THE EFFECTS OF SERUM ON SPERMATOZOA

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(Received for publication, November 4, 1946)

Blood serum is considered an excellent nutritive and protective medium for the preservation and the growth of animal cells and tissues. The effect of serum on spermatozoa, one of the animal tissues differentiated for motility and fertilization, has been neglected or only slightly investigated. This paper reports a series of experiments demonstrating a spermicidal effect of serum. The spermatozoa and serum of rabbits have been chiefly employed, but the effect of sera of various animals on the spermatozoa of different species has also been studied.

Methods

Blood was collected aseptically by heart puncture with or without anesthesia in the case of the rabbit, rat, and guinea pig. Bovine blood was collected by jugular puncture or from the neck vessels at slaughter. Human blood was drawn from the basilic vein. The serum was obtained by centrifugation of whole blood for 20 to 30 minutes at 2,000 R.P.M. Guinea pig and rat sperm suspensions were made by hashing the tail of the epididymis and the entire vas into normal saline (0.9 per cent NaCl). Semen of the bull and rabbit was collected with an artificial vagina (Walton, 1945; Macirone and Walton, 1938).

One or two drops of fresh sperm were added to 1 ml. of serum in a pyrex tube for testing. The motility of spermatozoa was examined at room temperature with high power magnification by putting a drop of the suspension on a slide. Observations were made immediately after the suspensions were prepared, usually in 2 to 5 minutes. The samples were examined half an hour later and then at 1 to 1½ hour intervals. The immobilization of spermatozoa was considered an indication of the spermicidal effect of serum.

The different grades of motility and the proportion of motile spermatozoa were recorded after each examination according to Chang (1943). The following designations are employed to describe the composite results of several readings for each sample in order to show the strength of the spermicidal factor.

5 +, all spermatozoa were killed immediately.

4 +, most spermatozoa were killed immediately.

3 +, a large number of spermatozoa were killed in 30 to 60 minutes.

2 +, a large number of spermatozoa were killed or inactivated in 2 hours.

+, small portion of spermatozoa were killed or most spermatozoa were inactivated in 2 hours.

−, no effect.

It should be noticed, here, that human, bovine, and rabbit sperms remain alive and

maintain normal motility, *i.e.* progressive movement, for about 5 hours in normal saline at room temperature. Guinea pig and especially rat sperms are rather weak. They remain alive only for about 3 hours but their motility slows down very quickly during this interval.

RESULTS

1. The Spermicidal Effect of Rabbit Serum on Rabbit Spermatozoa.—The sperms of eleven buck rabbits of various breeds were tested with sera collected from thirty-eight rabbits, most of which were females. All the serum samples showed spermicidal activity. That is, all the sperms were killed immediately when suspended in fresh pure rabbit serum. Sperms of a buck rabbit were

TABLE I

*Spermicidal Effect of Rabbit Serum on Rabbit Spermatozoa**

Pure serum or serum diluted with 0.9 per cent NaCl.

Test	Pure serum	½ serum	1/8 serum	1/16 serum
1	5+	5+	4+	+
2	5+	5+	3+	2+
3	5+	3+	2+	+
4	5+	4+	2+	−
5	4+	4+	2+	−
6	5+	4+	+	− (serum and sperm of the same rabbit)
7	5+	5+	−	−
8	5+	5+	2+	+
9	5+	4+	3+	−

* Refer to Methods for legends.

killed even by his own serum (Table I). The concentration or strength of this spermicidal factor in the sera was tested by dilution of serum with saline. Table I represents, not the whole data but part of them, to illustrate the findings.

Table I clearly shows that all the pure rabbit sera killed rabbit sperms immediately. The strength of this spermicidal factor varied in different samples. The pure serum and the sperms of the same animal were incompatible. Furthermore, it was found that there was no predictable relationship between the serum of one breed and the sperm of another breed so far as this incompatibility is concerned.

The pure serum not only immobilized the spermatozoa but actually killed them because the immobilization of sperms by serum was not reversible after either dilution or resuspension, following centrifugation, in saline or Tyrode's solution.

Pure rabbit serum also killed sperms recovered from the epididymis or sperms washed with saline and reconcentrated by centrifugation. It seems, therefore, that this spermicidal reaction of serum is not due to any reaction product formed by mixing seminal fluid and serum which may exert a lethal effect on spermatozoa. It is a direct action of serum on spermatozoa.

2. The Instability of the Spermicidal Factor in the Serum.—All fresh rabbit

TABLE II

Effect of Stored Serum on Rabbit Spermatozoa

Pure serum.

Samples		Days of storage							
		1	2	3	4	5	6	7	
1	Rabbit serum	5+		4+		−			
2				5+		4+		−	At room temperature
3		5+			−				
4		5+		5+			−		
5			4+		4+		−		
6		5+				3+		−	
7			5+			+		−	At 0–4°C.
8				5+		−			
9			5+		5+			−	
10				5+			4+	−	
11		4+	−						
12	Bovine serum	5+	5+					−	
13		5+	−						
14	Guinea pig serum	5+	−						
15			4+		−				
16	Rat serum	5+	+						
17		5+	−						

sera killed rabbit sperms but the spermicidal property of serum was easily lost after storage at room temperature or at 4°C. for 4 to 7 days. The data of Table II demonstrate this fact. They indicate again that the strength of the lethal factor varies in different samples. That is, some samples lost activity earlier than others even though all were stored in the same way (*cf.* samples 11 and 10).

The loss or the decomposition of this spermicidal factor during storage occurred not only in rabbit serum. It also happened in the sera of other animals; *i.e.*, cow, guinea pig, and rat. The fresh serum of these animals killed rabbit sperm immediately. But after 2 to 7 days' storage, the sera of these animals no

longer killed rabbit spermatozoa. Since the serum of different animals showed the same change in the same way during storage, it suggests that the spermicidal factors present in the sera of different animals are similar.

3. The Thermolability of the Spermicidal Substance in Serum.—It is clearly demonstrated in Table III that the spermicidal factor in the serum was destroyed by heating the serum at 56° to 57°C. for 5 to 10 minutes. At 40°C. a longer time was required to destroy this substance. In numerous tests it was found that heating for 10 minutes at 55°C. invariably destroyed the spermicidal

TABLE III

Effect of Temperature on the Spermicidal Factor in the Serum

Rabbit serum tested with rabbit sperm.

Temperature	Time of treatment	Results		Time of treatment	Temperature	Results	
		Treated	Untreated			Treated	Untreated
°C.					°C.		
					51	5+	
	5 min.	5+			53	4+	5+
	10 min.	5+			56	−	
40	20 min.	5+	5+				
	30 min.	5+		5 min.	61	−	
	24 hrs.	−			64	−	5+
	5 min.	5+			71	−	
52	10 min.	4+	5+		74	−	
	20 min.	5+					
	30 min.	5+					
	10 min.	−					
57	20 min.	−	5+				
	30 min.	−					

factor. The heat lability suggests that a serum protein may be the lethal substance.

The spermicidal factor against rabbit sperms present in the sera of the cow, guinea pig, and rat was also destroyed after heating for 10 to 20 minutes at 55°C. The factor present in the sera of rabbit, cow, guinea pig, and rats which killed bovine and guinea pig spermatozoa behaved in the same way. These results are another proof that the spermicidal factors in the sera of various animals are similar.

4. Limited Amount of Spermicidal Activity Present in the Serum.—All the spermatozoa, about 3 to 9 million, in one drop of rabbit semen were killed by 1 ml. of undiluted fresh serum. When a second drop of semen was added to the serum-sperm suspension, the sperms were not all killed if the concentration

of sperms in the semen was high. Furthermore, after 1 ml. of serum had killed all the sperms in one or two drops of semen, the supernatant fluid of the serum-sperm suspension no longer killed added sperms. It is obvious, therefore, that the spermicidal activity present in the serum is limited, and this substance can be used up by a certain number of spermatozoa.

Table IV indicates roughly that 0.1 ml. of fresh serum killed about 1.2 to 1.6 millions of spermatozoa. By calculation, 1 ml. of serum ought to kill 12 to 16 millions of sperms. One drop of semen ordinarily contained less than 12

TABLE IV

Quantitative Determination of Spermicidal Substance in Rabbit Serum

The number of sperms, in millions, killed in each sample is shown. One drop of semen was added in each sample.

Serum of	1 ml. pure serum		0.5 ml. serum and 0.5 ml. saline		0.2 ml. serum and 0.8 ml. saline		0.1 ml. serum and 0.9 ml. saline	
	Motility	No. of dead sperms	Motility	No. of dead sperms	Motility	No. of dead sperms	Motility	No. of dead sperms
188	None motile	—	None motile	—	3/5 motile	2.2	4/5 motile	0.98
191	" "	—	" "	—	4/5 motile	1.4	4/5 motile	0.84
189*	" "	14	" "	15	4/5 motile	3.2	4/5 motile	3.30
190	" "	8.5	3/5 motile	1	4/5 motile	0.6	4/5 motile	0.70
171	" "	4.8	None motile	5.1	Very few motile	3.7	2/5 motile	1.50
192	" "	3.6	" "	3.2	1/5 motile	2.0	4/5 motile	0.33
194	" "	4.7	" "	5.1	1/5 motile	3.3	2/5 motile	0.30
VI	" "	6.6	" "	6.0	1/5 motile	5.0	3/5 motile	1.60
R1	" "	7.6	" "	7.9	1/5 motile	5.8	2/5 motile	5.20
Mean of dead sperms.		7.1		6.2		3.0		1.60
No. of sperms killed per 0.1 ml. of serum.....				1.2		1.5		1.60

* Two drops of semen were added.

to 16 millions of sperms; therefore under our standard conditions all the sperms were killed. If two drops of semen which may contain more than 16 millions of sperms were added to 1 ml. of serum, the spermicidal effect would not be complete because the spermicidal factor was used up by a portion of the spermatozoa.

5. The Inactivation of the Spermicidal Factor in Serum by Sodium Citrate.— The spermicidal factor in the serum can be destroyed, not only by heat but also by certain chemicals; *e.g.*, sodium citrate. Table V presents the data obtained when serum was diluted half and half with various solutions. It is evident that sodium citrate destroyed the spermicidal factor in the sera of different species, although the extent of destruction may not have been the same in all cases. For instance, the destruction of the spermicidal factor in

various sera against bovine sperms or guinea pig sperms was not as complete as in the case of various sera against rabbit sperms. However, this may indicate that there is a differential resistance of sperms toward the spermicidal

TABLE V

Effects of Various Solutions on the Spermicidal Factor in Serum

0.5 ml. of serum diluted with 0.5 ml. of following solutions.

Test	Pure serum	H_2O	0.9 per cent NaCl	3 per cent Na citrate	Phosphate buffer*	7 per cent glucose	Unbuffered Ringer's solution	Tyrode's solution	Boric buffer pH 7	Remarks
1	5+	5+	5+	+	5+			5+		Rabbit serum against rabbit sperms
2	5+	4+	5+	−	5+	5+	5+	5+		
3	5+		5+	−		4+		5+		
4	5+		2+	−	2+		2+	4+	3+	
5	5+		5+	−	5+		5+	5+	5+	
6	5+		5+	+						Bovine serum against rabbit sperms
7	5+		5+	+				5+		
8	4+		4+	−						
9	5+		5+	−	5+				5+	Rat serum against rabbit sperms
10	4+		4+	−	Guinea pig serum					against guinea pig sperms
11	5+		5+	2+	Rat serum					
12	5+		5+	2+	Rabbit serum					
13	5+		5+	−	Bovine serum					
14	5+		5+	3+	Human serum					against bovine sperms
15	5+		5+	+	Bovine serum					
16	5+		5+	3+	Rabbit serum					
17	5+		4+	2+	Guinea pig serum					
18	5+		5+	2+	Rat serum					

* $Na_2HPO_4 \cdot 12H_2O$, 2.0 gm.
KH_2PO_4 0.20 gm.
H_2O 100 ml. (Phillips and Lardy, 1940).

factor. On this basis the sperms of guinea pig and bull would have a low resistance to the spermicidal factor as compared with rabbit sperms.

The spermicidal effect of serum diluted with solutions other than sodium citrate was not due to the reaction of these solutions with some serum constituents since in each instance, whatever the diluent, the spermicidal activity disappeared after heating at 55°C. for 10 minutes.

The destruction of the spermicidal factor in the serum by sodium citrate

cannot entirely be ascribed to the buffer capacity of sodium citrate owing to the fact that Phillips and Lardy's phosphate buffer (1940), Tyrode's solution, and boric buffer had no effect on the spermicidal factor of the serum. However, phosphate buffer (0.15 M of Na_2HPO_4 and 0.15 KH_2PO_4) prepared in the range from pH 7.2 to 6.2 partially inhibited the spermicidal factor in the serum. When serum was diluted half and half with these buffer solutions, most sperms were inactivated but they were not killed immediately. When serum was

TABLE VI

Effect of pH Value on the Spermicidal Factor in the Serum

Test	pH	Control 7.4–7.6	4.5±	5±	5.5±	6±	6.5±	7±	7.5±	8±	8.5±	9±
1		5+						5+				−
2		5+										−
3		5+		5+	5+	2+		5+	5+	5+	4+	2+
4		5+			3+	4+	5+	5+	5+	5+	5+	5+
5		5+	5+		2+	2+	5+	5+	5+	5+	5+	5+
6		5+		5+	5+	4+		5+	5+	5+	5+	5+

(0.2 ml. of saline added to 1 ml. of serum served as control. 0.02 to 0.2 ml. of 0.2 N HCl or of 0.1 N NaOH was added to each 1 ml. of serum to adjust pH value.)

TABLE VII

The Effect of Ultrafiltered Rabbit Serum on Rabbit Spermatozoa

Test	Rabbit serum before ultrafiltration or ultrafiltered serum		Untrafiltrate of rabbit serum	
	Pure	½ serum and ½ saline	Pure	½ ultrafiltrate and ½ saline
1	5+	5+	−	−
2	5+	5+	−	−
3	5+		−	
4	5+		−	

diluted with McIlvaine's buffer in the range from pH 7.8 to 6.0, the spermicidal factor was destroyed. This indicates that the destruction of spermicidal factor by McIlvaine's buffer is due to the presence of citrate ions as in the case of sodium citrate.

In connection with these findings, the effect of pH value on the spermicidal factor was studied by adding 0.02 to 0.2 ml. of 0.2 N HCl or of 0.1 N NaOH to 1 ml. of serum. The results are presented in Table VI. The spermicidal factor was active in the pH range from 6.5 to 8.0. At pH values of 5.5 to 6.0 and 8.5 to 9.0 this factor was partially destroyed or the reaction of this factor was slightly inhibited.

6. Large Molecular Size of the Spermicidal Factor.—Table VII reveals that

the spermicidal factor in the serum cannot be forced to pass through a cellophane membrane by ultrafiltration.

Serum dialyzed against water for 24 hours killed sperms invariably regardless of the treatment with heat. When 0.9 per cent of NaCl was added to the dialyzed serum, it no longer killed sperms (Table VIII). It is obvious that the lethal effect of the serum dialyzed against water is due to the absence of salt, not to the presence of the spermicidal factor. When serum was dialyzed against 0.9 per cent of NaCl, some samples killed sperms. The presence of the spermicidal factor was shown by heat treatment. Some samples dialyzed

TABLE VIII

Effect of Dialyzed Serum on Spermatozoa

Dialyzed against H_2O or saline for 24 hours at 4°C. External fluid changed four times.

Test	Control			Against water			Against saline		
	Pure	Pure heated	½ saline	Pure	Pure heated	½ saline	Pure	Pure heated	½ saline
1	−	−	−	5+	5+	−			
2	4+	−	3+	5+	5+	−	4+	−	5+
3	5+	−	5+	5+	5+	−	−	−	−
4	5+	−	5+	5+	5+	−	5+	−	3+
				NaCl added					
5	4+	−	5+	−	−	−	−	−	−
6	5+	−	5+	−	−	−	4+	−	4+
7	5+						4+		
8	5+	−	4+	−	−	−	−	−	−
9	5+						2+		
10				−	−	−			

against saline lost the spermicidal factor (Table VIII). It seems, therefore, that there is a tendency to destroy this factor during dialysis.

Dialyzed serum mixed with different proportions of ultrafiltrate of serum or mixed with equal parts of saline containing 0.2 to 0.3 per cent of $CaCl_2$ and 0.15 to 0.1 per cent $MgCl_2$ did not kill sperms. This indicates that the spermicidal reaction of serum is not closely related to these inorganic ions.

7. The Absence of the Spermicidal Factor in Various Plasma Protein Fractions.—Fractions of human,[1] bovine, and porcine plasma were tested to find out which of their proteins are responsible for the spermicidal factor in the serum.

[1] The products of plasma fractionation employed in this work were developed from blood, collected by the American Red Cross, and by the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts, under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

Whole human plasma, albumin, γ- and β-globulin, β-globulins, α-globulin and lipids, α- and β-globulin, fibrinogen, and γ-globulins, dissolved in saline according to their normal concentration in the plasma or prepared in 2 per cent solution were tested with human and rabbit sperm. Two per cent solutions of bovine and porcine plasma fractions and I, II + III, IV, and V[2] in saline were tested with rabbit sperm. At first it appeared that 1 to 2 per cent solution of plasma albumin (the normal concentration in the plasma is 2.9 per cent) killed sperms. Similarly 1 to 2 per cent lactalbumin solutions, but not egg albumin, in saline killed sperms too. But these fractions killed sperms even after they were heated at 55°C. for 10 minutes. Furthermore, these albumin fractions and lactalbumin were acidic when dissolved in saline (pH at 3.5 to 4.5). If the albumin fractions of various plasma, lactalbumin, or other plasma fractions which slightly inhibited the motility of sperms were dissolved in Tyrode's solution, phosphate buffer, or sodium citrate, the adverse effect disappeared. It was also observed that the adverse effect of albumin fraction and lactalbumin in saline disappeared when the pH value of these solutions was adjusted to 7.5 by adding NaOH. Thus the harmful effect of albumin and lactalbumin solutions was due to the acidity and not to the presence of the spermicidal factor we are looking for.

Since the spermicidal factor of fresh serum is very unstable and is destroyed by sodium citrate, it is natural that this factor cannot be found in purified plasma fractions because the plasma fractions were prepared from citrated blood.

8. The Absence of Serum Spermicidal Substance in Extracts of Other Organs.— One gm. of various organs, *i.e.* heart, lung, liver, spleen, kidney, brain, adrenal gland, pancreas, duodenum, stomach, cartilage, and lymph gland was ground with 5 or 10 ml. of saline and centrifuged. One drop of rabbit sperm was then added to 1 ml. of these fluids. It was found that extracts of heart, spleen, pancreas, and duodenum inactivated the spermatozoa. However, after these fluids were heated at 55°C. for 10 minutes inactivation of sperm still persisted. Therefore, the sperm-inactivating factor in these tissue fluids is different from that present in the serum.

Bile (0.5 to 1.0 per cent), before or after the treatment with heat, killed rabbit and human sperms. The spermicidal effect of bile, of course, is different from that of serum.

Concentrations of 1 per cent snake venom, 1 per cent papain, or 1 per cent trypsin in saline killed sperms in $\frac{1}{2}$ to 1 hour. However, the action of these substances differs from that of serum. These substances inactivated the sperms gradually and continuously whereas fresh pure serum killed sperm very

[2] I (mainly fibrinogen), II + III (mainly β- and γ-globulin), IV (mainly α- and β-globulin), V (mainly albumin).

suddenly, within 1 to 2 minutes, and those sperms left alive showed progressive movement and lived in the serum for a very long time; *i.e.*, as long as or slightly longer than sperm suspended in saline or Tyrode's solution.

9. The Similarity of the Spermicidal Factor to the Complement in the Serum.— This spermicidal factor found in the present study has several characteristics similar to the complement or "amboceptor" in the antibody-antigen reaction; for instance, disappearance after storage, thermolability at 55–56°C., different concentration between individuals and between species. In addition to these similarities, it was also found that the spermicidal factor was destroyed by Seitz filtration, by adding trypsin (proteolytic enzyme), or by adding snake venom. It was inactivated by sunshine (ultraviolet) or by adjusting pH value

TABLE IX

*Effect of Various Treatments on the Spermicidal Factor in the Serum**

Test	Control 0.2 ml. saline added	Seitz-filtered	0.2 ml. of 0.5 per cent trypsin	0.2 ml. of 0.5 per cent snake venom	30 min. under sunshine	5 mg. of			3 drops of		
						Casein	Kaolin	Cholesterol	Chloroform	Ether	Alcohol
1	5+	−	−		4+	5+			5+	5+	5+
2	5+	−	−	3+	4+	5+			5+	5+	5+
3	5+	−	+	−	4+	5+5+‡	5+	5+			
4	5+		4+	3+		5+	5+	5+			

* 1 ml. rabbit serum was the standard solution used; rabbit sperms were employed.

‡ Double entry means two tests were done.

to 9 or to 6 (Tables VI and IX). All these treatments are said to destroy complement in the serum (*cf.* Boyd, 1943).

These facts show that the characteristics of the spermicidal factor are similar to those of complement. However, cholesterol, kaolin, chloroform, ether, or alcohol which also inactivate complement have no effect on the spermicidal factor (Table IX). Thus, whether or not the spermicidal factor is identical with complement is still open to further research.

10. Failure to Recover the Spermicidal Factor from Sperms Killed with Serum.—Attempts were made to determine whether the spermicidal factor in the serum is recoverable from the dead sperms after treatment with serum. To 5 ml. of rabbit serum 0.1 ml. of rabbit semen was added. All the sperms were killed immediately. This sperm-serum suspension was centrifuged. After taking off the supernatant serum the dead sperms were resuspended in 2 ml. of saline. This sperm-saline suspension was shaken for 10 minutes and centrifuged. One drop of semen was added to the supernatant saline to see whether or not the spermicidal factor could be washed off the dead sperms. It was found that the supernatant saline had no spermicidal effect. This in-

dicates that the spermicidal factor cannot be recovered from the dead sperms under this treatment, which shows that the spermicidal factor in the serum reacted with some components of sperms to form other substances.

Considering that the spermicidal factor cannot be recovered and that it is similar to complement, which is a complicated protein system, it is rather doubtful that the spermicidal factor is an enzyme.

11. The Loss of Fertilizing Capacity of Rabbit Spermatozoa after Treatment with Pure Serum.—Seven superovulated female rabbits (Pincus, 1940) were inseminated with sperms suspended in pure rabbit serum or serum mixed with saline. Table X shows that the fertilizing capacity of rabbit spermatozoa was completely lost if they were suspended in pure serum which killed all of them.

TABLE X

Fertilizing Capacity of Rabbit Sperms after Treatment with Rabbit Serum

Sperm suspended in pure serum or serum diluted with saline, 1 ml. of sperm-suspension was inseminated into the vagina of each rabbit. Ova examined 32 hours later.

Serum	No. of sperms	Motility of sperms	Does inseminated	Cleaved eggs	Uncleaved eggs	Cleavage
						per cent
1/5	2 millions	3/5 motile	146	39	2	95
			148	34	19	64
			149	47	0	100
1/6	1.14 millions	1/5 motile	157	9	3	75
			159	10	4	71
Pure	3.25 millions	None motile	230	0	18	0
			231	0	28	0

However, if there were motile sperms still remaining in the suspension containing serum, though a large portion of sperms were killed, those still alive were able to fertilize the ova. These results indicate that once the spermicidal substance in the serum is used up by a certain amount of sperm there is no adverse effect of this serum on the fertilizing capacity of spermatozoa.

12. The Effect of Sera Collected from Different Species on the Spermatozoa of Different Species.—Fresh human, bovine, rabbit, guinea pig, and rat sperm specimens were tested when suspended in fresh serum of these animals. The results are presented in Table XI. Samples of human serum from eleven different individuals had no adverse effect on the sperms of five different individuals. Human serum samples had no effect on rabbit sperms except in one of ten samples. But human serum did kill bovine, guinea pig, and rat sperms. Bovine, rabbit, and rat sera killed all the sperms of these five species but they were relatively ineffective against human sperms. Guinea pig serum was

comparatively weak against rabbit and guinea pig sperms, and very weak against human sperms, but strong against bovine and rat sperms. It seems that the strength of the spermicidal factor present in the serum of different

TABLE XI

Effect of Serum of Different Species on the Spermatozoa of Different Species

Pure serum or serum diluted with saline. Each symbol represents one test.

Test		Human serum			Bovine serum			Rabbit serum			Guinea pig serum			Rat serum		
		Pure	½	1/8	Pure	½	1/8	Pure	½	1/8	Pure	½	1/8	Pure	½	1/8
	1	−−	−−		3+	3+		+	+		+	+		4+	4+	
	2	−−			3+			3+			+					
Human	3	−			3+			3+								
sperms	4	−−−			2+						−			4+		
	5							3+			−	−		3+	−	−
	6	−−	−−	−−	5+	4+	3+	5+	4+	4+	+	+	+	4+	4+	2+
	7	−	−	−				+	−	−	+	−	−			
Bovine	8	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+
sperms	9	5+	5+	5+	5+	2+	−	5+	5+	5+	5+	5+	5+	5+	5+	2+
	10	−−	−−		5+	5+		5+	5+		4+4+	4+4+		5+	5+	
	11	4+−	−−		4+	5+					2+					
Rabbit	12	−−−			5+						5+	5+	4+	5+	5+	
sperms	13				4+	2+		5+	5+	3+				3+		
	14	−	−	−	5+	5+	+	5+	4+	−	4+	2+	−	+	−	−
	15	−−	−−	−−	3+	2+	−	5+	+	−	5+	5+	4+			
	16							5+	5+	5+	4+	4+	2+	5+	5+	5+
Guinea	17				5+	5+	4+	5+	5+	5+	4+	4+	3+			
pig	18	5+	5+	5+	5+	5+	5+	5+	5+	5+	4+	4+	2+	5+	5+	5+
sperms	19	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+
	20	5+	5+					5+	5+					5+	5+	
Rat	21													4+	4+	4+
sperms	22	5+	5+	4+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	5+	4+

animals is in descending order as follows: bovine, rabbit, rat, guinea pig, and human.

On the other hand, the results can also be explained by assuming a differential resistance of sperms of different species. That is, the resistance of sperms of different species against this spermicidal factor may not be the same. On this basis human sperms have the highest resistance and the rabbit sperms are the next, while the resistance of bovine and guinea pig sperms is low. Rat sperms are the weakest because they died even in saline alone after 2 to 3 hours, which never happened with the sperms of other species.

DISCUSSION

The effect of serum on spermatozoa has not been thoroughly investigated by our procedures. Bernstein and Lazarew (1933) reported that bull sperm is agglutinated by blood serum of the cow and bull, but not if the serum is previously heated at 55–60°C. for 1 hour. This process, however, is reversible in about 24 hours. Sterile blood serum of horse and sheep was tried as a diluent for ram sperms by Gunn (1936) who found that there was no increase in sperm longevity though there was no harmful effect such as that described by Bernstein. Kato (1936) found that rabbit and horse sera have great power of agglutinating rabbit spermatozoa but he did not mention any other adverse effect of serum on spermatozoa. In fact, blood serum was considered as the most favorable diluent for cock sperms by Grodzinski and Marchlewski (1935).

The reasons why the previous investigators did not notice the spermicidal factor present in serum are: (1) this factor is unstable and is easily lost after only a few days of storage; (2) it can be demonstrated only when a small number of sperms are added to the serum because this substance can be used up by a certain number of sperms and has no residual effect on the remaining spermatozoa.

Agglutination of sperms was induced by all serum preparations and occurred in any serum containing suspensions in the pH range 5 to 8. Sperms also agglutinated in those solutions containing egg albumin, plasma fractions, trypsin, and all the different tissue extracts.

It seems that the agglutination of sperms is simply due to the presence of protein in the medium. It may be a general reaction between sperms and a colloidal substance such as protein. It is very unlikely that it is a specific reaction between sperm and some specific substances.

Agglutination is a general feature of live spermatozoa even if they are suspended in saline or Tyrode's solution for a longer time. The dead sperms very rarely agglutinate, though occasionally it was observed that dead sperms formed small lumps in certain media or in serum; *i.e.*, human sperms in fresh bovine serum. Whether or not the mechanism of lump formation of dead sperms is similar to the agglutination of live spermatozoa is open to question. However, the agglutination disappeared after the sperms had been dead for some time.

The tendency to agglutinate varies with the spermatozoa of different species. Guinea pig sperms agglutinated even in normal saline; they agglutinated earlier in all media than the sperms of other species did. The agglutination of human spermatozoa was very rarely observed in the present study even when they were suspended in fresh sera.

The antigenic properties of spermatozoa are now well established. Spermatozoa exhibit a considerable degree of organ specificity as well as species specificity (*cf.* Parkes, 1944). It is doubtful that the serum spermicide is an

antisperm antibody. Its instability makes this unlikely. That the serum of various animals should act on the diversity of sperms used in such similar fashion argues either for a rather widespread type of antibody or against any antibody.

An attempt was made to determine whether the strength of the spermicidal factor was higher in those animals which had been operated on, or vasectomized, or injected with gonadotropin. The results with serum from such animals did not show any significant differences from those obtained with normal serum.

It is interesting to note that spermatozoa and ova react to serum in an entirely different way. Pure fresh serum is the best medium for the culture of mammalian eggs *in vitro*, while pure fresh serum kills the sperms.

Although spermatozoa are one of the tissues of an animal their structure and function are unique. They behave more or less like protozoa. Perhaps their functional integrity is somewhat similar to that of lower organisms,as suggested by Hammond (1930), due to their best survival under partial anaerobic conditions. Since the normal serum of certain animals contains antagonistic substance for a number of protozoa,— for instance, human serum kills numerous trypanosomes as reported by Culbertson (1935),— it is most probable that serum kills spermatozoa in a similar manner.

It is a very remarkable fact that human serum does not kill human spermatozoa although it is lethal to the sperms of other species. The sera of the other animals tested kill their own sperms as well as the sperms of other species.

Histological examination of spermatozoa killed with serum revealed that the "gelea capitis" was usually absent on the anterior part of the sperm head and that there were granules attached to the spermatozoa. Since the detachment of "gelea capitis" is not necessarily an indication of the death of spermatozoa but rather an indication of a regressive change in the sperm (Blom, 1945), the histological finding explains very little about this spermicidal reaction. The mechanism of the action of this spermicidal factor on spermatozoa has to be studied by means of physiological or biochemical approaches.

The chemical nature of this spermicidal factor present in the serum; the mechanism by which it kills spermatozoa; the fact that it kills sperms from its own body; the biological significance of this reaction; and its relation to sterility and fertility; all these are questions for future research.

SUMMARY

A spermicidal factor was found in fresh human, bovine, rabbit, guinea pig, and rat sera. It kills the spermatozoa of its own species (except in the case of human serum) and the sperms of other species. It was unstable, thermolabile, and of large molecular size. It was present in limited quantity in the fresh serum and could be used up by a definite number of spermatozoa. It could be

destroyed by sodium citrate, by Seitz filtration, by trypsin, and by snake venom. This factor was not present in tissue extracts and various plasma protein fractions. The strength or concentration of this factor varies in different individuals and in different species. This factor has several characteristics similar to those of complement.

The writer is very grateful to Dr. G. Pincus for his constant encouragement, enthusiasm, and reading of the manuscript. Thanks are also due to Professor E. J. Cohn of Harvard for supplying the plasma fractions for tests in human spermatozoa, to Armour Laboratories of Chicago for supplying bovine and porcine plasma fractions, to Dr. R. Shaw for supplying bull semen, and to the Foundation of Applied Research of San Antonio, Texas, for a grant.

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