Sperm and Antibody Production in Rabbits Following Immunization with Sperm and Semen.* (29513)

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Although immunization of guinea pigs and rats with homologous sperm produces aspermatogenesis(1-3), immunization of rabbits did not produce histological changes in the testes(4).

In this study sperm counts were made as well as assays for antibody before and after immunization with homologous and heterologous semen and sperm.

Methods and materials. Dutch-belted and New Zealand white rabbits were maintained on rabbit ration with water *ad lib.* The room was maintained @ 74°F and lights were on from 7 A.M. to 7 P.M. daily. Rabbits were from 6 to 9 months old at the onset.

Semen was collected twice weekly using an artificial vagina (Holborn Surgical Instruments, London) and a teaser doe. Sperm counts were made with a hemocytometer.

Antigens. Rabbit antigens were prepared from pooled frozen semen. Several preparations were made: a) semen, b) sperm washed 7 times with cold 5% $NaHCO_3$ and concentrated 10-fold, c) seminal plasma, d) defatted sperm prepared by treatment with ethanol and then ether, e) very fresh semen (used within a half hour after collection). Amounts of sperm protein injected ranged up to 12 mg per rabbit (preps b and d).

Human antigens were prepared in a similar way.

Antigens were emulsified with one volume of Freund's complete adjuvant (Difco) on a Lourdes Magnamixer. Completeness of emulsification was checked by use of a conductivity bridge and by gross stability.

Antibody assays. Direct agglutination of sperm by antisera gave highly variable results, as described by others(5), even when the method of Kibrick *et al* was used(6). Therefore the methods employed in this study were the Ouchterlony technic and hemagglutination with coupled cells. For the former, 0.5% "Ionagar #2" (Consolidated Laboratories) in 0.1 M TRIS buffer @ pH 7.9 was used. For hemagglutination, the method of Ingraham(7) was used as modified in this laboratory(8). Human seminal plasma (HSP) could be used undiluted to couple the red cells. However, rabbit seminal plasma (RSP) required precipitation with 75% saturated ammonium sulfate followed by dialysis against saline to prevent spontaneous agglutination of cells. Sperm were washed 7 times with 5% $NaHCO_3$, brought to the original volume of semen, subjected to 5 cycles of freezing and thawing (−70° to + 37°) and sonerated 20 minutes on a Raytheon Model DF101. These fluids were concentrated 10× by dialysis against 20% PVP. After centrifugation, the supernatant fluids were used for coupling. Titrations and hemagglutination inhibitions were carried out as previously described(8) with 5 μg N/tube of dialyzed HSP or RSP used as inhibiting antigens.

Histological studies. Testes were fixed in Bouin's fixative for 18 hours, thoroughly washed, blocked and stained with hematoxylin and eosin.

Experimental. Baseline semen volumes and sperm counts were determined for 6 weeks. Antigen (a) was then injected into 2 intradermal sites, one subcutaneous site and one intramuscular site. Each month another antigen was similarly injected.

Seven rabbits received rabbit antigens and 3 rabbits received human antigens. The experiments were terminated after a total of 7 to 9 months at which time the rabbits were bled from the heart and testes removed for histological studies.

To study the immunological response more closely, 12 New Zealand rabbits were divided into 6 groups of 2 each. Each group was im-

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munized with a single antigen as follows: a) rabbit seminal plasma (12 mg solids/rabbit), b) washed and dried rabbit sperm (4.5 mg/rabbit), c) lipid extracted rabbit sperm (5.0 mg/rabbit), d) human seminal plasma (12 mg solids/rabbit), e) human sperm (1.5 mg/rabbit) and f) lipid extracted human sperm (5.0 mg/rabbit). All rabbits were bled after one month.

Results. The sperm counts were analyzed statistically by computer. Individual rabbits were compared using baseline values *vs* treatment values and the 2 groups (*i.e.* those receiving rabbit and those receiving human antigens) were also so compared. Treatment produced no significant decrease of sperm numbers in any of the rabbits. Semen volumes were likewise unaffected.

Antibody assays were carried out on 6 normal rabbits in addition to the immunized rabbits. None of the normal rabbits had detectable antibody to any of the 4 antigens (rabbit sperm, human sperm, RSP, HSP) by either Ouchterlony or hemagglutination (HA) technics. Similarly, none of the 22 immunized rabbits had HA antibodies to rabbit sperm. Two rabbits had low HA titers (20 and 56) to human sperm. These 2 rabbits had received multiple injections of human semen and sperm preparations. Both titers were abolished by addition of HSP indicating that the response was to small amounts of contaminating HSP rather than sperm. Four of the 22 rabbits had HA antibodies to RSP (range 20-320). All had received heavy immunization with RSP and titers were neutralized with RSP. All 9 rabbits which had received human antigens had HA antibodies to HSP, ranging from 160 to 70,200. All were inhibited by HSP although 4 had not received HSP at all, but had received sperm only. The highest titers were in those given large amounts of HSP. Unexpectedly, one rabbit which received rabbit semen and sperm had an HA titer of 128 to HSP but to no other antigen and this was inhibited with HSP.

The Ouchterlony tests showed only 5 positive plates out of the 22. Four were to HSP from rabbits receiving large quantities of HSP. The prominence of the lines and the number correlated with the HA titer. The serum with an HA titer of 70,000 had 3 lines, one with 13,000 had 2 lines and one with 10,000 had 1 line. Sera with HA titers of $<10{,}000$ showed no Ouchterlony lines. In addition the rabbit with the 70,000 HA titer produced a faint line with human sperm. However, addition of HSP to this serum abolished the reaction suggesting contamination of the injected antigen with HSP.

To study further the distribution of the antigen(s) in HSP, gel-diffusion tests were set up with 25% saline homogenates of various tissues as antigens. Eight human tissues were tested (adrenal, thyroid, kidney, lung, heart, pancreas, liver and brain). Mouse liver and 6 rabbit tissues (brain, liver, heart, kidney, lung and spleen) were tested in the same manner. Rabbit A23 serum was used as the source of antibody. This rabbit had received 28 mg of non-dialyzable HSP N in complete adjuvant and was bled one month later. The antiserum regularly produced 2 sharp lines against HSP. Of the 15 tissues tested, only human kidney gave strong reactions. Two sharp lines were formed, one of which was identical with one of the lines formed with HSP. In fact, when HSP was diluted to a 25% solution, more comparable to the kidney concentration, the lines were considerably stronger with kidney than with HSP. In addition to kidney, one broad weak band was formed to rabbit spleen. No similar band was present with HSP.

Histological examination of the testicles showed normal spermatogenesis irrespective of the immunizations given.

Discussion. These studies indicate that injections of rabbit and human sperm and/or seminal plasma injected repeatedly in complete adjuvant have no effect on spermatogenesis in the rabbit. This corroborates the study of Weil and Finkler(4) who injected rabbit sperm.

The antibody studies clearly suggest that this lack of effect is due to lack of antigenicity of either rabbit or human sperm in the rabbit. No positive immunological reactions to rabbit sperm were obtained and only a

few weak responses to rabbit seminal plasma were obtained. This challenges the widely accepted view that sperm of all species is a sequestered antigen which is antigenic when introduced into the systemic circulation. These results agree with those of Edwards (9). More unexpectedly, human sperm was not found to be antigenic since all reactions could be abolished with HSP. These findings support the view of Weil(10) that the predominant antigen in semen is in the seminal plasma and that reactions with sperm are due to their coating with this antigen. Hathaway and Hartree(11) found that Ouchterlony reactions to ram sperm *vs* rabbit antisera were abolished by prior addition of ram seminal plasma. This indicates that ram as well as human sperm is non-antigenic in the rabbit.

Summary. Rabbits were immunized with rabbit or human seminal plasma and/or sperm in complete adjuvant. Sperm counts and semen volumes were not depressed nor were testicular changes produced. Antibody studies using hemagglutination and Ouchterlony technics indicated that: a) rabbit sperm was not antigenic in rabbits, b) rabbit seminal plasma was weakly antigenic in some rabbits, non-antigenic in others, c) human sperm was not antigenic in rabbits, d) human seminal plasma was antigenic in rabbits, and e) human kidney shared a common antigen with human seminal plasma.

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Differences in Levels of Circulating Vasopressor Materials in Dogs with Acute and Chronic Renal Hypertension.* (29514)

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It was shown previously in this laboratory that the blood of rats with acute renal hypertension (1-14 days) yielded a vasopressor response when injected into normal recipient rats(1). In contrast, no pressor response could be obtained when blood from rats with chronic renal hypertension was tested in the same manner.

This paper concerns findings of a similar study in dogs with renal hypertension, which essentially confirm the results of our previous observations in rats.

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Methods. Method of producing hypertension. Eight dogs weighing from 15 to 20 kg were rendered hypertensive by constricting the main renal arteries with Goldblatt clamps. In 5 dogs both renal arteries were constricted and in 3 dogs the right kidney was resected and a clamp was applied to the left main renal artery. All operative procedures were done in one stage. The surgery was performed by Dr. Lester Persky, Professor of Urology, Western Reserve University.

Blood pressure recording. Blood pressure was recorded on a Hg. manometer by means of direct puncture of the femoral artery.