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Effect of Injecting Heterologous Glomerular Basement Membrane Preparations in Pregnant Sheep.* (27936)

RAYMOND WILLIAM STEBLAY (Introduced by M. Edward Davis)
Department of Obstetrics and Gynecology, University of Chicago, Chicago, Ill.

We have previously shown that injections of heterologous glomerular basement membrane (GBM) and Freund's complete adjuvant into adult female sheep every 2 weeks produces a fatal, fulminating glomerulonephritis within 27 to 90 days after the first injection, presumably by an autoimmune mechanism(1).

We decided to inject pregnant sheep in the last third of their pregnancy (gestation is approximately 150 days) to observe the effect of pregnancy on the course of the induced nephritis and to observe the effect of the injections of heterologous GBM on maternal and fetal kidneys. Toxemias of pregnancy occur only in the last third of human pregnancies.

This paper reports that immunization of sheep with heterologous GBM and Freund's adjuvant in the last third of their pregnancy produces a fatal glomerulonephritis in the mother without any demonstrable effect on the fetal kidneys.

Methods. Human, monkey and rat GBM were prepared by the method previously described(2). The GBM was homogenized in isotonic saline with the sonic oscillator† and made up to a concentration of 50 mg (wet weight) per ml. The homogenized suspension was slowly added to Freund's complete adjuvant‡ to form a 1:1 water-in-oil emulsion. The final concentration of GBM in the emulsion was 25 mg/ml.

Three Hampshire sheep in the last third of pregnancy were obtained from different herds through a local veterinarian. After having been found free of overt disease and with normal urine and blood urea nitrogen (BUN), the sheep were injected every 2 weeks with 7 ml of emulsion. Each sheep was injected with antigen from a single species. The injections were given by a combination of intramuscular, subcutaneous and intradermal routes at each administration. For example, 7 cc of emulsion was divided as follows: 3 ml intramuscularly in one hind leg, 2 ml subcutaneously in the axillary and inguinal regions on the same side, and 1 ml intradermally in the skin of the dorsum of the neck.

Periodic urinalyses and BUN determinations were performed on the sheep during the immunization. Serum cholesterol was determined prior to injection and at death.

At death complete autopsies were performed, bladder urine analyzed and the kidneys carefully examined in the gross and microscopically. Kidney tissue was fixed in ice cold 10% formalin and stained with hematoxylin and eosin, periodic acid-Schiff reagent with alcian blue and Masson's trichrome stain.

Results. The results are summarized in Tables I and II. All 3 pregnant sheep died from a fatal fulminating glomerulonephritis in 45, 46 and 54 days after the first injection of antigen. All the criteria for nephritis

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† Raytheon Sonic Oscillator Model S102A, 50 watt, 9 kc, Raytheon Manufacturing Co., Waltham, Mass.

‡ Freund's complete adjuvant: Arlacel A (Atlas Powder Co., Wilmington) 15.0 ml; bayol F (Penola Division, Detroit) 85.0 ml; *Mycobacteriumbutyricum* (Difco) 100 mg.

TABLE I. Comparison of Renal Histology, Blood Urea Nitrogen, Serum Cholesterol and Bladder Urine Protein with Day of Death and Antigen Injections in Pregnant Sheep.

Sheep No.	Antigen	Total amt* of antigen (wet wt), mg	Total No. of inj	Day of death	Bladder urinary protein	Renal histology‡	Preinjection blood Chemistries Blood urea nitrogen	Preinjection blood Chemistries Cholesterol	Chemistries at death Blood urea nitrogen	Chemistries at death Cholesterol
							mg %			
27	Human GBM	525	3	46†	3+	Chronic glom'tis§	23	80	420	125
5	Monkey GBM	700	4	54	3+	"	18	80	450	110
37	Rat GBM	700	4	45	4+	"	27	100	355	180

* Concentration of antigen in emulsion was 25 mg/ml and 7 ml of emulsion was given at each administration by multiple portals.
† This sheep was sacrificed in a moribund state.
‡ All kidneys grossly were swollen, gray brown and contained petechiae on cortical and cut surface.
§ Chronic glomerulonephritis.

in the sheep as outlined by us previously(1) were present and consisted of azotemia exceeding 350 mg %, petechiae on cortical and cut surfaces, proteinuria and histologic evidence of severe extracapillary glomerulonephritis. There was no edema present in the nephritic sheep during life or at autopsy. The serum cholesterol was not considered to be significantly elevated. They did not have a nephrotic syndrome. The nephritis did not appear to affect the usual course of the pregnancy nor did the pregnancy seem to affect the course of the nephritis in the mothers.

On the contrary, all the fetal and neonatal lamb kidneys were histologically normal. The lamb from sheep No. 27 suckled for 3 days after birth. The mother was sacrificed in a moribund state on the 3rd postpartum day with a BUN of 420 mg %. The lamb appeared well but was also sacrificed. The BUN of the lamb at death was 137 mg %.

Sheep No. 37 died in uremia 45 days after the first injection. Two fetal lambs, approximately term size, were found *in utero*. Both fetal kidneys were histologically normal but the mother had severe diffuse extracapillary glomerulonephritis.

Sheep No. 5 delivered a live lamb 41 days after the first injection. The lamb suckled for 3 days and died of unknown causes. The lamb kidney was normal. The mother had proteinuria at time of delivery and died in uremia 13 days later.

It may be concluded that whereas all the maternal kidneys showed severe diffuse glomerulonephritis, all the newborn and fetal kidneys were normal in appearance. The pregnancy did not alter the course of nephritis in the mother nor did the induced nephritis alter the pregnancy.

Discussion. Immunization of sheep in the last third of their pregnancy with heterologous GBM and Freund's adjuvant produced a fatal fulminating extracapillary glomeru-

TABLE II. Absence of Renal Lesions in Lambs of Mothers Immunized with Heterologous GBM in Last Third of Their Pregnancy.

Lamb No.	Days after 1st inj that lamb was born	No. days postpartum that lamb died	No. days lamb suckled	Lamb renal histology	Blood urea nitrogen of lamb (mg %)
27A	43*	3	3	Normal	137
5A	41	3	3	"	32
37A	45†	—	—	"	n.d.‡
37B	45†	—	—	"	"

* Gestation is approximately 150 days.
† Lambs 37A, 37B were found about term size *in utero* after mother died in uremia.
‡ n.d. = not done.

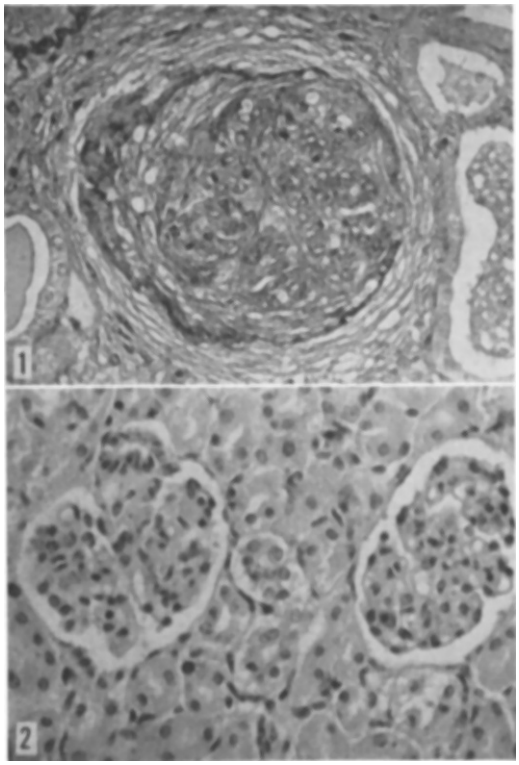

FIG. 1. Kidney from sheep No. 27 which was sacrificed in a moribund state 46 days after first of 3 injections of human GBM. Extensive fibroepithelial proliferation obliterating Bowman's space (crescent) and fusing with the extensive periglomerular tissue is present. Dilated tubules are present with casts. (Masson's trichrome stain. ×290.)

FIG. 2. Kidney from lamb of sheep No. 5. The lamb was found dead 3 days postpartum. There are slight postmortem changes, but normal glomeruli, tubules and Bowman's capsule can be seen. (Hematoxylin and eosin. ×315.)

lonephritis in all the maternal kidneys but failed to affect the fetal kidneys. We have discussed elsewhere(1) the reasons for believing this new experimental nephritis occurs by an autoimmune mechanism, that is, the kidney damage is mediated by sensitized cells and/or autoantibody.

Since the fetal kidney is not damaged *in utero*, this suggests that sensitized cells and/or antibody fail to reach the fetal kidney and affect it. Newborn sheep are said to be born agammaglobulinemic and to derive all their gamma globulin in one massive dose from their mother's colustrum within 36 hours after birth(3). The agammaglobulinemia in newborn sheep would presumably explain failure of transmission of antibody to the fetus. If the maternal disease was caused by sensitized cells, one would not expect renal damage in the fetus in the absence of any passage of significant number of cells. Large numbers of such cells do not presumably cross the placental barrier.

Two of the lambs managed to suckle colustrum for 3 days postpartum. The serum from one of these lambs (No. 27A) contained an elevated blood urea nitrogen. This was presumably derived by passive diffusion from its uremic mother *in utero* and/or in the colustrum. The lamb kidney was histologically normal so the elevated BUN represented inability to completely clear passively transferred BUN. The suckling neonatal lamb derives various antibodies from the colustrum but fails to obtain antibodies which will damage its own kidney.

Recent studies indicate that sensitized cells are probably the mediator of tissue damage in autoimmune rat nephrosis(4), thyroiditis(5), and allergic encephalomyelitis (6).

Sclare(7) has shown that injection of homologous thyroid extract and Freund's adjuvant into pregnant guinea pigs led to development of thyroiditis in the mothers. Circulating autoantibodies to thyroid tissue were found in the offspring but no permanent damage to the thyroid of the offspring was observed. In a somewhat different study(8) brain emulsion and Freund's adjuvant was injected into mice prior to pregnancy. About 8-9% of the mice had abnormalities of the developing nervous system. It was felt transmitted maternal antibodies caused the abnormalities. Encephalomyelitis was not observed in the offspring.

In the present experiment failure to transmit renal disease to the offspring may reflect failure to transmit the appropriate antibody and/or sensitized cells. Since fetal sheep do not passively receive any significant amount of gamma globulin *in utero*(3), it might be expected that the specific autoantibody was not transmitted. Failure to transmit the disease by suckling might also reflect failure to receive the appropriate antibody and/or not enough time to affect the neonatal glomeruli.

The existence or importance of autoantibody in the pathogenesis of the renal lesion is still open to question but the present experiment suggests that antibodies transmitted through colustrum do not mediate the disease. It might also be expected that if sensitized cells were the mediator of tissue damage that it would be unlikely that a sufficient amount of such cells were able to traverse the placenta barrier or be transmitted through colustrum.

Summary. (1) Three sheep developed a fatal nephritis in 45 to 54 days when immunized with heterologous GBM and Freund's adjuvant in the last third of their pregnancy. (2) Immunization of the mothers or suckling their colustrum (in 2 of the 4 lambs) did not produce a renal lesion in the lambs. (3) It appears that the agent which produces a fatal nephritis in the mother was either not transmitted *in utero* or in the colustrum or it was incapable of affecting the fetal or neonatal kidney. (4) The above observations are consistent with the hypothesis that this sheep nephritis is produced by an autoimmune mechanism mediated by sensitized cells and/or autoantibody.

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Effect of Staphylococcal Enterotoxin on Dermal Reactivity to Epinephrine.* (27937)

V. BOKKENHEUSER,† M. A. CARDELLA, E. A. GORZYNSKI, G. G. WRIGHT, AND E. NETER

Depts. of Bacteriology and Pediatrics, State University of New York at Buffalo Medical School; Laboratory of Bacteriology, Children's Hospital, Buffalo, N. Y.; and U. S. Army Biological Laboratories, Fort Detrick, Frederick, Md.

Recently it was reported that certain strains of *Staphylococcus aureus* contain a toxic product with biologic properties resembling those of bacterial endotoxins(1-6). Staphylococcal extracts alter dermal reactivity of the rabbit to epinephrine, cause hemorrhagic necrosis of sarcoma 180 of mice, are lethal to adrenalectomized mice, and cause death of 10-day-old chick embryos(1-6). Viable or killed staphylococci also produce some of these effects(1,5). The heat stability of the active principle of the extracts was reminiscent of staphylococcal enterotoxin. Accordingly, the present investigation was carried out to determine whether purified preparations of enterotoxin also alter dermal reactivity to epinephrine of rabbit and guinea pig.

Materials and methods. Partially and highly purified enterotoxins prepared from the S-6 strain of *Staphylococcus aureus* were employed. Preparations 7A, 8A, 9B, and 94 were prepared according to the methods of Bergdoll *et al.*(7), and supplied by Dr. Bergdoll of the University of Chicago. Preparations D2 and D6 were prepared by Dr. E. J. Schantz of Fort Detrick, according to somewhat modified procedures. The toxin content of the preparations was measured by the Oudin single diffusion method in tubes; purity was calculated as the ratio of the specific

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† U.S.P.H.S. Research Fellow.