Isoantigenicity of Rabbit Semen.* (25339)

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Isoantigenicity of spermatozoa and testis has been established for a variety of animals (1-4), and autoantibodies to spermatozoa have been found in man in a number of cases (4-9). In view of the close immunological relationship between spermatozoa and seminal plasma, which has been recorded for man(10), guinea pig(11), rabbit(12), and bull(13), we became interested in the isoantigenicity of seminal plasma and its relationship to the antigenic components common to seminal plasma and spermatozoa.

Methods. Semen was obtained from mature and fertile, pure bred Dutch belted rabbits (Rockland Farms) by means of the artificial vagina(14,15). Rabbits of mixed breed were used for immunization. After an attempt to elicit antibody response by intravenous injections had failed, antigen was administered deep into the subscapular region in form of a suspension in Freund's adjuvant (Difco). Into 1 ml adjuvant, 1 ml ¼ diluted seminal plasma or 1 x 10^8 thrice washed spermatozoa in 1 ml saline solution were incorporated. Six injections at 14 day intervals yielded maximal response. Rabbits that showed antibodies in test bleedings were exsanguinated, and the serum stored in dry ice. Immunological methods and preparation of organ extracts used during these experiments have been described(10,12). Absorption of antibody was obtained by adding 500 million washed spermatozoa to 1 ml immune serum. The mixture was incubated at 37°C with frequent shaking, left in the refrigerator over night, and the spermatozoa removed by centrifugation at 10,000 rpm. Organs of immunized and non-immunized (control) rabbits were fixed in formalin and examined after staining with hematoxylin and eosin.†

Results. Of 10 rabbits injected with seminal plasma in Freund's adjuvant, 5 (2 male and 3 female) produced antibody demonstrable by complement fixation test, hemagglutination, spermagglutination, and agar diffusion (Ouchterlony's technic). The homologous antisera reacted in complement fixation test with saline extracts of rabbit prostate, but did not react with saline extracts of rabbit testis, epididymis, seminal vesicle, kidney, liver, spleen, muscle, nor with rabbit or human serum, human seminal plasma and spermatozoa (Table I).

Isologous immune serum shows only one precipitation band (Fig. 1). It gave a reaction of identity with the strongest line obtained with heterologous immune serum and could be removed by absorption of the immune serum with either seminal plasma or washed spermatozoa. The cross reactivity of the isologous immune sera against seminal plasma with spermatozoa could also be shown by their ability to agglutinate spermatozoa in high titers (1/1024 or higher).

The complement fixation method did not furnish evidence concerning this point because normal rabbit serum fixes complement in the presence of rabbit spermatozoa in serum dilutions individually varying between 1/10 and 1/160. This was seen with every specimen of 30 rabbits tested, including preimmuniza-

TABLE I. Fixation of Complement by Anti-Rabbit Seminal Plasma Rabbit Immune Serum (Diluted 1/10) and Antigens (Rabbit).

Antigen dilution	A Seminal plasma 1/500	B Prostate 1/10	C Testis* 1/10	D Serum 1/100
1/1	4+	4+	—	—
1/2	4+	4+	—	—
1/4	4+	4+	—	—
1/8	4+	4+	—	—
1/16	2+	—	—	—
1/32	—	—	—	—

* Negative results were similarly obtained with extracts of epididymis, seminal vesicle, spleen, liver, kidney, and muscle; also with human seminal plasma, spermatozoa and serum. Antigen and antiserum alone, also antigen plus normal rabbit serum remained negative.

4+, no hemolysis; 2+, moderate (approx. 50%) hemolysis; —, complete hemolysis.

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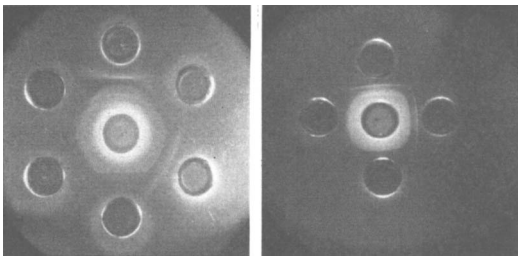

FIG. 1. Center troughs: Rabbit seminal plasma (undiluted). Outer troughs: Sera, undiluted. *Left Frame*, clockwise from top: guinea pig treated with rabbit seminal plasma (4 bands); rabbit treated with rabbit seminal plasma (not reactive); same, reactive (one band); rabbit treated with rabbit spermatozoa (not reactive); same; normal rabbit serum. *Right Frame:* guinea pig treated with rabbit seminal plasma (3 bands); rabbit treated with rabbit seminal plasma (1 band); the same sera absorbed with spermatozoa (no reactions).

tion bleedings from the rabbits subsequently injected with seminal plasma or spermatozoa and the sera of the Dutch belted rabbits serving as donors of the spermatozoa. No consistent increase in titer in terms of effective serum dilution or of concentration of spermatozoa could be observed in our serum specimens containing antibody according to all other criteria. The non-specifically reactive material was liberated from the spermatozoa by sonic vibration(see 10,12). The fluid in which the spermatozoa had been suspended during such treatment showed non-specific reactivity.

Eleven rabbits (5 male and 6 female) were injected with washed rabbit spermatozoa in Freund's adjuvant. No reactions significantly differing from those of pre-immune sera could be obtained in complement fixation tests with spermatozoa as antigen. Thus deprived of our most sensitive tool in testing for circulating antibody, we had to rely on other tests; they remained negative. They include agglutination with seminal and epididymal spermatozoa, hemagglutination, agar diffusion tests and complement fixation test with seminal plasma.

Histological examination of all the rabbits discussed above, including testes and epididymides, showed no degenerative or inflammatory processes.

Discussion. Rabbit seminal plasma is isoantigenic. The antibodies produced by rabbits injected with rabbit seminal plasma in Freund's adjuvant are of high specificity. Only one antigenic component could be detected with these antisera by the gel diffusion method. Thus it would appear that only one of the several (at least 5) antigenic components (12) demonstrable with heterologous immune sera is isoantigenic. The antigen involved is present also on spermatozoa obtained from semen. This could be proven by the ability of such spermatozoa to absorb the antibody from iso-immune sera and sperm agglutination.

However, no antibody formation was obtained in rabbits after 6 injections of 10^8 spermatozoa in Freund's adjuvant or after an extended course of intravenous injections. Why an antigen, which is highly effective in heterologous immunization with spermatozoa, should be ineffective isologically, is not clear. It may be worthy of exploration, because the same antigen is isologically effective in solute form, namely in the seminal plasma, under comparable experimental conditions.

Normal rabbit serum gives complement fixation with rabbit spermatozoa. The nature of this reaction needs to be clarified. It is in sharp contradistinction to the behavior of rabbit serum with the spermatozoa of other species(10) and to that of rabbit spermatozoa with the sera of guinea pig, mouse and man (12).

No degenerative changes are seen in the testes and epididymides of rabbits treated with spermatozoa or seminal plasma. We are grateful to Drs. Bishop and Katsh(16) for their permission to state that they also were unable to obtain degenerative changes in the testes of rabbits injected with testis emulsions in Freund's adjuvant. It would, therefore, appear that the rabbit does not produce the peculiar degenerative changes in the testis and epididymis which characterize the guinea pig's(3,16,17) and (to a lesser degree) the rat's(18) response to spermatozoa or testes suspensions.

Summary. 1) Rabbit seminal plasma contains at least one isoantigenically active component. 2) No isoantigenicity could be observed with rabbit spermatozoa under comparable experimental conditions even though the spermatozoa remove homologous antibody against seminal plasma and are agglutinated

by isologous anti-seminal plasma immune serum. 3) Normal rabbit sera fix complement with rabbit spermatozoa. 4) No degenerative damage to rabbit testis or epididymis could be produced by injections of spermatozoa or seminal plasma in Freund's adjuvant.

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Biological Effects of Hypoglycin A in Hydrocortisone Treated Adrenalectomized Rats.* (25340)

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Hassall and Reyle(1,2) succeeded in isolating 2 peptides, hypoglycin A and B, from fruit seeds of the tree, *Blighia sapida*, and reported them to cause hypoglycemia in rodents. Subsequent structural analyses revealed hypoglycin A to be an amino acid: cyclopropanepropionic acid, α-amino-2-methylene(3,4,5,6). Several authors reported on pharmacological and biochemical actions of hypoglycin A(7,8, 9,10). Attention in this report is directed to action(s) of hypoglycin A on several parameters of hydrocortisone-induced gluconeogenesis.

Methods. Adult male Sherman rats (140-170 g) were bilaterally adrenalectomized and maintained with 0.9% sodium chloride solution, along with high protein diet *ad libitum*. On fourth post-operative day the food was removed. Sixteen hours later the rats were injected subcutaneously with 1 mg of hydrocortisone suspended in 0.2 ml of a carboxymethylcellulose vehicle. Hypoglycin A was dissolved in water and 0.2 ml of the solution was injected intraperitoneally at time of steroid administration. Control animals received injections of the vehicles. No food or water was allowed during test period. Thirty minutes after initiating treatment, rats were injected intraperitoneally with 5 ml of 0.9% sodium chloride, and 2 rats placed in each metabolism cage. Four hours after steroid or drug administration animals were anesthetized with sodium pentobarbital and livers were excised and hydrolyzed immediately in 30% potassium hydroxide. During removal of liver, blood was drawn from the portal vein and analyzed for glucose by the Nelson method(11). Liver glycogen was determined by the anthrone method of Seifter, *et al.*(12).

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