

arbor viruses in tissue culture. Plaque-forming unit endpoint titrations were generally higher than the cytopathogenic endpoint (TCD₅₀) even when CPE was evident, or the endpoint (LD₅₀) in infant mice inoculated intracerebrally. It is suggested that the tube overlay technic may be an aid in large scale screening of clinical or field specimens and in conducting serum neutralizing antibody surveys for many arthropod-borne viruses.

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Investigation of Rejection of Canine Renal Homotransplants by Fluorescent Antibody Technic.* (24903)

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It is widely held that rejection of transplants of homologous tissue results from an acquired immunity expressed by host to donor tissue. However, identification and classification of this type of immune process has remained enigmatic. Attempts to relate circulating antibody with homograft rejection have been conflicting(1,2,3). No changes in serum complement(3) or properidin(4) have been found. It appeared worthwhile to study renal homotransplants in various phases of rejection for presence of recipient's globulin by fluorescent antibody technic of Coons and Kaplan(5). Utilization of so-called indirect modification of the latter also allowed for possible detection of circulating antibodies in recipient's serum.

Materials and methods. Renal homotransplantation was performed on adult mongrel dogs by uniform surgical procedure used in renal homotransplant investigations. The right kidney and a segment of ureter of donor was severed with a cuff of aorta and vena cava attached to renal artery and vein respectively. After removal of recipient's right kidney, anastomosis of the vascular cuff with recipient's aorta and vena cava just above tri-

furcation was performed using standard vascular anastomotic technics. The ureter was implanted into recipient's urinary bladder. Left kidney of recipient was removed either simultaneously or 24 hours later through separate flank incision. Renal homotransplants were obtained by sacrificing 10 dogs prior to exhibition of rejection as indicated by normal BUN, 8 in an early phase of rejection (moderately elevated BUN) and 8 with moderate to marked elevation of BUN, often of several days duration. Portions of transplanted kidneys and their renal arteries, as well as from untreated controls were quickly frozen on dry ice. Sections were cut at 5 μ in a cryostat and stained with antisera containing either anti-dog globulin (anti-DG), anti-dog albumen (anti-DSA), or anti-dog fibrinogen (anti-DF) which had been conjugated with fluorescein isocyanate. Antisera were obtained by immunizing rabbits with fractionated canine plasma proteins prepared by continuous flow electrophoresis. Technical controls consisted of sections similarly treated except that they were first flooded with their respective non-fluorescent antisera. In some instances sections were stained with non-fluorescent anti-DG prior to application of anti-DF. Alternate frozen sections as well as those prepared after routine formaline fixation were stained with hematoxylin and eosin. In the indirect

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study, sections of normal dog kidney or those prepared from donors remaining kidney were flooded with serum from 8 dogs with transplanted kidneys. Four of these animals exhibited overt transplant rejection and 4 were sacrificed 9 days after transplantation although there was no clinical or biochemical evidence of active rejection. Ultraviolet microscopy was performed with mercury lamp (Scopicon) utilizing Corning exciter filter # 5840 and Wratten 2A barrier filter.

Results. Morphologic alterations in homotransplanted canine kidneys removed prior to, at beginning and terminal phases of rejection were similar to those recounted by others (6,7). Prior to onset of rejection such kidneys appeared histologically normal or exhibited only moderate tubular degeneration and stromal infiltrate of histiocytes, lymphocytes, plasma cells and neutrophils. These changes appeared more severe in animals sacrificed during early periods of rejection and were often accompanied by focal or confluent focal zones of cortical necrosis. In addition, transplanted kidney of animals sacrificed in extremus with elevated BUN, exhibited marked neutrophil infiltration, broad zones of parenchymal lysis and occasional fibrinoid masses within interlobular renal arteries and their smaller branches and less frequently within glomerular tufts (Fig. 1). Sections of major renal arteries were histologically normal. Examination of sections of homotransplanted kidneys and major renal arteries by fluorescent antibody technic failed to reveal preferential localization of canine globulin although the latter was noted at expected sites in control sections. Specific fluorescence did not appear more intense in the inflammatory infiltrate of rejected kidneys than in similar cells not infrequently noted in interstitium of control kidneys. The fibrinoid material in interlobular and arteriolar branches of renal artery and less frequently in glomeruli of such kidneys disclosed specific fluorescence when stained only with anti-DF (Fig. 2).

No affinity for normal renal structure could be detected in sections examined with sera from animals exhibiting transplant rejection by indirect technic.

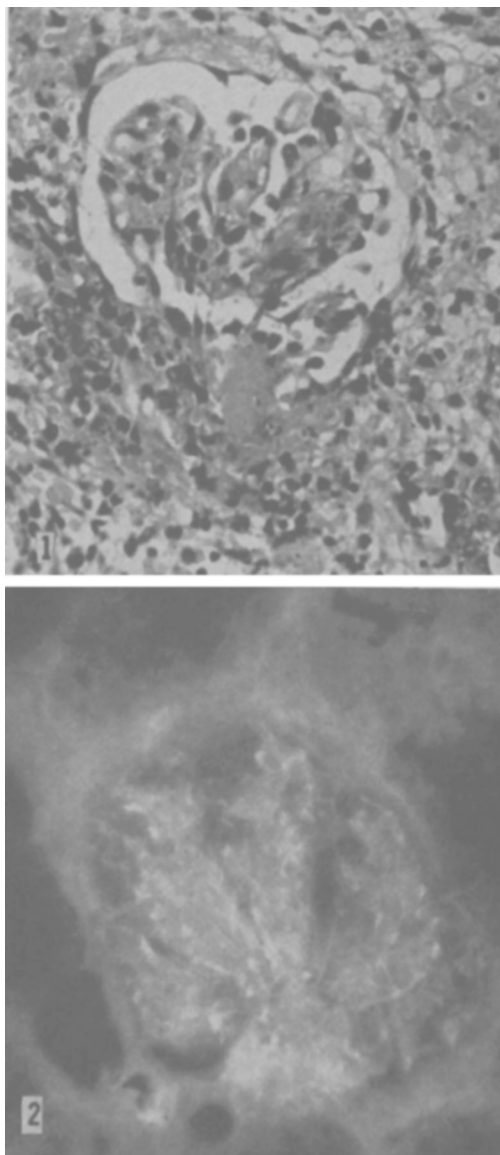


FIG. 1. "Fibrinoid" in glomerular arteriole with extension into capillary tufts. (H. & E. $\times 280$.)

FIG. 2. Fluorescence of material observed in 1 with anti-dog fibrinogen ($\times 280$).

Discussion. Failure to demonstrate abnormal concentrations of globulin in renal homotransplants is consistent with the failure of most workers to detect serum antibodies in this phenomenon, but does not unequivocally indicate their absence. It is conceivable that either antibody in extremely low concentration or antibody with little avidity might not

be detected by this technic. These findings do not exclude the possibility that homotransplant rejection may be of the tuberculin or cellular hypersensitivity type.

Macroscopic and histologic findings of various stages of renal homotransplant rejection are sequentially similar to those observed in renal cortical necrosis associated with certain obstetrical complications and less frequently in men, non-pregnant women and children(8, 9). It resembles the appearance of kidneys in generalized Shwartzman reaction. Demonstration of fibrin composition of vascular and glomerular fibrinoid in some examples of homotransplant rejection is also in keeping with the identification of this material by this immunohistochemical technic in the latter phenomenon by Vazquez and Dixon(10). However, unlike the Shwartzman reaction fibrin thrombi appear to represent a secondary manifestation, being relatively rare and only found in kidneys of those animals considered to be in the terminal phase. Also, we have not observed alterations in factors concerned with blood coagulation in animals experiencing renal homotransplant rejection, but have been considered of pathogenetic significance in the Shwartzman reaction(11). The lack of morphologic or immunohistochemical alterations of major renal arteries does not exclude the possibility that vasospasm of these structures may play a role in pathogenesis of lesions encountered in cortical necrosis as proposed by Sheehan and Moore(9) and demonstrated in renal homotransplantation rejection arteriographically by Dempster(7). Its primacy in this phenomenon remains to be elucidated, however.

Summary. No preferential localization of globulin was noted in rejected homotransplanted canine kidneys studied by fluorescent antibody technic. Indirect variation of this method similarly failed to disclose antibodies exhibiting an affinity for renal structure in serum of recipients exhibiting rejection of renal homotransplants. The fibrin nature of fibrinoid lesions occasionally noted in terminal phases of the rejection phenomenon have been demonstrated. Relationship of pathologic and immunohistochemical alterations of renal homotransplantation rejection and the generalized Shwartzman phenomenon is discussed.

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