Grids were then lifted to coat them with carbon. Excess water was removed from the grid and forceps by blotting with filter paper from the under side of the grid. Coated grids were then placed on a piece of filter paper, gridside down, and allowed to dry for at least an hour prior to use.

Results. The carbon disc substrates were made slightly larger than the standard grid on which they were deposited by employing 4 mm glass rod. This had the advantage of permitting the carbon film to fold around the edge of the grid as it emerged from the water and thus assist in banding carbon to grid.

Although the surfaces of the fractured glass rods were slightly contoured, this was not reflected in the surface of the carbon films when floated on water or when viewed in the electron microscope. As is the case with carbon films that are stripped from a mica surface, films made as described above appear rather rough to the naked eye as they float on the surface of the water.

Experiment showed clearly that only soft glass could be used as a substrate from which carbon could be floated off after deposition. Attempts to remove carbon from pyrex rod by flotation were completely unsuccessful. If sections of soft glass rod were made by cutting with a glass saw, these also proved unsatisfactory as a base on which to evaporate carbon.

The thickness of the carbon film deposited on the glass rods was evaluated by noting the color of the carbon deposited on a piece of white porcelain on which was placed a small drop of vacuum oil. Films could be successfully removed from the glass rods when the porcelain indicator showed a faint brown color indicating a thickness of about 100 Å of carbon.

In addition to the simplicity and convenience of this method of making carbon substrates it should also be pointed out that after deposition of carbon on glass rods these may be held for a period of at least one to two months before removing the carbon without any apparent deterioration in the properties of the film. It is important, however, to evaporate carbon on the glass rods shortly after they are cut. If rods are permitted to stand for about a day prior to evaporation of carbon on their surfaces, the films tend to shred and adhere to the glass.

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Urine and Serum Lysozyme Measurement in Renal Homotransplantation.* (30641)

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The major cause of failure in renal homotransplantation, aside from technical difficulties, is rejection of the homograft. Although several signs of ascertaining rejection of the transplant may be present, none is specific and the diagnosis usually is made on the basis of cumulative laboratory and clinical evidence. Recently, several complicated and time-consuming procedures for detection of homograft rejection have been advocated (1-3) but most have proved to be impractical.

Several investigators(4-6) have demonstrated that increased urinary lysozyme activity occurs in patients with a variety of types of kidney disease. Because of the rapidity (20 minutes) and ease of lysozyme assay and since such measurements have been proposed as "an important tool for estab-

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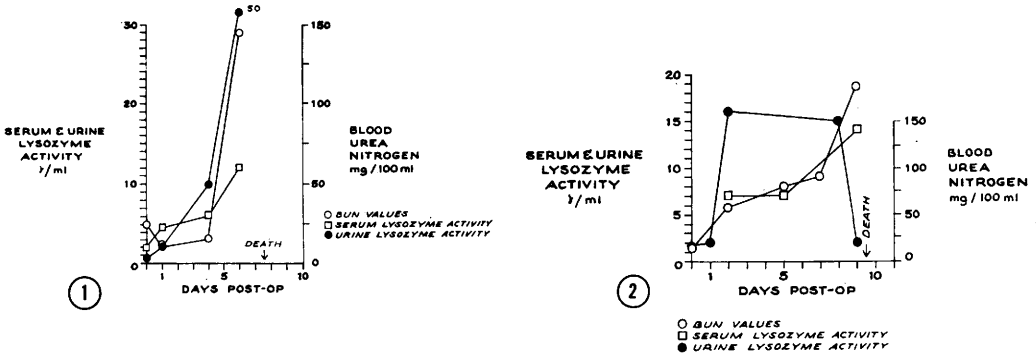


FIG. 1. Lysozyme (urine and serum) and BUN values after renal homotransplantation in dog #1. A marked increase in urinary lysozyme activity occurred on day 4 although no significant change in BUN was observed on this day.

FIG. 2. Lysozyme (urine and serum) and BUN values after renal homotransplantation in dog #2. A precipitous decrease in level of urinary lysozyme was observed immediately prior to death.

lishing the diagnosis and prognosis of renal lesions"(6) the efficacy of such analysis in predicting renal homograft rejection in dog and man was investigated.

Materials and methods. Concentrations of lysozyme in serum and urine of 10 dogs and in urine of 5 patients were assayed after renal homotransplantation according to the method of Smolelis and Hartsell(7).‡ In addition, routine tests of renal function (in dogs, BUN, and in man, BUN, serum creatinine, creatinine clearance, and quantitative urinary protein) were carried out according to standard techniques.

Results. Following kidney homotransplantation in dogs both serum and urinary lysozyme and BUN fluctuations were closely parallel. The levels of lysozyme increased in 7 dogs in which the graft was rejected and decreased and remained low in 3 animals in which it was accepted. In one animal (Fig. 1) significant increase in the level of urinary lysozyme, heralding a rejection, preceded a similar change in BUN value. In 2 of the 7 dogs in which the graft was rejected, the level of urinary lysozyme dropped precipitously shortly before death (Fig. 2). Urinary lysozyme levels fluctuated more widely, in general, than serum determinations and hence appeared to provide a more sensitive measure of homograft rejection or acceptance.

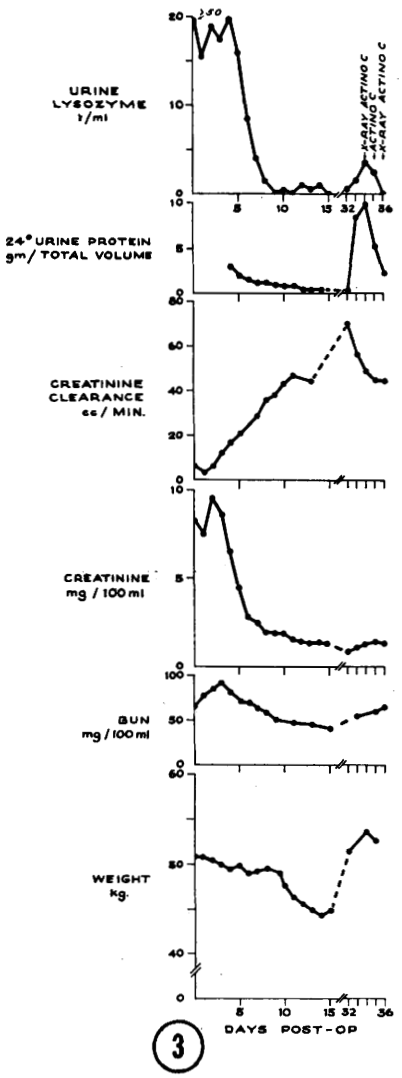
In the clinical cases, elevated levels of urinary lysozyme were present immediately after homotransplantation (Fig. 3). After the initial phase of injury, urinary levels of lysozyme returned to normal ($\leq$ 2 γ/ml) and remained so in the 3 patients in whom no "rejection crisis" occurred. In the 2 patients (Fig. 3 and 4) in whom a rejection crisis was verified by cumulative clinical and laboratory evidence, the level of urinary lysozyme again increased. There was a similar increase in total amount of protein excreted in 24 hours. The amount of lysozymuria was not directly proportional to that of proteinuria (Fig. 4). In one patient, J. C., (Fig. 4) in whom 2 rejection crises occurred, the most convincing laboratory evidence of the second episode was demonstrated by measurement of increased urinary lysozyme activity.

Discussion. The results of these experimental and clinical studies indicate that measurement of urinary lysozyme activity may provide a reliable method of detecting renal homograft rejection. Prockop and Davidson (6) have shown in animal experiments that little increase in lysozyme excretion occurs when glomerular damage is induced with an antibody preparation but that marked lysozymuria follows tubular lesions produced with $HgCl_2$. These investigators also report that a quantitative relationship does not exist between lysozymuria and proteinuria. Furthermore they observed, as we did in some of the dog experiments, that in the terminal stages of uremia when urine production is

‡ All the required materials for the assay were obtained from Difco Laboratories, Detroit, Mich.

minimal, low urinary lysozyme activity is present despite markedly elevated levels of BUN.

Kountz *et al*(8) reported that destruction of a renal homotransplant is an immunologic reaction mediated by immature plasma cells directed specifically against the intertubular blood vessels. Damage to these vessels results in a progressive decrease in total renal blood flow with subsequent tubular necrosis



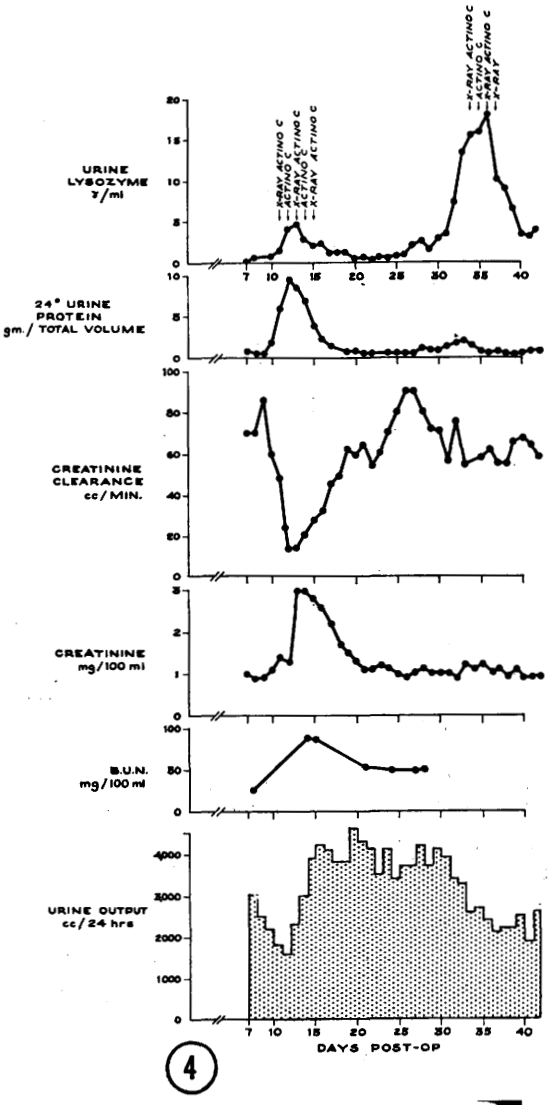


FIG. 3. Postoperative course after renal homotransplantation of a 14-year-old girl (B.R.) with chronic glomerulonephritis. On the 33rd, 34th and 35th days the increase in total amount of urinary protein excreted per 24 hr and the decrease in creatinine clearance verified a rejection crisis; note increase in urinary lysozyme activity during this interval. The crisis was successfully managed with x-ray and immunosuppressive therapy.

FIG. 4. Postoperative course after renal homotransplantation of a 27-year-old woman (J.C.) with chronic glomerulonephritis. The decrease in creatinine clearance and urine output and increase in total amount of urinary protein excreted in 24 hr observed on day 10 verified the beginning of the first rejection crisis; note subsequent increase in urinary lysozyme and serum creatinine activity. This crisis was managed successfully with x-ray and immunosuppressive therapy. Beginning on day 30, the gradual decrease in urine output and slight increase in total amount of urinary protein excreted per 24 hr heralded a second rejection crisis; note markedly abnormal levels of urinary lysozyme during the second, also successfully managed, episode.

and massive vacuolation in the cells of the proximal convoluted tubule. Oliguria proceeds rapidly to anuria with destruction of larger blood vessels and hemorrhage. Hume *et al*(9) showed that unless a major vascular insult such as thrombosis or infarction has occurred, tubular rather than glomerular lesions are present early in a rejection crisis.

Lysozymuria was noted in the rejection crises observed in this study. In view of the reported relationship between lysozymuria and renal tubular damage(6) it would appear that this investigation lends further support to the ascribed importance of tubular lesions in the rejection phenomenon.

Summary. Increased lysozyme activity in urine has been shown to be a reliable sign of renal homograft rejection. The ease and rapidity (20 minutes) of the measurement should make this assay a most valuable adjunct in the postoperative management of patients with kidney homotransplants.

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Adrenal Cortical Function in Experimental Alcoholism in Dogs.* (30642)

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Although evidence is available to suggest that ethanol administration stimulates adrenocortical secretion in several animal species (1,2,3), no work involving direct measurement of plasma steroids appears to have been done in dogs under the acute influence of this alcohol. Since some features of the disposition of both ethanol and the corticosteroids seem to occur in common in man and the dog, a series of studies on the interrelationships between experimental alcoholism and adrenal function was begun in dogs. The present communication is concerned with the effect of acute ethanol intoxication on steroid secretion as indicated by 17-hydroxycorticosteroid levels in the peripheral plasma of these animals.

Methods. Mongrel dogs of both sexes were used repeatedly and under a variety of conditions so that control and experimental data could be obtained from the same animals. Blood samples were obtained by venipuncture of the intact jugular vein before and 1, 2, 4, and 6 hours after one of the following intravenous infusions or injections: saline, 10 ml/kg; corticotropin (Wilson's ACTH solution), 1.2 units/kg; ethanol (redistilled absolute), 1.0, 2.0 or 3.0 g/kg (diluted to 20%, w/v, with saline); or hydrocortisone (free alcohol), 1 mg/kg. Saline and ethanol solutions were infused into a leg vein during the course of 10 to 20 minutes. ACTH solution was diluted with saline to a volume of 5 ml and administered by rapid injection. Hydrocortisone was dissolved in 5 ml of 5% ethanol in saline and injected in a similar manner.

Plasma was analyzed for 17-hydroxycorticoid content by the water-benzene partition

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