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THE SPECIFICITY OF SOME MAMMALIAN SPERMATOZOA

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Mammalian spermatozoa have been shown to possess species specificity (1). However, between the spermatozoa of two closely related species, namely the bull and the ram, close serologic relationship has been found (1). The question of whether or not serologic relationships (as well as differences) might also be found between the sperm-cells of less closely related species was left open by the work cited. The present investigation shows that relationship as well as specific difference may be demonstrated between spermatozoa even of different mammalian orders. A study of the specificity of isoimmune sera of two species has also been made.

PREPARATION OF SUSPENSIONS OF SPERMATOZOA

The testes of bulls and rams were obtained from the abattoir with all their coverings intact. The testes of rats and mice were excised by way of the abdominal cavity, and rabbits were castrated under aseptic conditions.

After all adherent blood clots were washed off, the *tunica vaginalis* was incised and the *cauda epididymidis* removed together with a long section of the *vas deferens*. If the testes had been removed through the abdominal cavity, and were no longer covered with the *tunica vaginalis parietalis*, the *cauda epididymidis* was cut off immediately. In rats and mice it was necessary to close the *vas deferens* with a clamp before cutting it off, as it is filled with spermatozoa which otherwise might flow out. Pieces of epididymis with the adherent *vas*, were placed in sterile saline until used. Saline solution was injected into the *vas* with a 10-ml. syringe. A blunt 22-gauge needle was used for all species, including the mouse. The *vas* was held on the needle by means of a clamp, whose jaws were covered with fine rubber tubing to prevent cutting of the tissue and backflow of the saline.

On injection of a small amount of saline, the *ductus epididymidis* protruded in certain places, one distended section after another appearing over the shiny surface of the epididymis. When the distension became great enough to threaten rupture of the duct, an incision was made in the last portion to fill up. The spermatozoa thus collected were washed once with saline and counted in a Levy blood-counting chamber. Manipulations were carried out with precautions to minimize contamination. Most of the suspensions were used immediately after obtaining them, particularly those used for injections. Others were kept in the refrigerator for no more than two days.

To avoid possible interference of blood-group antigens,—related substances being common among certain animals,—all human spermatozoa used were obtained from persons of group O. Yamakami (2) and at the same time Landsteiner and Levine (3) showed that spermatozoa can specifically absorb the alpha- and beta-agglutinins. The donors were asked to keep the semen in a test tube in the icebox, and to deliver it within twelve hours. The spermatozoa were washed twice with ten volumes of saline solution. It was impossible to obtain sufficient amounts of spermatozoa from necropsy-material, as the human epididymis apparently contains only a small number of spermatozoa. Similarly, the epididymis of the rabbit usually contains only a small number of sperm-cells.

PREPARATION OF ANTISERA

Female Chinchilla rabbits of about five to six pounds in weight were injected with suspensions of spermatozoa intravenously at two- to three-day intervals. Four to twelve injections were required to produce antisera of sufficient antibody-content. The number of injections depended upon the density of the spermatozoal suspensions. The animals were able to withstand, with no apparent untoward effects, the injection of 800 million bull spermatozoa in 1 ml. of suspension. Four to five such 1-ml. injections built up a titer equal to that obtained with ten to twelve 2-ml. injections containing 50 million per milliliter. Dense suspensions can be obtained economically from only a few species.

Antisera against blood serum were easily obtained with seven to eight intravenous injections of 1 ml. of serum each.

The animals were bled 8 to 10 days after the last injection. All of the antisera were inactivated at 56°C. for 30 minutes and were tested for potency by the complement-fixation technic. Guinea-pig serum preserved by the lyophile process (4) was used as the source of comple-

ment. Many of the antisera also were preserved in lyophile form until needed.

TISSUE-SPECIFICITY

The experiments of Landsteiner and van der Scheer (5) indicated that spermatozoa are tissue-specific. They compared these cells with suspensions of thymic tissue, tracheal epithelium, and

TABLE 1

Differentiation between serum and spermatozoa by means of antiserum

DILUTION OF ANTIGENS		ANTISERUM 1: 10					
		I. Rabbit vs. bull serum		II. Rabbit vs. bull spermatozoa		III. Saline control	
A Bull serum	B Bull spermatozoa 10^9/ml.	A Serum	B Sperm	A Serum	B Sperm	A Serum	B Sperm
1:50	1:1	0	tr	c	0	c	str
1:80	1:3	0	wk	c	0	c	ac
1:125	1:5	0	ac	c	0	c	c
1:250	1:10	0	c	c	0	c	c
1:415	1:16	0	c	c	0	c	c
1:625	1:25	0	c	c	0	c	c
1:1250	1:50	0	c	c	0	c	c
1:2080	1:80	0	c	c	tr	c	c
1:3125	1:125	0	c	c	wk	c	c
1:6250	1:250	0	c	c	ac	c	c
1:10,410	1:415	0	c	c	c	c	c
1:15,625	1:625	tr	c	c	c	c	c
0	0	c	c	c	c	c	c

0 indicates no hemolysis, tr = trace, wk = weak, str = strong, ac = almost complete, c = complete hemolysis (symbols for tables 1–7).

renal tissue. But the problem of tissue-specificity of spermatozoa cannot be considered settled until a comparison with a greater number of tissues has been made. The question of possible cross-reactions between spermatozoa and serum is of special importance. Hektoen and Manly (6) studied the specificity of human semen and found that antisemen also reacted with homologous serum. After adsorption of antisemen with homologous serum, distinct reactions remained for seminal fluid, as well as for homologous spermatozoa.

Antispermatozoal-serum, which should be more specific than antisemen, has been tested with the spermatozoa and the serum of the bull.

To constant volumes (0.25 ml.) of increasingly diluted normal bull serum and bull spermatozoa (initial concentration 100 million per milliliter) were added 0.25-ml. volumes of 1:10 rabbit vs. bull serum; to a second series, rabbit vs. bull spermatozoa, and to a third, saline solution; then 0.25 ml. of complement, 1:15. After incubation at 37°C. for one hour, 0.5 ml. of sensitized 5 per cent sheep-cells was added to each tube.

The results (table 1) show that the antispermatozoal serum reacts only with its homologous antigen, and similarly, the antiserum prepared against bull serum does not react with the spermatozoa. Corresponding results were obtained with antisera for human spermatozoa and human serum as well as for spermatozoa and serum of the rat. It is quite likely that the method of obtaining the spermatozoa in these experiments explains this clear-cut result, as well as the failure of antispermatozoal sera to produce any hemolysis of homologous red blood cells. The sperm-suspensions were practically free of blood.

ISOIMMUNIZATION

Von Dungern and his co-workers (7, 8), who attempted to immunize dogs with dog blood, stated that the antigenicity of a substance depends less on the fact that it is foreign to the species or to the body, than that it must be foreign to the circulation (*zirkulationsfremd*). Although this statement is apparently based on blood-group differences, it has a bearing on isoimmunization in general.

Uhlenhuth and Seiffert (9) summarized the work (up to 1930) on isoimmunization with the lens of the eye, and showed that no definite conclusions could be drawn even though there were reports of weakly positive immune responses. Witebsky and Steinfeld (10) showed that brain possesses an organ-specificity comparable to that of the lens but Lewis (11) found an exception in that the cross-reaction with testis was so marked that by his method no differentiation was possible between the two tissues.

Schwentker and Rivers (12) succeeded in isoimmunizing rabbits only after adding foreign portein to brain-suspensions or after allowing autolysis under sterile conditions. Hektoen and Schulhof (13) found that rabbits could be isoimmunized with thyroglobulin, but it is necessary to consider the possibility of antigenic changes in the thyroglobulin during the process of purification.

Numerous papers on isoimmunization with spermatozoa have been published. Dittler (14), Guyer (15), Pommerenke (16) and Mudd and Mudd (1) have reported the production of antirabbit "spermatotoxins" in rabbits. Metalnikoff (17), Adler (18), Savini and Savini-Castano (19), Kennedy (20), and Eiseman and Friedman (21) were able to produce the same results in guinea pigs, and McCartney (22) and Fogelson (23) obtained positive results in rats. Negative results were obtained in the case of rats and rabbits by Eiseman and Friedman, and Oslund (24) was not successful in isoimmunizing these animals or guinea pigs.

The aim of most of these investigators was the prevention of pregnancy. Various antigens, such as semen, macerated testis, and epididymis were used, and serologic testing was done by means of immobilizing spermatozoa with the resulting sera (immobilization-test). This test is not readily workable in large-scale experiments. Nevertheless, it has disclosed a positive iso-antibody-response when used by other workers. By the electrophoretic technic, Mudd and Mudd occasionally obtained positive results in rabbits. Insufficient dosage of antigen or too brief a period of immunization could account for negative findings.

Isoimmunization of rabbits

The technic of obtaining isoantisera against spermatozoa was the same as that used for producing heteroantisera. Female rabbits were injected nine times, by the intravenous route, with 1.5 to 3 ml. of a once-washed suspension of rabbit-spermatozoa (100 to 200 million cells per injection). The suspensions were prepared freshly for each injection.

The reactions of the isoantisera are given in tables 2 and 3. The technic of the test has been described. The serum of three out of four rabbits reacted powerfully with spermatozoa of their

own species. The serum of one of these immunized rabbits was no more potent than normal rabbit-serum. London (25) and Fitzgerald (26) found isoantibodies present in some normal sera, although in very low titer. The normal sera used in these studies

TABLE 2

Rabbits' isoimmune sera tested with decreasing amounts of spermatozoa

RABBIT SPERMATOZOAL SUSPENSION 8×10^7/ML. DILUTIONS	ISOIMMUNE SERA				NORMAL SERUM
	No. 36	No. 37	No. 38	No. 39	No. 56
1:1	0	0	0	0	0
1:3	0	tr	0	0	tr
1:5	0	ac	0	0	str
1:10	0	c	0	0	ac
1:16	0	c	0	0	c
1:25	0	c	0	0	c
1:50	tr	c	wk	tr	c
0	c	c	c	c	c

TABLE 3

Rabbits' isoimmune sera tested with decreasing amounts of antiserum

DILUTIONS OF ANTISERUM	SUSPENSION OF RABBIT-SPERMATOZOA (3×10^7/ML.)				
	Isoimmune sera				NORMAL SERUM
	No. 36	No. 37	No. 38	No. 39	No. 56
1:10	0	0	0	0	tr
1:16	0	tr	0	0	ac
1:25	0	wk	0	0	c
1:50	0	str	0	0	c
1:80	0	c	0	0	c
1:125	0	c	0	0	c
1:250	0	c	0	0	c
1:415	0	c	0	0	c
1:625	tr	c	tr	0	c
1:1250	wk	c	str	0	c
1:2080	str	c	ac	wk	c
0	c	c	c	c	c

never completely inhibited lysis in dilutions greater than 1:15 nor with a suspension of rabbit-spermatozoa containing less than 40 million cells per milliliter. Positive antibody-response was evidenced by titers many times higher than those of the ten

normal sera examined. Rabbit 56 furnished the most potent of these normal sera.

Isoimmunization of white rats

Confirmation of these results was obtained in another species. White rats were chosen for this purpose. Each of six females

TABLE 4

White rats' isoimmune-sera tested with decreasing amounts of spermatozoa

RAT SPERMATOZOAL SUSPENSION 3×10^7/ML. DILUTIONS	IMMUNE SERA						NORMAL SERUM	RABBIT VS. RAT SPERMATOZOA NO. 59
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6		
Undiluted	0	ac	0	0	0	0	c	0
1:1	tr	c	0	0	0	0	c	0
1:2	str	c	0	0	0	0	c	0
1:4	c	c	wk	tr	wk	wk	c	0
1:8	c	c	c	ac	c	c	c	tr
1:16	c	c	c	c	c	c	c	str
0	c	c	c	c	c	c	c	c

TABLE 5

White rats' isoimmune-sera tested with decreasing amounts of antiserum

DILUTIONS OF ANTISERUM	SUSPENSION OF RAT SPERMATOZOA (3×10^7/ML.)						
	Immune sera						Normal serum
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	
1:10	0	c	0	0	0	0	c
1:20	wk	c	0	0	0	0	c
1:40	str	c	tr	0	0	0	c
1:80	c	c	wk	tr	0	wk	c
1:160	c	c	str	str	0	ac	c
1:320	c	c	c	c	tr	c	c
0	c	c	c	c	c	c	c

(250–300 grams) received eight intra-abdominal injections of rat spermatozoa at 2- to 3-day intervals (about 5×10^7 cells in 1.0 ml.). Eight days after the last injection the rats were decapitated and the blood of each, collected separately, yielded 2 to 3 ml. of serum. The tests were done as usual except that 0.1-ml. volumes of the components of the primary mixture were used.

The data in tables 4 and 5 require little comment. Twelve normal sera were separately tested and found to be negative. The rabbit excels the rat as an antibody-producer to this iso-antigen and, as is well known, to hetero-antigens.

ALCOHOLIC EXTRACTS

As to the nature of the antigens involved, it has been found that many of the organ-specific substances are alcohol-soluble. For instance, Witebsky (10) attributed the organ-specificity of brain to lipoids, and suggested that they may be concerned in the antigenicity of lens-substance (27). Sachs, Klopstock, and Weil (28) successfully immunized rabbits with alcoholic extracts of rabbit-kidney. We have tried alcoholic extracts of spermatozoa instead of spermatozoal suspensions as antigen in the complement-fixation reaction. Large amounts of spermatozoa were dried by the lyophile-process and then ground in a mortar. Five milliliters of alcohol (70 or 95 per cent) were added to 100-mgm. lots of the powdered spermatozoa. These mixtures were kept in the refrigerator for 4 to 8 days with repeated shaking and then filtered through paper. There was no fixation of complement when these extracts were used as antigens. Soxhlet extracts were also negative.

SPECIES-SPECIFICITY

Spermatozoa of different species were tested for their reactivity with homologous and heterologous antisera (0.25-ml. volumes).

As shown in tables 6 and 7 it is possible to differentiate between the spermatozoa of different species either by diluting the spermatozoal suspension and adding a constant amount of antiserum, or by diluting the antisera and adding a constant amount of spermatozoa. In every case the homologous reaction is the strongest one, but cross-reactions between the different species are very marked. It is to be noted, however, that these antisera were chosen for their ability to give strong cross-reactions; other sera equally reactive with homologous spermatozoa, showed less cross-reactions.

It is especially significant that the rabbit iso-antisera were not exceptional. They also reacted more strongly with homologous spermatozoa, as shown clearly in table 7 (where the antiserum is diluted). It is therefore possible to distinguish spermatozoa of the rabbit from other sperm-cells by means of an iso-antiserum produced in the rabbit.

Normal sera taken from the rabbits before immunization seldom showed a reaction with any spermatozoa. In those cases where

TABLE 6

Species-specificity of spermatozoal antisera tested with decreasing amounts of spermatozoa

SUSPENSION OF SPERMATOZOA 10^3/ML.	SUSPENSIONS OF SPERMATOZOA															
	A Bull				B Human				C Rabbit				D Rat			
	Rabbit serum vs. spermatozoa of															
	Bull 32	Man 44	Rabbit 39	Rat 3	Bull 32	Man 44	Rabbit 39	Rat 3	Bull 32	Man 44	Rabbit 39	Rat 3	Bull 32	Man 44	Rabbit 39	Rat 3
1:1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:5	0	0	0	0	0	0	0	tr	0	0	0	0	tr	0	0	0
1:10	0	0	tr	wk	tr	0	tr	wk	0	0	0	tr	wk	tr	tr	0
1:16	0	tr	str	str	wk	0	ac	ac	tr	0	0	wk	ac	wk	str	0
1:25	0	wk	str	ac	ac	0	ac	c	wk	tr	0	str	c	str	ac	0
1:50	0	ac	ac	c	c	0	c	c	c	str	tr	c	c	c	c	tr
1:80	tr	c	c	c	c	wk	c	c	c	ac	wk	c	c	c	c	wk
1:125	str	c	c	c	c	c	c	c	c	c	ac	c	c	c	c	c
0	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c

the normal serum showed a reaction it was never demonstrable in dilutions higher than 1:15, nor with sperm suspensions of a count of less than 50 million per milliliter.

Cross-reactions

Mudd and Mudd showed that spermatozoa contain a species-antigen. Our experiments indicate that spermatozoa show a species-specific dominance, but that cross-reactions between the

TABLE 7

Species-specificity of spermatozoa tested with decreasing amounts of antiserum

DILUTIONS OF ANTISERUM	ANTISERUM															
	I Rabbit vs. bull spermatozoa No. 32				II Rabbit vs. human spermatozoa No. 44				III Rabbit vs. rabbit spermatozoa No. 39				IV Rabbit vs. rat spermatozoa No. 3			
	Suspensions of spermatozoa (5×10^7/ml.) of															
	Bull	Man	Rabbit	Rat	Bull	Man	Rabbit	Rat	Bull	Man	Rabbit	Rat	Bull	Man	Rabbit	Rat
1:10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:40	0	0	0	0	0	0	0	0	0	0	0	0	tr	0	wk	0
1:80	0	0	tr	wk	0	0	0	0	tr	0	0	0	wk	tr	str	0
1:160	0	tr	wk	c	0	0	tr	wk	wk	tr	0	tr	str	wk	ac	0
1:320	0	wk	ac	c	tr	0	str	ac	str	wk	0	str	c	ac	c	0
1:640	0	ac	c	c	str	0	c	c	ac	str	0	c	c	c	c	0
1:1280	0	c	c	c	c	0	c	c	c	ac	0	c	c	c	c	tr
1:2560	wk	c	c	c	c	0	c	c	c	c	tr	c	c	c	c	str
1:5120	str	c	c	c	c	str	c	c	c	c	ac	c	c	c	c	ac
0	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c

TABLE 8

Cross-reactions between bull and human spermatozoa

	ANTISERUM														
	Rabbit vs. bull spermatozoa											Rabbit vs. human spermatozoa			
	No. 4	No. 5	No. 6	No. 19	No. 21	No. 22	No. 31	No. 32	No. 33	No. 34	No. 35	No. 13	No. 43	No. 44	No. 45
Bull spermatozoa*.....	2	2	2	2	2	2	2	2	4	2	2	20	20	20	30
Human spermatozoa*.....	20	10	50	20	20	20	20	50	50	20	20	1½	2	2	2
Titer with bull spermatozoa†.....	1250	1250	625	640	625	640	2560	5120	6250	5120	2560	25	50	320	125
Titer with human spermatozoa†..	125	250	80	160	160	320	160	320	160	160	320	1250	2100	3125	625

	Normal rabbit serum									
	No. 19	No. 20	No. 21	No. 22	No. 23	No. 24	No. 34	No. 35	No. 43	No. 44
Bull spermatozoa*.....	—‡	—	—	—	—	50	—	50	—	50
Human spermatozoa*.....	—	—	—	50	—	50	—	—	—	—
Titer with bull spermatozoa†.....	—	—	—	—	—	15	—	10	—	10
Titer with human spermatozoa†..	—	—	—	10	—	10	—	—	—	—

* The smallest amount of spermatozoa (in million per ml.) that gave complete fixation of complement in the presence of antiserum diluted 1:10.

† The highest dilution of antiserum that gave complete fixation of complement in the presence of a fixed amount of a suspension of spermatozoa (50 million per milliliter).

‡ Negative — indicates no reaction with a suspension containing 50 million spermatozoa per milliliter.

cells of different species also occur. Cross-reactions are prominent between closely related animals, and less marked in the case of more distantly related species. Strong antigenic resemblance between the spermatozoa of bull, sheep and deer, and of mouse and rat has been noted. In these cases, the ratio of the titer of the antiserum, when tested with homologous and heterologous spermatozoa, was between 2 to 1, and 5 to 1.

Cross-reactions between the spermatozoa of bull and man were not quite as strong. However, all of the rabbit vs. bull spermatozoa (11 sera) gave marked reactions with human spermatozoa, and the converse reaction, rabbit vs. human spermatozoa (4 sera) with bull-spermatozoa was also distinctly positive. These cross-reactions are correlated to some degree with the potency of the serum. An antiserum with an homologous titer of 250 will rarely, if ever, show cross-reactions stronger than those given by some normal rabbit sera. When the titer is higher than 500, cross-reactions are easily detectable, although there is no precise ratio between the highest dilution at which the serum reacts and the degree of cross-reactivity. Table 8 shows the cross-reactions of bull and human spermatozoa.

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SUMMARY

1. Antispermatozoal sera react with spermatozoa and not with serum of the same species; antisera against serum react with serum, and not with spermatozoa.

2. Isoimmunization of female rabbits and female white rats was successful.

3. Spermatozoa are dominantly species-specific; antisera against spermatozoa reacted best with homologous sperm cells. Nevertheless distinct cross-reactions occurred with heterologous spermatozoa.

4. Isoantisera reacted like heteroantisera. It is therefore possible to differentiate between spermatozoa of different species by means of antispermatozoal isosera.

5. Cross-reactions are stronger in closely related species, but

they also occur between more distantly related species. They are distinctly positive between the spermatozoa of bull and man.

6. Blood-group substances are not causes of these cross-reactions.

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