsackie B_4 virus(1), it was noticed that, besides in the valves and other tissues, antigen was found in the renal glomeruli and tubules of several chronically infected mice. Glomerulonephritis and tubular necrosis were noted on histologic examination in those animals where the presence of virus could be confirmed. It has long been thought that some renal glomerular diseases have an immunologic basis as demonstrated by the presence of gamma globulin and a component of complement believed to represent evidence of a hypersensitivity mechanism(2,3). However, little is known concerning the primary etiologic agent responsible for the provocation of hypersensitivity reaction in such cases.

A report of autoimmune reaction and the presence of virus-like particles in germ-free NZB mice by East *et al*(4) suggests the possible role of virus infection in "triggering" an autoimmune disease. Renal lesions similar to chronic membranous glomerulonephritis or lupus nephritis were frequent in these mice (5,6). The present report concerns the localization of globulin and Coxsackie B_4 antigen in the kidney of experimentally infected mice by use of the immunofluorescent technique.

Materials and methods. 36 HaM/ICR mice weighing 15-20 g were inoculated intraperitoneally with 0.1 ml of monkey-kidney culture fluid containing 10^5 $TCID_{50}$ of Coxsackie virus B_4(7). 24 littermates as controls were injected intraperitoneally with 0.1 ml of virus-free monkey-kidney culture fluid. The experimental and control animals were killed at weekly intervals for 8 weeks following the injection. Kidneys were then studied for histopathology and immunofluorescence of Coxsackie B_4 viral antigen and deposited globulin.

The direct immunofluorescent technique(8) was used to identify the viral antigen and

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globulin in the kidney tissue. Hyperimmune rabbit antiserum against Coxsackie B_4 virus and goat antiserum against mouse globulin, both labeled with fluorescein, were used along with frozen sections of kidneys from the experimental and control animals. Rabbit antiserum against cell proteins of the virus-free monkey-kidney culture medium also labeled with fluorescein was used for control staining. Special precautions were taken to reduce nonspecific background staining(9).

The specificity of the immunofluorescent staining was confirmed in several ways: a) renal tissue from control animals displayed no specific fluorescence of either the viral antigen or globulin except a weak nonspecific fluorescent staining in the glomeruli and the tubular epithelial cells; b) the specific fluorescent staining of viral antigen was absent when the labeled rabbit antiserum against proteins of virus-free monkey-kidney culture medium was substituted; c) the staining reaction of globulin was absent when fluorescein-labeled heterologous serum (sheep antiserum against rabbit globulin) was substituted for labeled goat antiserum against mouse globulin.

Results. At the end of the 1st and 2nd weeks after inoculation of Coxsackie B_4 virus there were no conspicuous histologic changes in the renal tissue. Distension and engorgement of glomerular tufts with red blood cells were the only consistent findings. Coxsackie virus antigen was not noticed on immunofluorescence study and only weak fluorescent staining was seen in a few glomeruli when the labeled goat antiserum against mouse globulin was employed.

At the end of the 3rd and 4th weeks after infection some of the glomeruli were apparently swollen with adhesion of the capillary tuft to Bowman's capsule. However, no cellular exudate was noted either in the glomeruli or tubular interstices. Spotty, apparently intracytoplasmic immunofluorescence specific for Coxsackie B_4 virus was found in a few glomeruli of the infected mice. Fluorescent stain for globulin in glomeruli became more prominent. Seemingly, globulin was deposited along the basement membrane of glomerular tuft.

5-8 weeks after Coxsackie B_4 virus inoculation increased cellularity of most of the glomeruli with capillary congestion and basement adhesion was noted (Fig. 1). Focal dense aggregation of plasmal and lymphocytic cells was frequently seen in the tubular interstices (Fig. 2) and in the surroundings of major blood vessels of hilum. More often, specific immunofluorescence for globulin and the virus was seen in the affected glomeruli (Fig. 3). The fluorescent viral antigen seemed to be localized mostly in the cytoplasm of endothelial cells of the glomeruli. The fluorescent-positive globulin displayed a globular pattern and irregular crescent formation in the glomeruli (Fig. 4).

Discussion. The most significant finding of the present study was the demonstration of both the specific viral antigen and immunoglobulin in the renal glomeruli of Coxsackie B_4 virus infected mice. The similar concurrent presence of the viral antigen and immunoglobulin may have an etiologic or pathogenic role in certain types of glomerulonephritis in man.

Approximately 20% of cardiac output flows through the kidneys. Consequently, during viremia filtering, trapping and fixing of the virus particles in the kidney is extremely likely. Since kidney cells of certain mammals such as primates present good tissue culture material for many viruses infectious for man and animals, viruses that enter kidney tissue could probably grow and damage the kidneys seriously.

There are 2 possible sources of globulin deposited in the glomeruli. One could be a circulating antigen-antibody complex, the antigen bound immunoglobulin which is trapped in the capillary tufts of glomeruli and other areas of the kidney. The precipitation of antigen-antibody complex in glomerular tufts is considered to be responsible for renal damage in malaria infection(10,11). Another source could be the autoimmune globulin sensitive to renal glomerular tissue. East *et al*(4) suggested that the virus could act directly as an endogenous antigenic stimulus to abnormal immunologic activity or, by acting directly, modify either tissue antigen or lymphoid cell function and thereby evoke autoimmune reactions. The fact that histologic examination of the kidney of several infected mice 7 and 8 weeks after infection revealed dense accumu-

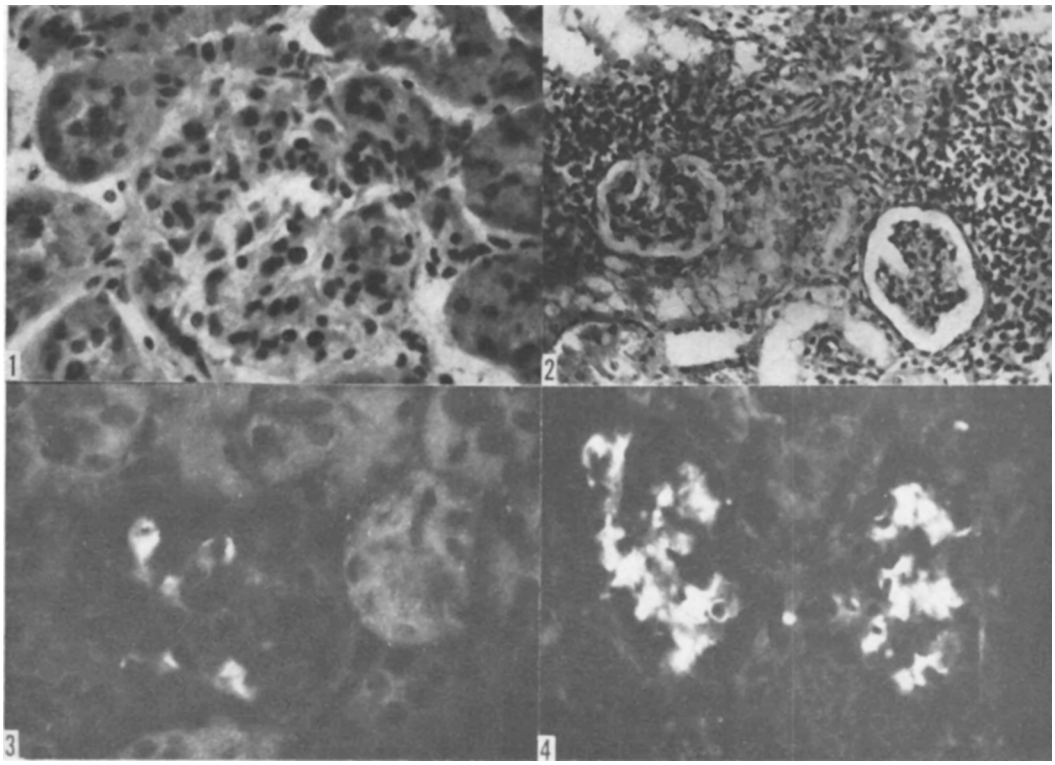

FIG. 1. An enlarged glomerular tuft completely fills the capsular space. Note hypercellularity of the glomerulus and adhesions in Bowman's capsule. From an infected HaM/ICR mouse 6 weeks after inoculation of Coxsackie B_4 virus. Hematoxylin-eosin stain × 780.

FIG. 2. Heavy aggregation of plasmal and lymphocytic cells in the renal interstices with destruction of tubules. Note the thickened Bowman's capsule of the glomerulus at the left side and hypercellularity of the right one. From a Coxsackie B_4 virus infected HaM/ICR mouse 7 weeks after inoculation. Hematoxylin-eosin, × 200.

FIG. 3. Bright, spotty, intracytoplasmic fluorescence apparently present in the endothelial cells of a glomerular tuft stained with anti-Coxsackie B_4 virus rabbit serum labeled with fluorescein. From a Coxsackie B_4 virus infected HaM/ICR mouse 6 weeks after inoculation. × 780.

FIG. 4. Bright glomerular fluorescence localizing deposited globulin stained with anti-mouse-globulin goat serum labeled with fluorescein. From a Coxsackie B_4 virus infected HaM/ICR mouse 7 weeks after inoculation. × 480.

lation of lymphocytic and plasmal cells in the renal interstices and in the surroundings of major blood vessel walls provides further evidence of a characteristic tissue response during hypersensitivity phenomenon.

Studies of renal disease by immunofluorescent methods have demonstrated gamma globulin, component of complement and fibrin in a variety of glomerular diseases. These materials have been thought to play a significant role in the pathogenesis of the glomerular diseases(2,3). However, a type of nephrotropic virus has not been considered as a primary etiologic source of the disease. Most adult people with chronic nephritis give no history of previous acute renal disease. There seems to be no fully established connection between post-streptococcal acute nephritis in children and chronic nephritis in adults(12). It is generally assumed that chronic renal disease in adults begins with an unrecognized attack of acute nephritis during childhood precipitated by streptococcal infections. The frequent occurrence of viral infection during childhood and later periods of life and our recent observations of clinically occult Coxsackie B viral myocarditis in infants and children at routine autopsies(13,14) introduce a strong possibility that inadequately defined or unrecognized viral infections of the kidney may be etiologically concerned with renal disease in the later life of these patients.

Latent viruses were able to be recovered by cell cultures from kidney tissue of children (15). Chronic and latent infections with "slow" viruses such as the CHINA viruses can produce many peculiar infections in mammals(16) that resemble diseases in man. Aleutian disease in mink which is transmittable by a filtrable agent manifests glomerular lesion with precipitated materials of possible immunologic basis in the capillary tufts similar to membranous and lupoid glomerulonephritis(16,17). This disease was also reported in man(17). A type of hemorrhagic fever is accompanied by bilateral massive damage of kidneys(18). In the light of these observations it is possible that Coxsackie B viruses are responsible for some renal diseases in animals and man.

Summary. Coxsackie B_4 virus may be highly nephrotropic. It may produce a viral nephritis and provoke an autoimmune-like reaction in the affected renal tissue of the experimentally infected mice. In this study, by means of immunofluorescent technique, both the specific viral antigen and immunoglobulin deposit were demonstrated in the glomeruli of infected mice within 5 to 8 weeks after inoculation with Coxsackie B_4 virus. It is suggested that the virus may play a significant etiologic role in the pathogenesis of acute and chronic renal disease in man, of which disease the etiologic source is often unknown.

1. Sun, S. C., Colcolough, H. L., Burch, G. E., DePasquale, N. P., Sohal, R. S., The immunofluorescent study of histopathogenesis of coxsackie B_4 viral valvulitis in mice. Proc. Soc. Exp. Biol. & Med., 1967, v125, 157.

2. Freedman, P., Markowitz, A. A., Gamma globulin and complement in diseased kidney, J. Clin. Invest., 1962, v41, 328.

3. McCluskey, R. T. Vassalli, P., Gallo, G., Baldwin, D. S., An immunofluorescent study of pathogenic mechanism in glomerular disease. New Engl. J. Med. 1966, v274, 695.

4. East, J., Prosser, P., Holborow, E. J., Jaquet, H., Autoimmune reactions and virus-like particles in germ-free NZB mice. Lancet, 1967, v1, 755.

4. Helyer, B. J., Howie, J. B., Spontaneous autoimmune disease in NZB/BL mice. Brit. J. Haemat., 1963, v9, 119.

6. Hicks, J. D., Burnet, F. M., Renal lesions in the "auto-immune" mouse strains NZB and F1 NZBXNZW. J. Path. Bact., 1966, v91, 467.

7. Burch, G. E., DePasquale, N. P., Sun, S. C., Hale, A. R., Mogabgab, W. J., Experimental coxsackie virus endocarditis, J.A.M.A., 1966, v196, 349.

8. Coon, A. H., Kaplan, M. H., Localization of antigen in tissue cells. J. Exp. Med., 1950, v91, 1.

9. Cherry, W. B., Goldman, M., Carski, T. R., Moody, M. D., Fluorescent antibody technics in the diagnosis of communicable disease, Washington, D.C., United States Public Health Service, Publication 1960, 729.

10. Ward, P. A., Conran, P. B., Immunopathologic studies of Simian malaria. Milit. Med., 1966 (supp.), v131, 1225.

11. Dixon, F. J., Comments on immunopathology, Milit. Med. 1966 (supp.), v131, 1233.

12. Barnett, H. L., Paediatric nephrology: Scientific study of kidneys and their disease in infants and children. Arch. Dis Child., 1967, v41, 229.

13. Burch, G. E., Sun, S. C., Colcolough, H. L., Sohal, R. S., DePasquale, N. P., Coxsackie B viral myocarditis and valvulitis identified in routine autopsy specimens by immunofluorescent techniques. Amer. Heart J., 1967, v74, 13.

14. Burch, G. E., Sun, S. C., Chu, K. C., Sohal, R.S., Interstitial myocarditis and coxsackie B viral myocarditis in infants and children. A comparative histologic and immunofluorescent study of 50 autopsied hearts. JAMA, in press.

15. Benyesh-Melnick, M., Rosenberg, H. S., Watson, B., Viruses in cell cultures of kidney of children with congenital heart malformation and other diseases. Proc. Soc. Exp. Biol. & Med., 1964, v117, 452.

16. Karstad, L., Aleutian Disease. A slowly progressive viral infection of mink. Current Topics in Microbiology and Immunology, New York, Springer-Verlag, Inc., 1967, v40, 9.

17. Chapman, I., Jimenez, F. A., Aleutian-mink disease in man. New Engl. J. Med., 1963, v269, 1171.

18. Smorodintsev, A. A., Kazbintsev, L. I., Chudakov, V. G., Virus Hemorrhagic Fevers. (Virusnye gemorragicheskie likhoradki) Published for the National Library of Medicine, U.S. Public Health Service through the National Science Foundation, Washington, D.C.

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