Antigens of Rabbit Semen.* (24188)

ALFRED J. WEIL AND ALEXANDER E. FINKLER
Department of Bacteriology, Bronx Hospital, New York 56, N. Y.

It has long been known that spermatozoa are antigenic and possess a high degree of immunological specificity(1-5). Strong antigenicity was also reported for seminal plasma of man(4-6) and several animal species(3,5. 7). Both for spermatozoa and for seminal plasma evidence was obtained that more than one antigenic entity is involved. That spermatozoa and seminal plasma have in common powerful and seemingly preponderant antigens has recently been established both for man(4) and guinea pig(5). In man, at least, indications are that the common antigens do not have their origin in the testes, but in the adnexal glands of the male genital tract. The antigenic material, however, is so firmly adherent to spermatozoa, that it has hitherto been impossible to separate it from these cells by physical means. Further study of the immunological relationship between spermatozoa and seminal plasma seemed to be indicated, with emphasis on the search for the site of production of these antigens.

Methods. Dutch belted rabbits purchased from Rockland Farms served as semen donors. Semen was obtained by means of the artificial vagina, first developed by Macirone and Walton(8) and introduced in this country by Gregoire, Bratton and Foote(9)†. Rubber balloons from Anderson Co., Akron, cut at the tip, provided satisfactory linings. Procedures of complement fixation test, sperm-agglutination, and hemagglutination with tanned erythrocytes were identical with those described before(4). The Ouchterlony technic(5,10) was used for demonstration of antigen-antibody reaction by agar-diffusion. Controls with antigen alone and antigen plus preimmunization serum were included in the tests and always found non-reactive. Rabbit semen contains spermatozoa in the order of 200 million/ml. Small numbers of large cells with round nuclei and numerous spherical bodies of approximate size of cocci are also present. Their nature is unknown. It is worth noting that these spherical bodies are not affected in any visible way by addition of immune serum in the slide agglutination test with spermatozoa. Immune sera of high antibody titer were obtained in guinea pigs after 2 intramuscular injections at 2 week interval of 0.5 ml of a mixture of equal parts of Freund's adjuvant (Difco) and either seminal plasma (diluted 2/5 with physiological saline solution) or washed spermatozoa in saline (adjusted to contain 10 million cells/dose.) Serum was collected 3-4 weeks after last injection. Sera of 4-6 guinea pigs were pooled after preliminary individual tests for titer. Suspensions of spermatozoa in physiological saline solution were treated in the Magnetostriction Oscillator (Raytheon, 9 kilocycles, 50 W). By alternating centrifugation at low and high speeds 3 fractions were obtained, namely (a) spermatozoal heads and a few detached tails; (b) tails plus the spherical particles mentioned before; and (c) a clear supernatant fluid.

Results. Complement fixation in serum dilutions varying between 1/100 and 1/1600 was obtained. Anti-seminal plasma and anti-spermatozoal sera were indistinguishable in reactivity with the 2 antigens. Complement fixation was observed with seminal plasma in dilutions varying between 1/1600 and 1/12800 or slightly better (Table I).

Our immune sera against rabbit seminal plasma and spermatozoa did not show cross reactivity with human seminal products and vice versa. These observations further confirm data on species specificity of seminal antigens in the literature, including the recent data of Katsh(11). The occasional findings of this author suggesting slight cross-reactivity in maximally sensitized guinea pigs merit further critical exploration.

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† We are most grateful for the opportunity of learning their procedure at Cornell Univ. Agric. Exp. Station.

TABLE I. Fixation of Complement by Guinea Pig Immune Serum (Diluted 1/20) and Antigens (Rabbit).

Antigen dilution	A Seminal plasma 1/200 Anti-sem.	A Seminal plasma 1/200 Anti-sp.	B Spermatozoa 10^6/0.1 ml Anti-sem.	B Spermatozoa 10^6/0.1 ml Anti-sp.	C Testis 1/10 Anti-sem.	C Testis 1/10 Anti-sp.	D Epididymis 1/10 Anti-sem.	D Epididymis 1/10 Anti-sp.	E Seminal vesicle 1/10 Anti-sem.	E Seminal vesicle 1/10 Anti-sp.	F Seminal vesicle content 1/10 Anti-sem.	F Seminal vesicle content 1/10 Anti-sp.	G Prostate 1/10 Anti-sem.	G Prostate 1/10 Anti-sp.	H Kidney 1/10 Anti-sem.	H Kidney 1/10 Anti-sp.	I Liver 1/10 Anti-sem.	I Liver 1/10 Anti-sp.	J Serum 1/100 Anti-sem.	J Serum 1/100 Anti-sp.
1/1	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	—	—	—	—
1/2	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	3+	,,	,,	—	—	—	—
1/4	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	3+	—	2+	2+	—	—	—	—
1/8	,,	,,	,,	,,	3+	,,	1+	3+	,,	,,	,,	,,	—	—	—	—	—	—	—	—
1/16	,,	,,	,,	,,	—	3+	—	—	,,	—	,,	,,	—	—	—	—	—	—	—	—
1/32	,,	,,	,,	,,	—	—	—	—	3+	—	,,	,,	—	—	—	—	—	—	—	—
1/64	,,	2+	3+	4+	—	—	—	—	—	—	,,	,,	—	—	—	—	—	—	—	—
1/128	1+	(+)	1+	1+	—	—	—	—	—	—	,,	3+	—	—	—	—	—	—	—	—
1/256	—	—	—	—	—	—	—	—	—	—	1+	—	—	—	—	—	—	—	—	—

Antiserum and antigen controls, also antigen plus normal rabbit serum negative.
Anti-sem. = Anti-seminal plasma serum; Anti-sp. = Anti-spermatozoal serum.
4+, no hemolysis; 3+, slight (approx. 30%) hemolysis; 2+, moderate (approx. 50%) hemolysis; 1+, strong (approx. 70%) hemolysis; (+), almost complete; —, complete hemolysis.

If heavy suspensions of thrice washed rabbit spermatozoa (100 million/ml or more) in saline are kept in the refrigerator 1 to 8 weeks, the clear supernate obtained by centrifugation is reactive in complement fixation test with anti-seminal plasma or anti-spermatozoal sera in dilutions of 1/64 to 1/128. The spermatozoa of the sediment showed no measurable loss of reactivity.

The fractions obtained after sonic vibration of spermatozoa gave complement fixation to approximately equal extent with anti-seminal plasma and antispermatozoal sera. No immunological differentiation was observed.

Attempts to use methods of adsorption failed, due to development of anticomplementary properties. The anticomplementary action could not be diminished by 2 hours' centrifugation at 33,000 g (16,000 rpm in Lourdes high speed angle centrifuge).

High organ specificity of immune sera is exemplified by data given in Table I. The organ extract employed in these tests was prepared as previously described for material of human origin(4). In striking contrast to the slight cross-reactivity of organ extracts and lack of cross reactivity with rabbit serum, the fluid obtained from seminal vesicles was reactive in dilutions comparable with those of seminal plasma. This fluid was obtained by removal of the content from seminal vesicles (which are rather large in the rabbit) with a fine hypodermic needle and syringe. Some turbidity was removed by centrifugation. Under the microscope the sediment showed numerous spherical bodies similar to those described above for semen, and a very few spermatozoa. The clear supernate was not anticomplementary.

Indications of a difference in reactivity between anti-seminal plasma and the anti-spermatozoal immune sera were also absent in hemagglutination tests with tanned human erythrocytes on which seminal plasma was adsorbed (for technical data see 4 and 12). Agglutination was observed in serum dilutions ranging up to 1/40,000. No systematic difference was seen between the 2 kinds of immune sera.

Tanned erythrocytes treated with the con-

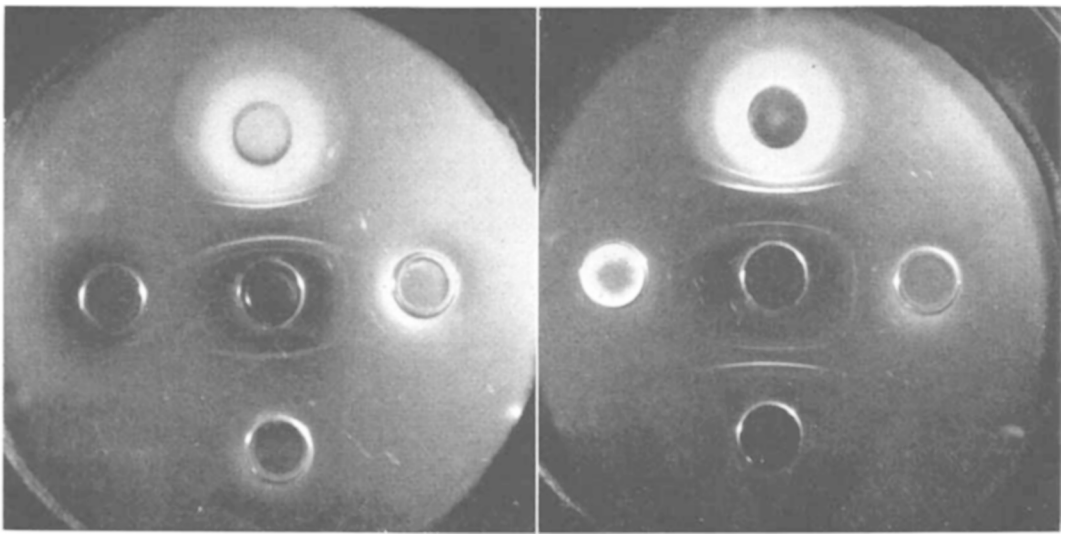

FIG. 1. Centers: Anti-rabbit seminal plasma serum (undiluted). Outer troughs: Antigens undiluted, rabbit. *Left frame:* top, seminal plasma; right, testis; bottom, epididymis; left, blood serum. *Right frame:* top, seminal plasma; right, seminal vesicle, wall; bottom, seminal vesicle, content; left, prostate.

tent of seminal vesicles were agglutinated by our immune sera in dilutions up to 1/1600. However, tanned erythrocytes treated with various organ extracts were not agglutinated by sera in dilutions of 1 25.

Both anti-seminal plasma and antispermatozoal immune sera strongly agglutinated spermatozoa from whole fresh semen and also spermatozoa thrice washed with physiological saline solution. The slide agglutination method(4,13) was used. With fresh semen, serum dilutions up to 1 4096 gave definite agglutination. With washed spermatozoa, the titers were lower, ranging from 1/16 to 1 512. These findings differ from those of Smith (3) who observed higher agglutination titers with washed spermatozoa than with fresh semen. Again, no difference could be detected in the behavior of the 2 kinds of immune serum employed. Agglutination was essentially of the "tail" type, as also reported by Smith(3).

In the absence of spermatozoal antigen in soluble form, immune precipitation by the agar diffusion method is at a disadvantage for purposes of testing spermatozoal antigen. However, the method yielded 2 contributions of interest for our problem: Antiseminal plasma and anti-spermatozoal sera gave the same precipitation lines with seminal plasma, namely 2 heavy ones close to and curved toward the trough containing the antigen and 3 (2 of them very close together) near and curved toward the antiserum (Fig. 1). Thus by this technic also, the 2 kinds of antiserum remain indistinguishable in their reactivity with seminal plasma. Secondly, antiseminal plasma immune serum can be completely exhausted by spermatozoa, and anti-spermatozoal serum, in turn, by seminal plasma as far as reactivity in the Ouchterlony test (with seminal plasma as antigen) is concerned. The value of this evidence is somewhat restricted by the fact that the agar diffusion method is less sensitive than the complement fixation test.

The data obtained by complement fixation method on organ specificity of immune sera were supplemented in an illuminating way by the results with the agar diffusion technic (Fig. 1). As far as cross reactions with organ extracts can be demonstrated, they are related to one single precipitation line, namely the second one (counted from trough containing antiserum). There is one important exception; with the extract from seminal vesicle, all 3 lines close to the antiserum containing trough are produced. The content of

the seminal vesicles produced 4 lines obtained with seminal plasma, *i.e.*, all of the reactions of seminal plasma except the one indicated by innermost (nearest to antiserum trough) weak line. Serum, kidney and liver extracts did not produce lines of precipitation.

Conclusions and summary. 1) Comparison of data with those on human semen presented previously(4) shows a close similarity in the immunological properties of seminal products of man and rabbit. In both species, seminal plasma and spermatozoa contain powerful antigens, so closely related that they have resisted differentiation. 2) These antigens are highly species and organ specific. Such cross-reactions as could be obtained, according to evidence provided by agar diffusion method, are due to one of the 5 components demonstrable by this procedure. The multiplicity of antigenic components corresponds with that previously shown in man and guinea pig (4-6). 3) Data obtained from human semen specimens lacking spermatozoa suggested that the antigenic material does not originate with spermatozoa. This view is further strengthened by the low degree of cross reactivity of rabbit sera with extracts from testes and epididymis, and, still more strongly, by evidence that extracts of seminal vesicle wall contain some antigenic components reactive with anti-seminal plasma and anti-spermatozoal immune sera. The seminal vesicle fluid of the rabbit contains in abundance nearly all antigenic components demonstrable with our sera. 4) The effective antigens found in seminal plasma and spermatozoa of semen appear to originate in the seminal vesicle.

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Inhibition of Carbonic Anhydrase: Effect on Tissue Gas Tensions in the Rat* (24189)

JOHN C. MITHOEFER AND JOHN S. DAVIS (Introduced by H. Rahn)
(With technical assistance by E. Moffat and R. Hanor)
Cardio-Pulmonary Laboratory, Mary Imogene Bassett Hospital, Cooperstown, N. Y.

The established importance of carbonic anhydrase in CO_2 transport implies impairment in CO_2 elimination when the enzyme is inhibited. This controversial problem, has been discussed, and evidence presented for impaired CO_2 transport with resultant retention of this gas following carbonic anhydrase inhibition in the dog(1). The evidence presented, however, did not demonstrate a rise in venous or tissue CO_2 tension following inhibition of carbonic anhydrase, a change implicit in the mechanism of CO_2 retention from any cause. Such demonstration was not possible because usual technics for measurement of venous CO_2 tension are inapplicable in the presence of carbonic anhydrase inhibition. When carbonic anhydrase is inhibited a dis-

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