STUDIES ON THE ANTIGENIC STRUCTURE OF SOME MAMMALIAN SPERMATOZOA

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Numerous studies of the antigenic composition of various bacteria have been made, but rarely has it been possible to identify certain antigens with definite morphological features of the cells. Exceptional cases are the members of the typhoid-paratyphoid group. Since the possibility of such a correlation of immunizing action with structural components depends somewhat on the degree of morphological differentiation it should be possible to carry out such analyses in the case of certain types of cells other than bacteria. Because of their extreme differentiation, involving almost complete localization of cytoplasmic and nuclear portions, mammalian spermatozoa are particularly suited to this type of analysis. The study of antigenic structure described in this paper was further facilitated by the discovery that the spermatozoa can be broken readily into heads and tails by sonic vibration, and that the two components can thereafter be separated by centrifugation. Several different antigenic materials have been demonstrated in the two fractions by subsequent serological procedures.

Materials and Methods

The preparation of spermatozoal suspensions as well as methods for preparation of antisera have been fully described (1).

Separation into Heads and Tails.—Separation of mammalian spermatozoa into heads and tails apparently has not been successful previously, although efforts to accomplish such cleavage have been made. Miescher (2) and, subsequently,

several others have separated heads from tails of piscine spermatozoa by the simple expedient of centrifuging in distilled water. The heavy heads broke off and stratified while the material of the tails stayed in the supernatant, but mammalian spermatozoa were not susceptible to this manipulation.

To fragment the gametes we have made use of the magnetostriction oscillator used by Chambers and Flosdorf (3) for the extraction of labile antigenic substances from *Streptococcus hemolyticus* and *Eberthella typhi*. The cell suspensions were exposed directly to vibrations at 9000 cycles per second of sufficient intensity to promote vigorous cavitation in the fluid. Temperature of the suspension was kept below 20°C. at all times by water cooling the inside of the nickel vibrating element. Descriptions of similar oscillators and the cooling arrangement have been published previously by Gaines (4) and by Chambers and Gaines (5).

After brief exposure to sonic cavitation it was found that practically all bovine spermatozoa in a heavy suspension were split into heads and tails. Final separation of the two components was accomplished by slow centrifuging. The heads settled down first and the majority of tails stayed in the supernatant liquid. By washing the first sediment four to six times an almost pure head suspension was obtained. After prolonged slow centrifuging of the first supernatant it was almost free of heads and subsequent high speed centrifugation for 30 minutes threw down the tails or at least the larger fragments of them. There was an obvious difference in the color of the sediments, the heads being milk white and the tails yellowish.

By the method described it was possible to obtain suspensions of either heads or tails of bull spermatozoa with 1 to 4 per cent of the unwanted part. The suspensions were counted in a Levy blood-counting chamber but the tail count cannot be considered exact since the tails usually were more or less fragmented. Only the larger fragments were counted. The supernatant liquid was not entirely clear after sedimentation of the tails and probably still contained small tail particles. Serologically the tails and the supernatant fluid behaved identically. It was therefore reasonable to assume that smaller amounts of antigen would be observed in the tail suspensions than would be found in the intact tails.

While the breaking of bull, dog, or rabbit spermatozoa was fairly complete after 7 minutes of vibration, 15 minutes were required for guinea pig cells, and 20 minutes for human. Final separation of guinea pig heads and tails was difficult as both came down at nearly the same rate during centrifuging. The best results which could be obtained showed about 12 to 15 per cent of the unwanted part. In human spermatozoa on the other hand it was very easy to get the pure head suspensions as the tails were entirely dispersed by the sonic

process. In this case it was necessary to separate the heads from the seminal crystals which settled first. Instead of a suspension of tails the supernatant liquid was used. However, it was very difficult to obtain the large amounts of human spermatozoa necessary for these experiments and the same limitation hampered to a lesser degree the use of spermatozoa of dogs and rabbits. Because of these difficulties bull spermatozoa and their parts were used most extensively and the male gametes of other species usually were employed only when confirmation was sought.

Smears prepared from the various suspensions and stained with methylene blue or Gram's stain revealed no differences in the surface of the heads before and after separation from the cells. Only guinea pig spermatozoa lost their acrosoma. Cleavage occurred uniformly at the junction of midpiece and head except in human spermatozoa. In the latter case a small part of the midpiece tended to remain with the head. No further morphological changes were detectable upon dark field examination of fresh preparations.

Serological Tests.—Tests of antigenic activity were carried out by means of the complement fixation technique. Equal volumes (0.25 ml.) of dilutions of antigen, antiserum, and complement were mixed together and incubated for 1 hour at 37°C. Then 0.5 ml. of sensitized sheep red blood cells (2½ per cent) were added. Two units of complement and two units of amboceptor were always employed. Normal serum controls were included in all tests but never showed any reaction in the dilutions used and therefore are omitted from the tables.

Absorptions were performed in the following way. Antiserum diluted one to ten was added to sedimented spermatozoa or to one of their parts, and the mixture was incubated for 30 minutes at 37°C. After centrifuging, an equal volume of the corresponding suspension was added to the supernatant portion and this mixture was allowed to stand overnight in the refrigerator. After centrifuging again at high speed for 1 hour the supernatant material was removed and incubated for 20 minutes at 56°C.[1] The unabsorbed serum control was treated similarly except for the addition of saline solution in place of the spermatozoal suspension. Final dilution of each serum was 1:20.

Slide agglutination and preparation of antibody solutions are described in the text.

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[1] The long centrifugation was necessary in order to get rid of smaller antigen-antibody complexes which of course would fix complement in the test as detectable by the saline control of the serum. Even then we encountered occasionally a distinct fixation of complement in these controls. This was due especially to the sera absorbed with homologous spermatozoa as in these cases a tremendous number of cells was required to remove all antibodies.

EXPERIMENTAL

1. Homologous Reaction.—In order to compare the reactions of the different fractions of spermatozoa with the homologous antisera the following experiment was performed. Five antigens were used, *i.e.*, 1, native bull spermatozoa; 2, vibrated bull spermatozoa; 3, heads of bull spermatozoa; 4, tails of bull spermatozoa; 5, the supernatant remnant of vibrated bull spermatozoa. All suspensions contained 10^8

TABLE I

Comparison between Bull Spermatozoa and Their Component Parts in the Homologous Reaction

Dilution of suspension	Rabbit *versus* bull spermatozoal serum 32				
	Suspension of bull spermatozoa or their parts 10^8 per ml.				
	Whole cells	Sonically vibrated	Heads	Tails	Supernatant
1: 1	0	0	0	0	0
1: 3	0	0	0	0	0
1: 5	0	0	wk	0	0
1:10	0	0	c	0	0
1:16	0	0	c	0	0
1:25	0	0	c	tr	0
1:50	wk	0	c	str	0
1:80	c	wk	c	c	str
0	c	c	c	c	c

0 = no hemolysis.
tr = trace of hemolysis.
wk = weak hemolysis.
str = strong hemolysis.
ac = almost complete hemolysis.
c = complete hemolysis.

cells or parts per milliliter and the supernatant solution was so diluted as to correspond to an equal concentration. Suspensions 1, 2, and 5 were derived from the same stock suspension of spermatozoa in order that the figures might be strictly comparable. However, under the conditions of preparation, 3 and 4 had to be counted separately. All the antigens were serially diluted and a constant volume of rabbit *versus* bull spermatozoal serum 32 in a dilution of 1:20 was added.

The data obtained (Table I) showed that the heads contained less

antigen than the tails or the supernatant solution. There was regularly a slight increase of the antigenicity of the vibrated spermatozoa as compared with the native cells. This may be due to a greater dispersion of the antigen.

If instead of the different antigens the antiserum was serially diluted and a constant amount of the suspensions was added no striking dif-

TABLE II

Effect of Absorption with Whole Spermatozoa or Their Component Parts on the Reaction of a Homologous Antiserum with These Antigens

Dilution of serum	Rabbit *versus* bull spermatozoal serum 34															
	A Unabsorbed				B Absorbed with whole bull spermatozoa				C Absorbed with heads of bull spermatozoa				D Absorbed with tails of bull spermatozoa			
	Suspension of bull spermatozoa or their parts 5×10^7 per ml.															
	Whole cells	Heads	Tails	Saline	Whole cells	Heads	Tails	Saline	Whole cells	Heads	Tails	Saline	Whole cells	Heads	Tails	Saline
1:20	0	0	0	c	0	0	0	0	0	wk	0	c	0	0	0	0
1:35	0	0	0	c	wk	wk	wk	wk	0	ac	0	c	0	0	0	tr
1:50	0	0	0	c	c	ac	c	c	0	c	0	c	0	0	wk	str
1:100	0	0	0	c	c	c	c	c	0	c	wk	c	0	0	ac	c
1:160	0	0	0	c	c	c	c	c	wk	c	c	c	0	0	c	c
1:250	0	0	0	c	c	c	c	c	c	c	c	c	0	wk	c	c
1:500	0	0	0	c	c	c	c	c	c	c	c	c	str	ac	c	c
1:810	0	0	tr	c	c	c	c	c	c	c	c	c	c	c	c	c
1:1250	wk	wk	str	c	c	c	c	c	c	c	c	c	c	c	c	c

ference between the different parts of spermatozoa could be observed (compare Table II, column A).

By use of an absorption technique, however, it was possible to show that heads as well as tails possess antigens of their own (Table II).

If a rabbit *versus* bull spermatozoal serum was absorbed with the whole bull spermatozoa no more complement fixation took place with either one of the parts save for the inhibition by the absorbed serum itself. After absorption with heads of bull spermatozoa there remained a distinct reaction with the whole cells and the tails, while

conversely, absorption with the tails left a marked reaction with the whole cells and heads.[2]

In order to identify further antigenic properties the different suspensions were submitted to heating at 100°C. for 20 minutes.

It is obvious from Table III that both heads and tails contained a heat-stable antigen. This substance is apparently identical in both parts of the cell as antibodies against it were absorbed by either heated heads or heated tails. Absorption with heads or tails in the native state, which left either the tail-specific or head-specific anti-

TABLE III

Demonstration of a Heat-Stable Antigen Common to Heads and Tails

Dilution of antiserum	Rabbit *versus* bull spermatozoal serum 32											
	Unabsorbed				Absorbed with 100°C. heads				Absorbed with 100°C. tails			
	Suspension (5 × 10⁷ per ml.) of											
	Heads		Tails		Heads		Tails		Heads		Tails	
	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°
1:20	0	0	0	0	0	wk	0	str	0	ac	0	c
1:35	0	0	0	0	0	str	0	c	0	c	0	c
1:50	0	0	0	0	0	c	0	c	0	c	0	c
1:100	0	0	0	0	0	c	0	c	0	c	0	c
1:160	0	0	0	0	0	c	0	c	0	c	0	c
1:250	0	0	0	tr	0	c	0	c	0	c	0	c
1:500	0	tr	0	ac	0	c	0	c	0	c	ac	c
1:810	0	ac	0	c	tr	c	str	c	wk	c	c	c
1:1250	0	c	wk	c	str	c	ac	c	ac	c	c	c
1:2500	wk	c	str	c	c	c	c	c	c	c	c	c

bodies, likewise removed the antibodies against the heat-stable substance. The absorbed sera did not react with heated heads or tails. The head-specific as well as tail-specific antigens are therefore heat-labile. These experiments were inadequate to determine the presence or absence of heat-labile factors common to both parts, but their existence is very likely since the remaining reactions after the absorption with the native parts are of relatively low titer in comparison

[2] The reaction with the supernatant remainder of vibrated spermatozoa equals that of the tails and was therefore left out of the table.

with the strength of the reaction after the absorption with the heated parts.

Such common factors appear to be responsible for somewhat indefinite results in the attempts to immunize rabbits with heads or tails of bull spermatozoa (Table IV).

While the head sera reacted to a much weaker degree with tails than they did with the homologous heads, the tail sera showed equally strong reactions with heads and tails. However, one must keep in mind that the suspensions of heads and tails never were entirely free from the other part. Since it seemed possible that the

TABLE IV

Comparison between Antisera against Whole Cells, Heads, and Tails

Dilution of antiserum	Antiserum against								
	Bull spermatozoa 34			Heads of bull spermatozoa 76			Tails of bull spermatozoa 78		
	Suspension of bull spermatozoa or their parts 5 × 10⁷ per ml.								
	Whole cells	Heads	Tails	Whole cells	Heads	Tails	Whole cells	Heads	Tails
1:50	0	0	0	0	0	0	0	0	0
1:100	0	0	0	0	0	str	0	0	0
1:160	0	0	0	0	0	c	0	0	0
1:250	0	0	0	0	0	c	0	0	0
1:500	0	0	0	tr	0	c	0	tr	wk
1:810	0	0	0	str	tr	c	0	str	str
1:1250	0	0	0	ac	ac	c	tr	c	ac
1:2500	str	tr	wk	c	c	c	ac	c	c

impurities of the suspensions used for the immunization might be responsible for the results with the tail sera we prepared other antisera with the supernatant remainder of vibrated spermatozoa. This material was found to react identically with the tail suspension. Because such preparations were free of heads we had reason to hope for antisera which would react dominantly with tails, but such was not the case since these sera reacted in the same way as the tail sera. The heat-stable common substance was found to be not entirely responsible for these cross-reactions.

The absorption experiments have shown that specific antigens exist in the heads and in the tails. We have also studied the effect of the

different antibodies by the slide agglutination reaction. For this purpose it was found to be advantageous to use motile spermatozoa, suspended in saline solution of pH 7.2 to 7.4. We were able to prepare the suspensions 2 to 3 hours after the bulls had been killed. Usually it was found necessary to incubate the suspension for a short time at 37°C. by which procedure the spermatozoa acquired their full activity. A drop of such a suspension was mixed with a drop of antiserum dilution on a slide and examined immediately under the microscope. Several different types of agglutination were thus observed in the different and variously absorbed sera.[3]

Absorption experiments were performed in the same way as shown in Table II. After the effect of the absorption had been established by the complement fixation test the sera were used for the agglutination reaction. When the unabsorbed serum was used fine strings of spermatozoa were formed immediately with attachment from head to head or tail to tail (Figs. 1 and 2).

Whether there occurred also a head to tail agglutination could not be surely established. When the spermatozoa were still living the heads were bent outward from the strings still moving and only other heads might stick to these heads. When the motion ceased the heads altered their position so that they often came into close proximity to the tails, but that seemed to be no antibody effect.

When the serum was absorbed with whole cells so that no reaction could be demonstrated in the preliminary titration no agglutination took place.

After the absorption of the serum with heads, only antibodies against the tails were left and these caused an immediate agglutination of the tails with all the heads at the outside of the agglutinate (Fig. 3).

When the spermatozoal suspension was a little older but the cells still very actively motile, this type of agglutination reached a point where the tails were caught only at the ends so that around the small initial clumps a wheel-like formation occurred. The heads building

[3] In photographing these types we encountered several difficulties. The agglutinates are tridimensional and it is therefore impossible to get all cells into focus. When some of the agglutinates are most typical, the spermatozoa are still very motile; after the activity ceased or the cells were killed the cells bent or the clumps were altered.

the outer circle were not attached to each other as was quite obvious in the still living preparation. Pictures of this type could not be taken (see footnote 3), but Figs. 6 and 7 show such wheels with the addition of a head to head agglutination as produced by an anti-tail serum.

In this formation the "acidophile body" (6) at the end of the middle piece seemed to play an important rôle. In fresh spermatozoa this ring was mostly in place; when the suspensions aged a little the acidophile bodies slid down along the tails and small clumps of them as well as of whole spermatozoa seemed to form the centers of the wheels. This type was seen very occasionally in heterologous spermatozoal sera, never in normal rabbit serum.[4]

When the serum had been absorbed with tails, the heads clumped together forming long rows, the tails still striking vigorously (Fig. 4).

This type of agglutination has to be considered with reservations since a similar type may occur in normal rabbit serum or serum of several other species. However, a definite difference in the size of the agglutinates was observed, they being smaller in normal serum than in antiserum. Furthermore the normally occurring agglutination was usually not found in dilutions higher than 1:10, while the antisera reacted up to 1:100 or greater dilution. In cases where the normal serum gave an agglutination the complement fixation test too showed slightly positive results.

Corresponding types of agglutination were observed with antisera obtained by injecting heads or tails of bull spermatozoa into rabbits. The head sera which reacted dominantly with heads in the complement fixation reaction (Table IV) showed correspondingly in higher dilution only the head type of agglutination. The tail sera fixed complement to the same degree with heads and tails (Table IV) but nevertheless they showed in the beginning of the agglutination the tail type. In low dilution a net-like formation was built immediately with all heads bent outward. This formation then shrank and the finer strings were disrupted, thereby clumps of different sizes were formed. Additional spermatozoa were caught at the end of the tail so that a rim of cells

[4] Sampson (7) saw a similar formation when spermatozoa of *Katharina tunicata* were exposed to homologous egg secretions or cytolized heterologous spermatozoa.

with the heads outside could be seen around the clumps (Figs. 5, 6, and 7). Often only a few cells or some of the acidophile bodies formed the center. After a while such clumps collided and a head to head combination resulted.

In order to confirm the results obtained by slide agglutination the same tests were carried out with antibody solutions. They were prepared by splitting off the antibodies from the antigen-antibody complexes at 56°C. according to the method described by Landsteiner and Miller (8) for the preparation of hemagglutinins. Heads or tails of bull spermatozoa were mixed with antiserum dilution and allowed to stand for 2 hours at room temperature. The fragments were sedimented and washed twice and resuspended in saline, incubated for 10 minutes at 56°C. with repeated shaking, then centrifugalized immediately while warm. The complement fixation reaction showed the presence of antibodies in the supernatant fluid. With antibodies split off from heads a slightly dominant reaction with heads was observed, while the antibodies obtained from tails showed the converse. The presence of common factors in the two parts has been pointed out before. The slide agglutination with these antibody solutions revealed types of agglutination corresponding to those given by the sera from which the antibodies were obtained.

2. *Heterologous Reaction.*—In a previous paper (1) it had been shown that antisera against spermatozoa possess a specific dominance for spermatozoa of the species from which the cells were obtained. The sera always reacted best with the homologous sperm cells, but more or less distinct cross-reactions occurred with heterologous spermatozoa.

To determine whether or not the cross-reactions were confined to only one of the parts, *i.e.* heads or tails, the following experiment was performed. Antisera prepared against spermatozoa of different species were allowed to interact with either heads or tails or supernatant solution (human), of bull, guinea pig, and human spermatozoa. Some of the results are given in Table V.

As shown in this experiment all antisera, no matter whether homologous or heterologous, reacted to about the same degree with the heads of bull and guinea pig spermatozoa when the antigen was increasingly diluted. Only the bull spermatozoal sera showed a weaker response

with guinea pig sperm heads. This is due to the lack of certain antibodies in these sera as will be explained later. A better homologous reaction was obtained with the heads of human spermatozoa.[5]

With the different tails as antigens we found a strong species-specific dominance although the degree of cross-reactivity of the different antisera varied. There were some heterologous antisera found, which did not react with tails at all.

TABLE V

Cross-Reactions of Heads and Tails of Spermatozoa of Different Species with Homologous and Heterologous Antisera

	Suspensions obtained from											
	Bull				Guinea pig				Man			
	Antiserum (1:10) against spermatozoa of											
Suspensions of heads (10^8 per ml.)	Bull 35	Man	Rabbit	Guinea pig	Bull 35	Man	Rabbit	Guinea pig	Bull 35	Man	Rabbit	Guinea pig
1:1	0	0	0	0	0	0	0	0	0	0	0	0
1:3	0	0	0	0	0	0	0	0	tr	0	0	0
1:5	0	0	0	0	tr	0	0	0	str	0	tr	tr
1:10	tr	wk	tr	wk	ac	0	0	0	c	0	str	wk
1:16	wk	ac	wk	ac	c	tr	wk	tr	c	tr	ac	str
Suspensions of tails (2 × 10^8 per ml.)												
1:1	0	0	0	0	0	0	tr	0	tr	0	0	str
1:3	0	0	0	0	wk	0	wk	0	wk	0	wk	ac
1:5	0	0	tr	tr	ac	0	wk	0	str	0	str	c
1:10	0	wk	wk	wk	c	wk	c	0	ac	0	c	c
1:16	0	ac	ac	ac	c	c	c	0	c	0	c	c
1:25	0	c	c	c	c	c	c	0	c	0	c	c
1:50	tr	c	c	c	c	c	c	wk	c	tr	c	c

One might expect the heads to be definitely specific because of their biological function. The results obtained with the heads were therefore at first surprising. The heads do possess, however, a species antigen because the titer of an antiserum was always markedly higher with its homologous heads than with heterologous ones. Furthermore

[5] Preparations of heads of human spermatozoa were shown to include fragments of tails in a higher percentage since the breaking does not always occur between head and midpiece.

a specific antigen in the heads could be shown simply by heating the heads for 20 minutes at 100°C. (Table VI).

The heating destroyed the heterologous reaction completely leaving a distinct homologous one. Spermatozoa or parts of them derived from other species also reacted only with their homologous antiserum after being submitted to heating. The heat-stable antigen is therefore species-specific.

When a heterologous antiserum was increasingly diluted and constant amounts of the different parts of the bull spermatozoa in suspension were added a striking difference in titer of the antiserum with the

TABLE VI

Influence of Heating on the Cross-Reactions

Dilution of suspensions	Antiserum (1:10) against spermatozoa of															
	Bull 33		Man		Rabbit		Guinea pig		Bull 33		Man		Rabbit		Guinea pig	
	Suspension of 10^8 per ml. heads of bull spermatozoa								Suspension of 10^8 per ml. tails of bull spermatozoa							
	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°	Native	100°
1:1	0	0	0	ac	0	c	0	ac	0	0	0	c	0	c	0	c
1:3	0	0	0	c	0	c	0	c	0	0	0	c	0	c	0	c
1:5	0	0	0	c	0	c	0	c	0	0	0	c	tr	c	tr	c
1:10	tr	tr	wk	c	tr	c	wk	c	0	tr	wk	c	wk	c	wk	c
1:16	wk	str	ac	c	wk	c	ac	c	0	str	ac	c	ac	c	ac	c
1:25	ac	c	c	c	ac	c	c	c	0	ac	c	c	c	c	c	c
1:50	c	c	c	c	c	c	c	c	wk	c	c	c	c	c	c	c

different parts was observed. Table VII illustrates an experiment of this kind.

There was a marked increase in titer after the spermatozoa had been vibrated, and this increase was apparently due to some change in the heads since neither tails nor supernatant gave such an increase. Corresponding results were obtained with spermatozoa of guinea pigs, dogs, and rabbits in the native and vibrated state. The only explanation for this phenomenon seems to be that a new antigen has come to the surface during sonic treatment. The cytological appearance of the vibrated cells has already been described, and it will be apparent that the only place where such a new surface could have appeared

was the site of breaking. Only in the case of guinea pig spermatozoa was there evidence that the acrosoma had been torn away.

Other mechanical procedures such as washing several times, vigorous shaking, or grinding, also increased the titer of the heterologous antiserum when the damaged spermatozoa were used as antigen. In such suspensions a considerable number of broken off heads were observed.

If a new antigen were responsible for this increase in titer, one should be able to demonstrate it by means of an absorption technique. Table VIII illustrates an experiment of this kind.

Rabbit *versus* human spermatozoal serum was absorbed with whole

TABLE VII

Influence of Sonic Vibration on the Titer of Heterologous Antisera

Dilution of antiserum	Rabbit *versus* human spermatozoal serum					Rabbit *versus* rabbit spermatozoal serum				
	Suspension of bull spermatozoa or their parts 5 × 10⁷ per ml.									
	Native	Sonically vibrated	Heads	Tails	Supernatant	Native	Sonically vibrated	Heads	Tails	Supernatant
1:20	0	0	0	0	0	0	0	0	tr	str
1:35	0	0	0	0	0	0	0	0	wk	ac
1:50	0	0	0	0	0	0	0	0	str	c
1:100	0	0	0	tr	0	0	0	0	c	c
1:160	tr	0	0	wk	tr	tr	0	0	c	c
1:250	wk	0	0	str	wk	wk	0	tr	c	c
1:500	ac	0	0	ac	c	c	wk	wk	c	c
1:810	c	0	wk	c	c	c	str	c	c	c
1:1250	c	tr	ac	c	c	c	c	c	c	c

bull spermatozoa or with heads or tails of bull spermatozoa. The results show clearly that after absorption with the whole cells the antiserum still gave a strong reaction with the heads of bull spermatozoa though a weaker one than the unabsorbed serum. Absorption with heads took out all antibodies. Absorption with tails decreased the titer with the whole cells but left the reaction with the heads apparently unaffected. This result constitutes definite evidence that a new antigen had come into action through the vibration. Corresponding results were obtained when spermatozoa of other species were used as absorbents. Other experiments show that absorption of an antiserum with whole dog spermatozoa for instance still leaves antibodies

against vibrated dog spermatozoa. After the sera were absorbed with heterologous cells or parts of them the reaction with homologous spermatozoa was either not detectably or only slightly decreased in titer.

The heterologous absorption experiment differed in some respect from the homologous one shown in Table II. It has to be noted that the homologous serum did not give a reaction with heads after absorp-

TABLE VIII

Effect of Absorption with Whole Spermatozoa or Their Component Parts on the Reaction of a Heterologous Antiserum with These Antigens

Dilution of serum	Rabbit *versus* human spermatozoal serum															
	A Unabsorbed				B Absorbed with whole bull spermatozoa				C Absorbed with heads of bull spermatozoa				D Absorbed with tails of bull spermatozoa			
	Suspension of bull spermatozoa or their component parts 5 × 10⁷ per ml.															
	Human sperm	Bull sperm	Heads	Tails	Human sperm	Bull sperm	Heads	Tails	Human sperm	Bull sperm	Heads	Tails	Human sperm	Bull sperm	Heads	Tails
1:20	0	0	0	0	0	wk	0	str	0	str	ac	str	0	0	0	tr
1:35	0	0	0	0	0	str	0	str	0	ac	c	ac	0	0	0	wk
1:50	0	0	0	0	0	ac	0	ac	0	c	c	c	0	0	0	ac
1:100	0	0	0	tr	0	c	0	c	0	c	c	c	0	wk	0	c
1:160	0	tr	0	wk	0	c	0	c	0	c	c	c	0	ac	0	c
1:250	0	wk	0	str	0	c	tr	c	0	c	c	c	0	c	0	c
1:500	0	ac	0	c	0	c	str	c	0	c	c	c	0	c	0	c
1:810	0	c	wk	c	0	c	c	c	0	c	c	c	0	c	wk	c
1:1250	0	c	ac	c	0	c	c	c	0	c	c	c	0	c	c	c
1:2500	0	c	c	c	0	c	c	c	tr	c	c	c	0	c	c	c
1:4150	wk	c	c	c	str	c	c	c	ac	c	c	c	wk	c	c	c

tion with the whole cells. Six different sera prepared against bull spermatozoa were absorbed with homologous whole cells but not one of them showed a reaction with heads of bull spermatozoa afterwards. Even when the serum was absorbed with the minimum amount of spermatozoa necessary for complete elimination of the reaction with the whole cells the reaction with the heads was gone. On the other hand when vibrated bull spermatozoa or heads of them were injected into rabbits antibodies were formed against this particular head antigen.

These antibodies were left in the serum in spite of absorption with the whole cells.

The results in cross-reactivity obtained so far are summarized in another form in Table IX which shows clearly that one may differentiate between three different cross-reacting antigens.

Antibodies against the first antigen (I) are absorbed by the whole cells and the heads but not by the tails; against the second one (II) they are absorbed only by the heads; and against the third (III) they are removed by all three, the whole cells, the heads and the tails.[6] Antigens I and III are on the surface of the cell, I is only on the surface of the heads, and it is not displaced by sonic vibration. Antigen II is believed to be covered by outside material in the whole cells

TABLE IX

Summary of the Results of Table VIII, Showing Three Cross-Reacting Antigens

Antigens	Rabbit *versus* human spermatozoal serum			
	Unabsorbed	Absorbed with whole bull spermatozoa	Absorbed with heads of bull spermatozoa	Absorbed with tails of bull spermatozoa
Whole bull spermatozoa	+	−	−	+
Heads of bull spermatozoa	+	+	−	+
Tails of bull spermatozoa	+	−	−	−

and therefore does not react in the native sperm cell. Only after breaking off the heads does this antigen II come into action. Antigen III is probably reactive only on the surface of the tails.

This conception explains why the absorption with heads removed the antibodies against antigens I and II, but does not hold for the disappearance of antibodies against antigen III. The absorption of anti-III by the heads might be due to traces of antigen III on the heads or might be caused by the admixture of tails in the head suspension. As mentioned before 1 to 4 per cent of the unwanted part were always counted in the different suspensions, and the following con-

[6] Further evidence for a cross-reacting substance III lies in the fact that some heterologous antisera do not react at all with tails of bull spermatozoa though they give good reactions with whole cells and heads.

siderations might account for the loss of antibodies against antigen III: 4 to 5 $\times$ 10^9 heads of bull spermatozoa were required to absorb 0.3 ml. of rabbit *versus* human spermatozoal serum, which means that 4 $\times$ 10^7 to 2 $\times$ 10^8 tails were present as impurities. On the other hand only 2 to 3 $\times$ 10^8 tails were necessary to remove from the same amount of this serum the entire cross-reaction with the tails. Therefore it is very likely that the impurity was responsible for the removal of anti-III by the head suspension.

To obtain further evidence of the cross-reacting antigens in bull spermatozoa it seemed desirable to remove if possible, the antigen I

TABLE X

Comparison between Specific and Cross-Reacting Antigens

Antigens	Rabbit *versus* vibrated bull spermatozoal serum 75							
	Unabsorbed	Absorbed with						
		Whole spermatozoa of			Heads of bull sperm		Tails of bull sperm	
		Bull	Man	Dog		And whole dog sperm		And whole dog sperm
Whole bull spermatozoa.....	+	−	+	+	+	+	+	+
Heads of bull sperm.........	+	+	+	+	−	−	+	+
Tails of bull sperm..........	+	−	+	+	+	+	−	−
Whole human spermatozoa..	+	−	−	±	±	−	+	−
Whole dog spermatozoa.....	+	−	−	−	±	−	+	−
Vibrated dog spermatozoa...	+	+	+	+	±	−	+	+

from the surface of the heads without destroying antigen II. However even prolonged sonic vibration (1 hour instead of 7 minutes) was not successful. Digestion with various enzymes as pepsin, trypsin, and papain likewise proved to be unsuccessful.

So far it has been shown in the homologous reaction that in bull spermatozoa there is a head-specific as well as tail-specific heat-labile antigen besides a heat-stable species-specific substance common to heads and tails. In the heterologous reaction three different cross-reacting antigens could be established, two in the heads, one in the tails. It remained to show the relation between the homologous and heterologous reactions.

For this purpose we selected an antiserum prepared against vibrated bull spermatozoa since it gave the reaction with the inside material.

It is obvious from Table X that:

1. Absorption with whole cells of the bull left a reaction only against the inside material not only of homologous bull spermatozoa but also of vibrated heterologous spermatozoa. There is no reaction with homologous or heterologous whole cells.

2. Absorption with heterologous whole spermatozoa removed the reactions with the absorbent, but did not detectably alter the reactions with the various homologous antigens. There was also a reaction left with vibrated heterologous spermatozoa due to the inside material.

3. Absorption with heads of bull spermatozoa left a distinct reaction with homologous bull spermatozoa and tails and only a doubtful reaction with heterologous spermatozoa. When additionally absorbed with heterologous whole cells only the homologous tail-specific reaction was left.

4. Absorption with tails of bull spermatozoa removed the reaction with the absorbent but only slightly altered the other homologous and heterologous reactions. When additionally absorbed with heterologous whole cells the homologous head-specific reaction remained and also the reaction with the inside material of the heads of homologous and heterologous spermatozoa.

SUMMARY

1. A method has been described for separation of heads and tails of mammalian spermatozoa.

2. By means of absorption technique applied to homologous spermatozoal sera, head-specific and tail-specific antigens could be demonstrated. Both are heat-labile.

3. A heat-stable antigen was found to be common to both heads and tails. This substance is species-specific.

4. Antibodies against the head- and tail-specific antigens led to two different types of agglutination as shown by the slide method.

5. Using heterologous antisera against spermatozoa three different cross-reacting antigens could be observed, two in the heads, one in the tails.

6. One of the head-antigens is not active in the native cell; it comes

to action only after breaking the cell. Antibodies against this substance were not found in antisera against native bull spermatozoa but were formed when vibrated spermatozoa or heads were injected into rabbits.

7. The cross-reactions can be removed from an antiserum leaving the head- as well as the tail-specific reaction intact.

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EXPLANATION OF PLATE 11

FIG. 1. Net-like agglutination in the native whole cell antiserum. × 40.

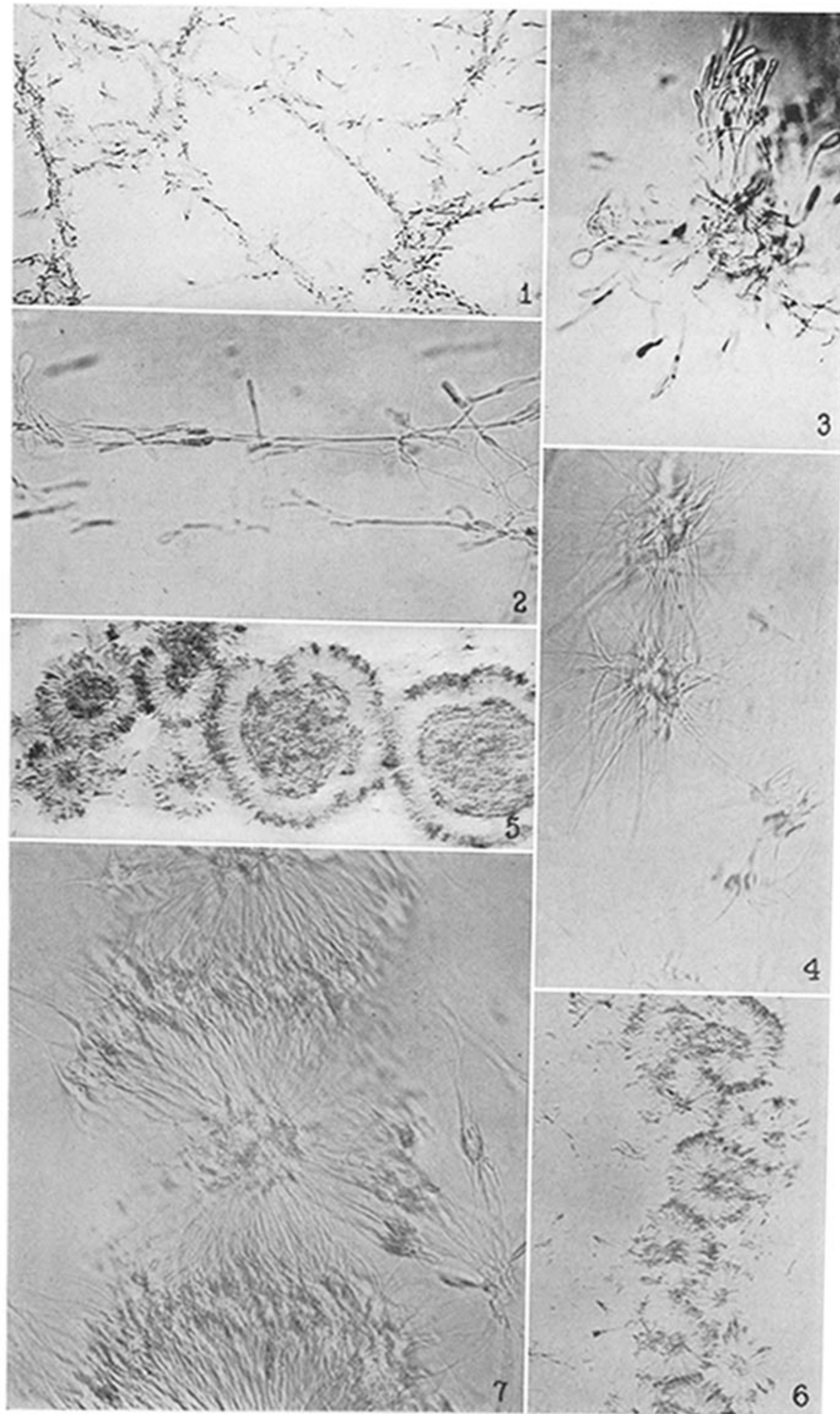
FIG. 2. One of the strings under higher power. × 320.

FIG. 3. Tail type of agglutination. × 320.

FIG. 4. Head type of agglutination. × 320.

FIGS. 5 and 6. Agglutination of bull spermatozoa in a native antiserum against tails with clumps showing the rim of heads at the outside. Both figures × 80.

FIG. 7. The same under higher power. × 320.



(Henle *et al.*: Antigenic structure of spermatozoa)