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Prolonged Exposure to Nephritogenic Beta-Hemolytic Streptococcus in Intraperitoneal Diffusion Chambers.* (28915)

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The role of the streptococcus in the pathogenesis of glomerulonephritis has been investigated using diffusion chambers containing beta-hemolytic streptococci, nephritogenic in man(1-4). In these experiments, the chambers remained in the peritoneal cavity for 24 to 72 hours and were then removed. Preliminary experiments in this laboratory with rats and rabbits suggested that the glomerular lesions were enhanced by allowing the chamber to remain *in situ* for periods exceeding the customary 48 hours. A similar observation had previously been noted in mice surviving prolonged streptococcal infection (5). This report deals with results obtained following insertion of diffusion chambers into the peritoneal cavity of rats and rabbits for prolonged periods.

Materials and methods. Diffusion chambers were fashioned from Plexiglass sheets and Millipore paper (H.A. pore size 0.45 μ) as previously described(1). 0.5 ml of a 24-hour broth culture of nephritogenic hemolytic streptococcus type A_{12}, strain No. 35,‡ was delivered into a sterile chamber. The chamber was then inserted free into the peritoneal cavity of 20 rats and 6 rabbits where it remained until the animal was sacrificed.

Twenty-four-hour and 2-hour "fresh" rabbit urine specimens were examined daily for excess proteinuria and hematuria. Blood samples were obtained weekly from the lateral ear vein of the rabbits for estimation of urea nitrogen.

The rats were sacrificed in pairs at 2, 7, 14, 21, 28, 35 and 42 days following insertion of the diffusion chambers. The rabbits were sacrificed in pairs at 21, 28 and 35 days following insertion of the chambers.

At sacrifice the peritoneal surface was cultured immediately on exposure. Heart blood was then collected aseptically, similarly cultured, and the serum separated. The diffusion chamber was recovered and the contents cultured. All cultures were plated on blood agar, incubated at 37°C, and read after 24 hours.

The animals were carefully autopsied and sections obtained from formalin-fixed kidney, heart, lung, liver, spleen, and lymph nodes were stained with hematoxylin and eosin. In addition, appropriate kidney sections were stained by the periodic-acid Schiff technique. Blocks of all rat kidneys were quick frozen and stored at —20°C until used. The sera of rats obtained at sacrifice were tested for rat kidney-localizing activity using the indirect "sandwich" fluorescent technique on both normal and experimental rat kidneys.

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Results. 1. *Urinalysis:* Rabbit urine collected daily from Day one to Day 35 of the experiment showed no consistent proteinuria in excess of normal controls nor hematuria although occasional red blood cells were seen. Urinary volume remained normal. Blood urea nitrogen remained normal in five rabbits. In the sixth rabbit, the blood urea nitrogen rose minimally to 30 mg % at 5 weeks following insertion of the chamber.

2. *Bacteriology:* At sacrifice the peritoneal surface and fluid and heart blood were consistently sterile on culture. The contents of all recovered chambers cultured a heavy growth of virulent (mat colony) beta-hemolytic streptococci. Thus the preparation appeared to be adequate.

3. *Pathology:* At autopsy, splenomegaly, splenic congestion and generalized lymphadenopathy were universal. The chamber was usually surrounded by dense adhesions. In 3 rats a small well localized abscess in direct continuity with the chamber was found, suggesting the chamber had leaked following formation of the adhesions. These animals appeared edematous and ill as did a small number of rats in whom no abscess was found. Except for moderate congestion, the kidneys were not remarkable nor were there gross lesions in the organs examined. On microscopic examination of the kidneys there were spotty, subtle changes in the glomeruli consisting of thickened basement membranes, disarray of normal architecture, and rarely a suggestion of hypercellularity in the tuft. In many animals the glomerular changes could not be distinguished from normal controls. The tubules, however, showed widespread degenerative changes consisting of necrosis of tubular cells and the presence of hyaline and granular casts. These changes varied from mild to severe and were essentially identical with those described earlier in mice(3,4).

4. *Fluorescent antibody studies:* Gamma globulin, specific for kidney, could not be demonstrated when the sera from rats with intraperitoneal chambers were layered on kidney sections obtained from normal controls and the experimental rats.

Discussion. The pathogenesis of glomerulonephritis in man remains unknown although its relationship to streptococcal infection appears to be established. Streptococcal antigens have been demonstrated to localize and persist in the glomeruli of rats and mice for longer periods than they do in other tissues(6,7). The belief that rat and human kidney and nephritogenic streptococci share common antigenic determinants continues to evoke interest(1,8). The nephropathy produced by prolonged exposure to beta-hemolytic streptococci in diffusion chambers may bear a relationship to the tubular damage in human glomerulonephritis as previously suggested(3) and presumably is mediated by streptolysin S as shown by Tan *et al*(9). A similar toxic action may explain the transient hematuria and proteinuria that not uncommonly follow streptococcal pharyngitis. The present results demonstrate that prolonged exposure of rats and rabbits to streptococci (nephritogenic in man) does not produce glomerulonephritis nor circulating antibody with affinity for renal tissue. The rat and rabbit kidney undoubtedly differ greatly from human kidney in their antigenic constitution and streptococci nephritogenic in man need not necessarily have an effect on the glomeruli of rats and rabbits. The point to be emphasized is that the tissue damage produced can be explained without evoking an immune response. Furthermore, the tissue damage resulting, presumably in release of tissue proteins (antigens), need not necessarily result in induction of an immune response directed to that organ. An explanation for the disparity between the present report and previous reports dealing with rats (1) may lie in the different strain of animals used.

Summary. Diffusion chambers containing beta-hemolytic streptococci, nephritogenic in man, implanted in the peritoneal cavity of rats and rabbits for intervals ranging from 2 to 42 days did not produce definite glomerular lesions and, in particular, the lesion characteristic of glomerulonephritis. Tubular damage was noted in all animals. These results are somewhat at variance with previous reports of experiments performed with rats and mice(1,2,5) but similar to the results of others using mice(3,4,9).

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Effect of Ethanol on Plasma Glucose and Insulin in the Fasted Dog.* (28916)

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Recent studies have demonstrated that the hypoglycemic potential of ethanol may be unmasked in normal human subjects by administering ethanol after 2 to 3 days of prior fasting(1-4). In the search for a more suitable experimental preparation for the study of "alcohol† hypoglycemia," we have investigated the effects of ethanol upon blood sugar in the dog. Although most previous studies have failed to disclose an acute hypoglycemic action of ethanol in this species (5-8), we felt that our efforts might be facilitated by the institution of preliminary dietary deprivation. The present studies have indicated that ethanol can acutely lower blood sugar in the fasted dog and that such "alcohol hypoglycemia" in dogs, as in humans(1,2) is not attended by elevations of plasma insulin.

Materials and methods. Food was withheld for 1.5 to 8.0 days from 8 male and 1 female mongrel dogs. Initial weights ranged from 13 to 32 kg. During the period of fasting, the animals were housed in individual cages, weighed daily, and permitted unlimited access to water. For the individual experiments, the animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg) except in one instance, where chloralose was employed. Supplemental amounts of pentobarbital were administered as necessary during the studies.

Following induction of anesthesia, the left femoral artery was cannulated, and a catheter was introduced into the right femoral vein for infusion of 0.85% saline, via Bowman pump, at the rate of 1.9-2.0 ml/min. The control infusion of saline was continued for a minimum period of 120 minutes to stabilize blood sugar. For the subsequent 120 minutes, 15% ethanol (absolute ethanol in isotonic saline, vol/vol) was infused. In most experiments, sodium tolbutamide (0.5 g) was introduced through the infusion tubing 60 or 120 minutes after cessation of alcohol administration, and intravenous glucose (0.5 g/kg) was given one hour later. The tolbutamide and glucose were designed to contrast the effects of known insulinogenic agents with those of ethanol.

Control specimens of arterial blood were obtained 30 and 5 minutes prior to the start of alcohol infusions, and experimental samples were secured at timed intervals thereafter

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† The terms "ethanol" and "alcohol" are used interchangeably in subsequent portions of text.