Antigens of Human Seminal Plasma.* (22558)

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The spermatozoa of man and other animals contain antigens of marked specificity(1-4). However, human seminal plasma has not been examined by immunological technics.† As to animals, Smith(4) produced one antiserum against rabbit seminal plasma in a goat, which reacted with seminal plasma, but did not agglutinate rabbit spermatozoa, whereas antispermatozoal sera did crossreact with spermal plasma. The immunological properties of human seminal plasma and the peculiar relations of the antigens of seminal plasma to those of human spermatozoa are the subject of this report.

Technics. Human semen specimens were obtained from private and clinic patients.‡ Except for agglutination tests necessitating the use of motile spermatozoa, specimens could be preserved in the refrigerator for several weeks without noticeable impairment of immunological activity. Dilutions were made in 0.85% NaCl solution, except where otherwise noted. Separation of seminal fluid and spermatozoa was done in a high speed angle centrifuge (Lourdes) by spinning for 10 min. at 10,000 rpm. The sediment was washed by repeated centrifugation with saline, until no antigen could be detected in the supernatant fluid by complement fixation test. The spermatozoa thus collected always contained small amounts of cellular (epithelial?) detritus and a few leukocytes. For sonic disintegration a Raytheon Magnetostriction Oscillator (9 kilocycles, 50 W.) was used. Spermagglutination tests were made according to the method outlined in(6). Precipitation tests were made by layering undiluted immune serum beneath varying dilutions of antigen. After reading for ring formation, the tubes were shaken and further readings were made after incubation for 2 h at 37°C and finally after standing in the refrigerator overnight. Tests with the agar diffusion method utilized the procedure outlined by Vaughan and Kabat(7). Complement fixation tests were performed according to the standard Kolmer method with 2 "full" units of complement. Immune sera were produced in rabbits either by a series of intravenous injections, or by 3 intramuscular injections of equal volumes of antigen and Freund's adjuvant.§ No difference in the behavior of the antisera obtained by these 2 methods was noted. Roosters were immunized by a series of intravenous injections.

Serum specimens obtained before immunization and sera of other untreated animals were used as controls. As no reactivity was seen in any of our tests with these controls or with the antigens used, they are not mentioned further in the text or the tables. Agglutination tests with fresh or tannic acid treated erythrocytes for adsorption of antigen were made according to the method given in (8).

Observations. Regardless of whether whole semen, seminal plasma or washed spermatozoa were injected, the rabbit immune sera, in serum dilutions individually varying between 1/80 and 1/640, reacted in complement fixation tests with seminal plasma in dilutions up to 1/100,000 or slightly more. This is exemplified in Tables I and II. The same sera also reacted with washed spermatozoa. One to four million spermatozoa (in 0.1 ml saline solution) sufficed to yield complete inhibition of hemolysis with 0.01 to 0.0006 ml immune serum. Rooster immune sera did not give complement fixation.

Both rabbit and rooster immune sera pre-

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† Ross *et al.*(5) mention preparation of immune serum against seminal plasma, but data on work done with it have not been found.

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TABLE I. Fixation of Complement by Rabbit Immune-Serum against Whole Human Semen (Diluted 1/20) and

Antigen dilution	a Whole semen 1/1000	b Seminal plasma (supernate from a) 1/1000	c Spermatozoa (6×10^6/0.1 ml)	d Last washing fluid from c 1/5
1/ 1	4+	4+	4+	—
1/ 2	4+	4+	4+	—
1/ 4	4+	4+	—	—
1/ 8	4+	4+	—	—
1/ 16	4+	4+	—	—
1/ 32	4+	4+	—	—
1/ 64	2+	2+	—	—
1/128	—	—	—	—

In this and the following tables, degree of hemolysis is represented as follows: 4+, no hemolysis; 3+, slight (approximately 30%); 2+, moderate (approximately 50%); 1+, strong (approximately 70%); (+), almost complete; —, complete hemolysis.

TABLE II. Fixation of Complement by Rabbit Immune Sera (Diluted 1/10) and

	Seminal fluid 1/1000		Washed spermatozoa 1×10^6/0.1 ml	
Antigen dilution	a Anti-seminal plasma serum	b Anti-spermato-zoal serum	c Anti-seminal plasma serum	d Anti-spermato-zoal serum
1/ 1	4+	4+	4+	4+
1/ 2	4+	4+	4+	4+
1/ 4	4+	4+	4+	4+
1/ 8	4+	4+	4+	4+
1/ 16	4+	4+	(+)	—
1/ 32	4+	4+	—	—
1/ 64	4+	4+	—	—
1/128	—	—	—	—

cipitated seminal plasma in dilutions up to 1/1000 or 1/10,000. Precipitation by the rooster sera was slower than with rabbit sera. Their reactions were not accelerated or increased by raising the NaCl content of the system to 4 or 8%.

Precipitation of seminal plasma was also obtained by the agar diffusion method with all the sera from both animal species. The zones observed with rooster sera were less clear cut than those with rabbit sera. With the latter, 3 regions of precipitation could usually be seen, namely a zone of heavy precipitation which moved with extreme slowness; a zone of precipitation moving with greater speed and often subdivided in many (up to 10) bands located very close to each other; and finally a faint band moving more rapidly than the second one. Individual specimens of seminal plasma show a considerable variation in the number of bands produced and in the speed with which they move with the same immune serum. This handicapped the identification of bands considerably. No consistent differences could be ascertained between the behavior of sera against seminal plasma and of those against spermatozoa.

Pre-treatment and other control rabbit or rooster sera agglutinated equally fresh and tannic-acid treated human erythrocytes (Group O) in dilutions between 1/5 and 1/20. (Rooster serum may also cause partial hemolysis in dilutions of this order.) Treatment of fresh erythrocytes with seminal plasma did not result in agglutination with immune sera beyond that seen with the controls. However, erythrocytes treated with tannic acid and then brought in contact with seminal plasma were agglutinated by our immune sera in dilutions of 1/10,000 or better. Again, there was no significant difference in the reactions of anti-seminal plasma and anti-spermatozoa immune sera.

TABLE III. Fixation of Complement by Rabbit Immune Sera (Diluted 1/10) and Antigens (Human).

Antigen dilution	A Seminal plasma 1/1000		B Serum 1/100		C Kidney 1/5			D Liver 1/5			E Spleen 1/5			F Atrophic testis 1/5		
	Anti-seminal plasma	Anti-spermatozoa	Anti-serum	Anti-seminal plasma	Anti-spermatozoa	Anti-serum	Anti-seminal plasma	Anti-spermatozoa	Anti-serum	Anti-seminal plasma	Anti-spermatozoa	Anti-serum	Anti-seminal plasma	Anti-spermatozoa	Anti-serum	Anti-serum
1/ 1	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
1/ 3	4+	4+	3+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+
1/ 10	4+	4+	—	4+	2+	4+	4+	2+	4+	4+	4+	4+	4+	—	4+	4+
1/ 30	4+	4+	—	2+	—	4+	(+)	—	4+	(+)	—	4+	—	—	4+	4+
1/ 100	4+	4+	—	—	—	3+	—	—	2+	—	—	1+	—	—	4+	4+
1/ 300	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1/ 1000	—	not done	—	—	—	—	—	not done								
1/ 3000	—		—	—	—	—	—									
1/10,000	—		—	—	—	—	—									
1/30,000	—		—	—	—	—	—									

Attempts failed to remove cross-reacting antigen from the spermatozoa both by extended washing in saline and by treatment by sonic vibration. After 5 min. treatment in the oscillator, the spermatozoa had lost their tails. After more extended treatment—up to one hour—the number of spermatozoal heads collectible by centrifugation became very small (exact counts were impossible because of clumping), and the remaining heads were visibly deformed. This effect became particularly pronounced if sonic treatment was carried out in 8% rather than in 0.85% NaCl solution. However, the heads remained just as reactive in the complement fixation tests as were the original spermatozoa.

When rabbit antisera were absorbed with spermatozoa, the antibodies were completely removed. The matter of differential absorption needs further study.

We had occasion to examine the ejaculates of 3 persons which did not contain spermatozoa due either to obstruction of the vas deferens or to failure of spermatogenesis. These ejaculates were just as active in their reactions with our antisera as were the seminal fluids of normal individuals.

The cross-reactivity of our antisera with other materials of human origin was studied. About half of 15 sera did not react in precipitation and complement fixation tests with human sera. The others showed reactivity varying from traces to reactions at considerable dilutions, but never more than about 1/100 of the reactivity of antisera versus human serum. In agar diffusion tests, the sera nonreactive in complement fixation did not produce precipitation at all. The most cross-reactive sera showed one weak, slowly moving band and a very faint one moving slightly faster. Anti-human serum immune sera showed only very scanty cross-reactivity with seminal plasma, but the most reactive of them (which is the one shown in Table III) showed in agar precipitation 3 well defined bands with seminal plasma. Of these the middle one exhibited several sub-zones similar to the ones described for the homologous system.

Saline extracts from human organs were

prepared by suspending finely minced tissue freshly obtained from autopsies in 10 times their (wet) weight of saline solution containing 1/10,000 merthiolate, whirling for 5 min in a Waring blendor, and filtering through paper. These extracts could be used in 1/5 dilution in complement fixation tests without anticomplementary action. Extracts have been made from kidneys, livers, spleens, and from the atrophic testes of an 82-year-old man. Results of complement fixation tests with these extracts are exemplified in Table III together with those with an anti-human serum immune serum. Cross reactivity as judged by these tests is slight. Similarly, precipitation tests with these extracts against anti-seminal plasma and anti-spermatozoal immune sera in fluid systems and by agar diffusion were entirely negative.

No information is as yet available concerning the chemical nature of the antigenic material in seminal plasma. Most of the *in vitro* activity is lost after 5 min. boiling undiluted or diluted 1/10 in saline solution. A small remainder stayed reactive even after 2 hours boiling. Only traces of material reactive *in vitro* could be extracted with organic solvents, such as alcohol, acetone, ether, or petroleum ether.

Two specimens of human serum containing the autoagglutinative antibody against spermatozoa described by one of us(6) were found not reactive in complement fixation tests with seminal fluid and with spermatozoa. No precipitation was observed in fluid systems with seminal plasma as antigen. However, in the agar diffusion test, the sera of both patients yielded with seminal plasma a weak and slowly moving zone, which was not seen if other human sera were employed.

Discussion. Seminal plasma contains highly antigenic material. It was impossible to distinguish by immunological methods between seminal plasma and spermatozoa with the technics employed. It remains uncertain whether the spermatozoa have antigenically active components of their own. A re-evaluation of the formerly collected data on the antigenic behavior of spermatozoa in the light of the evidence recorded here is indicated. It is likely that the antigenic material originates from the fluid products of the genital tract rather than from the spermatozoa because ejaculates free of spermatozoa show the same immunological behavior as does seminal plasma from normal semen. The observation that extracts from degenerated testes showed no more cross reactivity than other organs indicates also that the testis is not a major source of antigen. The testes from which the extract was prepared showed many immature germinal cells, though very few mature spermatozoa.

The data available make it likely that more than one antigenic component is present both in the seminal plasma and in or on the spermatozoa. The major portion of these antigens is of pronounced organ-specificity. It is remarkable that about half of our immune sera did not give any cross-reaction with human serum as antigen. A quantitative appraisal cannot, of course, be attempted as long as the general nature of the antigens involved remains unknown. Tests for species specificity have not yet been done. Arrangements are being made for collecting animal semen for this purpose. The data presently available on the species specificity of spermatozoa (1,2,4) would seem to be in need of supplementation by data on the immunological behavior of seminal plasma. The observations of Smith(4) suggest that what has been found with human semen must not be necessarily true for other animals.

The lack of reactivity of the sera with human spermagglutinating antibody in the sensitive complement fixation test is of interest. The agar diffusion test yielded a weak, but unmistakable zone of precipitation. This makes it likely that one of the antigenic components reactive with our immune sera is also related to the agglutinating antibody found in a few human beings.

Summary. Rabbits and roosters injected with whole human semen, seminal plasma and washed spermatozoa produced antisera which showed a high degree of specificity for seminal antigens as compared with human serum and organ extracts. However, a distinction be-

tween seminal plasma and spermatozoa could not be obtained with immunological technics. Evidence which would make it seem likely that the dominant antigenic material is derived from the fluid constituents of the semen rather than from the spermatozoa is presented. Human spermagglutinating sera do not give complement fixation tests with seminal plasma or spermatozoa, but they produce a zone of precipitation with seminal plasma as antigen in the agar diffusion test.

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Hemodynamic Effects of Vasopressor Agent (Metaraminol)* on Hypotension in Dogs Produced by Endotoxin. (22559)

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The mechanism of hypotension which occurs during the course of overwhelming infections has been studied intensively both in patients and experimentally in animals(1-5). The very striking hemodynamic changes which are sometimes observed in patients with bacteremia due to Gram-negative organisms have been attributed to the action of endotoxins which are liberated on dissolution of bacteria(6) and experimental shock may be readily produced by the administration of endotoxins derived from various Gram-negative bacteria. We had previously observed that intravenous injections of endotoxins, such as those obtained from *Escherichia coli* or *Brucella melitensis*, uniformly cause a prompt decline in arterial blood pressure in the anesthetized dog(7). This marked fall of blood pressure was shown to be the result of a corresponding fall in cardiac output without decrease in peripheral resistance. When total venous return was measured, the decreased cardiac output was found to be due to a marked deficiency in venous return(5,8). These changes were further correlated with a sharp increase in liver and intestinal weights (9). Our observations led to the tentative conclusion that the initial cardiovascular disturbances caused by various Gram-negative endotoxins in the dog are the end result of massive venous pooling in visceral organs(8).

The value of vasopressor agents in the treatment of shock has been controversial. Rashkind and co-workers(10) reported that norepinephrine normally increases venous return in the dog. Von Euler(11) expressed the opinion that norepinephrine has important vasoconstrictor effects on both arterial and venous vasculature thereby favoring effective circulation in patients with shock. Clinical experience with a related sympathomimetic amine, metaraminol (Aramine), has shown that this new vasopressor substance was effective in the management of shock associated with bacteremia(12). In an attempt to gain further insight into the influence of vasopressor agents on the course of shock, the effect of metaraminol on venous return in dogs was

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