Production of Renal Disease in the White Mouse with Streptococcal Infection.* (25862)

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In man group A streptococcal infection usually precedes onset of acute glomerulonephritis and frequently precedes an exacerbation of chronic nephritis. Several investigators have reported renal disease in animals infected with streptococci or inoculated with streptococcal products, but none of these systems contributed to our understanding of human nephritis and the search for suitable model continues. In this laboratory a new experimental renal disease has been observed in mice inoculated with living streptococci. The circumstances for production of this experimental disease and the extent of the process, characterized by albuminuria, hypoalbuminemia, generalized edema, and histological changes are described.

Materials and methods. Infections were produced with streptococcal strains D58 (type 3), D58/XXD2, GL8 (type 19), and ADA (type 14). These bacteria were originally isolated in other laboratories from humans without renal disease and maintained for several years by repeated subculture without animal passage except for the D58/XXD2 strain which was passed in one mouse. *Staphylococcus aureus* strains 80 and 81, originally isolated from carbuncles, were obtained from a phage-typing laboratory. *S. aureus* 3859 and *E. coli* 597 were recently isolated in hospital laboratory from septic processes. The animals used were adult, male, white mice of Webster strain from Harvard Medical School colony. They usually weighed 17 to 23 g at beginning of experiment. Injections were made subcutaneously in the dorsal aspect of either hindquarter. Inocula were prepared by suspending the centrifuged sediment of 18-22 hour broth cultures in fresh broth or sterile, light mineral oil. Concentrations of inocula were expressed either as number of organisms/animal injected, as determined by optical density at 640 mμ, or as concentration relative to original culture. With the latter method an inoculum designated 5:1 refers to the sediment from 5 ml of culture suspended in 1 ml of new medium. Paper electrophoresis of serum was performed with veronal buffer, pH 8.6 and 0.1 μ. Electrophoresis of urinary proteins was performed in 4 buffers: veronal, pH 8.6, phosphate, pH 7.0, and acetate at pH 5.5 and 4.0.

Results. Observations with streptococcal infections. Animals injected with 10^8 D58 or D58/XXD2 organisms developed marked swelling of inoculated quarter in 1-2 days. Local swelling usually persisted for duration of experiment. Most animals showed weight loss during first 2 to 4 days of infection. Thereafter many showed rapid weight gain, culminating in recognizable abdominal swelling between 5th and 10th day. Of 215 animals inoculated with large numbers of the D58 strain, 123 developed ascites as demonstrated by paracentesis or at autopsy (Table I). At times edema was extensive with weight gain of ⅓ or more of preinoculation body weight.

TABLE I. Incidence of Ascites.

Inoculations	Dose range	No. animals inj.	No. with ascites*
Streptococcus			
D58	.5 ml 4:1 to 33:1	73	40
"	$1.2\text{-}9.5 \times 10^8$	127	83
"	$2.0\text{-}8.2 \times 10^7$	15	0
GL8	.5 ml 33:1	5	2
ADA	.5 ml 33:1	9	1
S. aureus 3859, 80, or 81	$7\text{-}35 \times 10^9$	70	0
E. coli 597	$1.8\text{-}1.9 \times 10^{10}$	20	0
Oil, broth, and heat killed streptococci		33	0

* Ascites proven by autopsy or paracentesis. All animals had 1 or more attempted paracentesis between 5 and 14 days after initiating infection.

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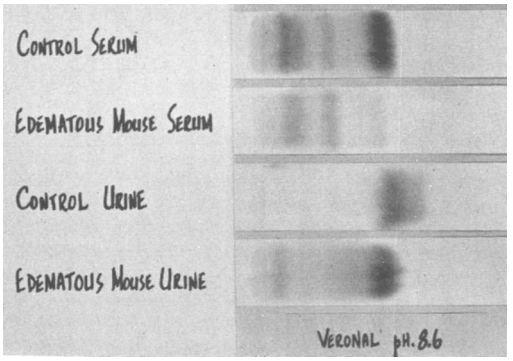

FIG. 1.

Paper electrophoresis demonstrated a decrease in albumin concentration in sera of all edematous animals studied. In 4 groups of edematous animals, 12 were successfully bled. Their average albumin concentration was 12.1%, range 2.1-19.5%. Six control animals had an albumin concentration of 40.0% with range of 20.7-56.4% (Fig. 1).

Since moderately large amounts of protein were found in urine of normal mice, partial characterization of urinary proteins from normal and edematous animals was undertaken to determine whether qualitative differences existed between the 2 groups. Electrophoresis at pH 8.6, 5.5, and 4.0 demonstrated 2 components in normal urinary protein and both moved faster than serum albumin. At all pH values urinary protein of edematous animals had mobilities comparable to serum proteins, with largest amount of material corresponding to albumin (Fig. 1). Ultracentrifugation also distinguished between urines of the 2 groups. Protein of normal animals had a sedimentation constant of 2.4, whereas the major component of edematous animals' urine and mouse albumin had constants of 4.6 to 5.0.

At autopsy kidneys of all edematous animals were pale, as were those of some animals without edema. Necrosis of proximal convoluted tubules and hyaline casts in the collecting tubules were seen by light microscopy during 2 to 14 days after initiating infection. In many instances tubular necrosis was so extensive that the tubules were denuded of cellular elements. This tubular lesion was believed to account for the gross pallor. Electron microscopic examination of glomeruli from kidneys of edematous animals at 13 days revealed fusion of epithelial foot processes. In animals sacrificed 30-40 days after inoculation thickening, fusion, and irregular dilatation of glomerular capillaries were observed by light microscopy. Evidence of generalized infection was usually absent. In several experiments lung weights of animals with generalized edema were comparable to those of controls.

Studies of a few animals infected with GL8 and ADA strains of streptococci established that a similar experimental disease may be associated with infection by strains other than the D58 (Table I).

Other animals were injected with heat killed streptococci, sterile broth, or sterile oil, and none developed edema. Serum and urinary proteins were normal by electrophoresis in several instances and microscopic changes were not found in the kidneys. Penicillin treatment of infected animals prevented occurrence of edema when initiated at time of injection of streptococci but did not prevent its development when given 24 hours or more after the bacteria.

Cultures made from kidneys of infected animals were usually sterile in absence of blood stream invasion (Table II).

Observations with other infections. Twenty animals infected with approximately 10^{10} *E. coli* cells and 70 mice infected with 10^9 or 10^{10} *S. aureus* organisms never developed ascites or edema. Concentration of albumin averaged 29.4% of total protein with range of 13 to 38% in sera of 18 of these mice. Urinary proteins in 6 pooled collections from 15 staphylococcus infected animals and in 4 collections from 13 *E. coli* infected animals

TABLE II. Cultural Data.

Infecting organism	No. with edema cultured	β-hemolytic streptococci recovered: Blood No. pos.	Blood No. done	Kidneys No. pos.	Kidneys No. done
D58 or D58/XXD2	26	0	41	1*	39
GL8	2	3	3	3	3
ADA	1	0	5	1	5

* Blood from this animal was not cultured.

were also normal by electrophoresis. Microscopic examination of representative kidneys from animals infected with these organisms were normal.

Discussion. Several investigators reported histological changes in kidneys of animals infected with streptococci or inoculated with streptococcal products(1-8). However association of albuminuria, hypoalbuminemia, and generalized edema with experimental streptococcal infection represents a new observation and suggests that group A streptococcal infection in these animals induced a nephrotic syndrome. The finding of an abnormality in the glomerular ultrastructure similar to that seen in spontaneously occurring nephrosis and nephrosis induced by other agents supports such an interpretation(9-13). However extensive tubular necrosis is not a feature of the nephrotic syndrome and the role of the tubular lesion in this experimental disease must be considered.

Renal shutdown as a mechanism for production of edema in these animals is unlikely since fluid was not forced, lung weights were not increased in edematous animals, and hypoalbuminemia was present in all edematous animals whose sera were studied electrophoretically. The possibility that a decrease in tubular reabsorption of protein was responsible for serum protein changes is also unlikely(14,15). Based on studies of albumin clearance 3 groups of investigators calculated that maximum possible reabsorption of protein by the tubules was small compared with that appearing in urine in pathological states (16,17,18). It appears reasonable to assume that the observed proteinuria is not the result of a tubular lesion but reflects increased glomerular permeability. The majority of evidence therefore favors the interpretation that tubular necrosis is not essential to the clinical picture but is either superimposed on or concomitant with a glomerular lesion responsible for an otherwise clear cut nephrotic syndrome.

In addition to characterizing clinical and pathological features of the experimental disease these studies suggest that growth of injected streptococci was necessary for induction of the disease. Thus inoculation of heat killed organisms failed to induce the experimental disease and penicillin treatment initiated at time of infecting inoculation prevented its development. The further observation that infection with large numbers of staphylococci or coliform organisms failed to induce renal changes suggests that properties of the infecting organism determine the occurrence of this nephropathy.

Summary. Development of albuminuria, hypoalbuminemia, and generalized edema was observed 5-10 days after initiating streptococcal infection in the male Webster white mouse. Animals with this clinical picture had pale kidneys which showed tubular necrosis by light microscopy, and glomerular changes by electron microscopy. Animals surviving 4 to 6 weeks also showed glomerular changes by light microscopy. It is proposed that these observations may be interpreted as production of the nephrotic syndrome by streptococcal infection with a superimposed or concomitant tubular lesion.

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Relative Effectiveness of B_{12} and Dimethylbenzimidazole B_{12}-Coenzyme in Thymine Methyl Biosynthesis.* (25863)

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We have recently reported that Vit. B_{12} stimulates conversion of formate to 5-methyl group of thymine by chick bone marrow cells (1). Further studies have indicated that the vitamin functions in reduction of one-carbon compounds between formate and formaldehyde levels of oxidation(2,3). Barker *et al.*(4) reported isolation of coenzyme forms of B_{12} which catalyze conversion of glutamate to β-methylasparate by certain microorganisms. In this system B_{12} is inactive. It appeared desirable to determine effectiveness of the coenzyme form of B_{12} in stimulating conversion of formate to thymine methyl.

Methods. Handling of chicks, preparation of bone marrow cell suspensions, details of incubation and formation of the acetol osazone from thymine were the same as previously described(1). Bone marrow cells from B_{12}-deficient chicks were incubated with formate-C^{14} and deoxyuridine and various concentrations of Vit. B_{12} or 5,6 dimethyl-benzimidazole B_{12}-coenzyme(4). The results are reported as per cent increase in conversion of formate-C^{14} to thymine as a result of addition of B_{12} or the coenzyme.

Results. Each result given in Table I is the average of at least 2 experiments. It is quite clear that the coenzyme form of B_{12} was less effective than the crystalline vitamin in stimulating conversion of formate to the 5 methyl group of thymine. This observation is in agreement with other reports indicating that the coenzyme form of B_{12} does not stimulate methionine biosynthesis under conditions in which the vitamin is effective(5,6). It appears that Vit. B_{12} has at least 2 distinct metabolic roles, one in reduction of one carbon compounds and the other in biochemical rearrangements catalyzed by coenzymes first described by Barker *et al.*(4).

TABLE I. Influence of Vit. B_{12} and 5, 6 Dimethylbenzimidazole B_{12} Coenzyme (DMBC) on Conversion of Formate C^{14} to Thymine Methyl by B_{12}-deficient Chick Bone Marrow Cells. (Results expressed as % increase in conversion of formate to thymine methyl.)

mγ/ml	B_{12}	DMBC
15	35	9
150	66	34
1500	90	43
3750		47

Summary. Vit. B_{12} was more effective in stimulating conversion of formate to thymine methyl by chick bone marrow cells than was 5,6 dimethylbenzimidazole B_{12}-coenzyme.

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