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Immunological Assessment of Surface Changes of Rabbit Sperm Undergoing Capacitation

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Sperm capacitation may involve the removal of seminal plasma factor(s) from the surface of the sperm. To determine the presence of seminal plasma components and in order to follow their release or alteration, antibodies to rabbit seminal plasma were produced in guinea pigs. The agglutinating ability of rabbit spermatozoa when mixed with antiserum was followed after washing the sperm cells for extensive periods. Eighteen hours of washing did not diminish the agglutination reaction. However, when ejaculated spermatozoa were incubated *in utero*, the agglutination of the sperm cells induced by the antiserum to seminal plasma diminished with time *in utero*. Additional data indicated that a higher percentage of cleaved ova resulted following insemination *in vitro* with uterine spermatozoa recovered at increasing intervals after coitus (9, 44, 55, and 74% at 3, 6, 12, and 18 h, respectively). Furthermore, utilizing the binding of ^{14}C-labeled antibodies against rabbit seminal plasma, the sperm-bound seminal plasma components were shown to be bound tightly to the sperm and were not removed during 24 h of incubation *in vitro* in a defined medium. However, when washed ejaculated sperm are incubated in uterine fluid, the uptake of ^{14}C-antibodies falls off with increasing time of incubation, up to 65% decrease with 6 h of incubation. These findings may provide a basis for a rapid quantitative assay for sperm capacitation.

INTRODUCTION

The need for sperm capacitation has been known for more than 20 years (Chang, 1951; Austin, 1951); however, our understanding of what causes sperm capacitation at the molecular level has been vague. This is in part due to the lack of a quick, specific assay for changes which the sperm undergo during the process of capacitation. It has been found that components from seminal plasma can decapacitate sperm, which could normally fertilize ova (Chang, 1957; Bedford and Chang, 1962; Weinman and Williams, 1964), and it has also been shown that seminal plasma components are present on the surface of ejaculated rabbit spermatozoa (Weil and Finkler, 1959; Weil and Rodenberg, 1962; Weil, 1965; Shulman, 1969; Menge and Potzman, 1967; Hekman and Runke, 1969). In this light, Metz (1967) suggested that it "would be of some interest to determine if spermatozoa are still coated with the sperm coating antigen after passing through the female reproductive tract." It is possible that removal or alteration of these seminal plasma components may be part or all of the capacitation process. In addition, ovum cleavage has been observed following insemination *in vitro* with rabbit sperm pretreated with uterine fluid (Brackett *et al.*, 1972).

In this work, then, an immunological approach has been used to follow the removal or alteration of the seminal plasma components present on the surface of the ejacu-

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lated spermatozoa, to relate these changes to the process of capacitation, and to quantitate the change in seminal plasma components bound to ejaculated sperm during incubation of the sperm in uterine fluid. In order to achieve this goal, a radioimmunoassay was developed, which should be useful in further studies of sperm capacitation of various species.

MATERIALS AND METHODS

Ejaculated rabbit sperm were obtained from New Zealand White bucks of proven fertility using an artificial vagina. The ejaculates were washed in six times the ejaculate volume in synthetic medium (Brackett, 1969) without bovine serum albumin (BSA). The sperm were recovered and concentrated by centrifugation at 734 *g* for 5 min at 22°C. The sperm were resuspended in a small volume of synthetic medium without BSA and counted in a hemocytometer, and the sperm cells were adjusted to a concentration of approximately 75×10^6 sperm/ml by addition of synthetic medium without BSA. Dilutions of this suspension were read on a spectrophotometer at 620 nm to estimate turbidity, and all further estimates of sperm concentration were made by turbidity determination. Since the turbidity of ejaculates of the same sperm concentration varies, the measurement of turbidity was compared with counts made with a hemocytometer for each ejaculate. Rabbit epididymal sperm was collected from excised testes through an incision in the cauda epididymis after distention of the epididymis with medium introduced via the vas deferens.

Antisera were prepared to seminal plasmas from which sperm was removed by centrifugation. For this work, seminal plasma is the fluid or nonsperm portion of semen. This includes contributions from the epididymis and male accessory glands (Mann, 1964), and since the seminal plasma was obtained as the supernatant of centrifuged semen, it may also contain some easily liberated sperm antigens. Antiserum to rabbit seminal plasma was obtained by immunization of guinea pigs with rabbit seminal plasma. Emulsifications of seminal plasmas in complete Freunds adjuvant (1:1) were injected subcutaneously; a second injection was made in 4 weeks. Blood was collected a week later by cardiac puncture, and the antiserum was prepared by the method of Brown (1966). Double diffusion experiments were done by the method of Ouchterlony (1948).

The γ-globulin (γG) fraction from the serum was obtained by addition of an equal volume of saturated $(NH_4)_2SO_4$ solution to the serum (Weir, 1967). The γG fractions of preimmunization serum and of antiserum were labeled with [^{14}C]formaldehyde by reductive alkylation as described by Oliphant and Brackett (1972). Immunoelectrophoresis was carried out on microscope slides, using 3.5 ml of 1% agarose–0.005 *M* sodium phosphate, pH 7.0, per slide. Electrophoresis was done at room temperature for 2 h at 140 V constant voltage. Diffusion of electrophoresed seminal plasma with antiserum was carried out over 1 week. Gels were then extensively washed in saline and stained with 0.1% Amido Schwarz–7% acetic acid.

Sperm agglutination was carried out by mixing 0.05 ml of diluted antiserum or control serum and 0.05 ml of a sperm suspension. Each sample was shaken on a vortex mixer 5 sec prior to incubation at 4°C. The optimal time of incubation was determined experimentally. Agglutination was estimated from + to 4+. One + was recorded when any, up to approximately 25%, of the sperm were linked head to head. When 25–50% agglutination was observed, 2+ was recorded. When estimates were made of 50–75% and 75–100% of the sperm cells agglutinated, 3+ and 4+ were recorded, respectively. Negative rating was made when no agglutination was observed.

Incubation of sperm with the ^{14}C-antiserum and ^{14}C-preimmunization serum varied according to the experiment; however, 0.05 ml of a 1:20 dilution of the antiserum was routinely used and incubation was carried out at 4°C. Following incubation the sperm were washed twice in 3.0 ml of 0.15 *M* sodium chloride, recovered by centrifugation at 12,000 *g* for 10 min at 4°C and finally suspended in 1.0 ml of 0.15 *M* sodium chloride. Sperm concentration was determined by turbidity measurements at 620 nm, and an 0.8-ml aliquot was used for counting in a scintillation counter (usually no more than 5% of the sperm were lost during washing).

Spermatozoa incubated in estrous rabbit uteri were introduced by natural mating. At various times after mating, the does were killed by cervical dislocation. Sperm were recovered by flushing the uterine horns with 4.0 ml of synthetic medium without BSA. Sperm collected from the uterine horns were concentrated by centrifugation for the agglutination assay. For incubation of sperm in uteri of pseudopregnant rabbits, the does were surgically opened and 10^6 washed ejaculated sperm in 0.05 ml were injected directly into each uterine horn. The pseudopregnant state was confirmed by observation of corpora lutea on the ovaries of these does at surgery, which was 9 days after an intravenous injection of 75 IU HCG ("A.P.L.," Ayerst). These sperm were recovered in the same manner as estrous rabbit uterine sperm.

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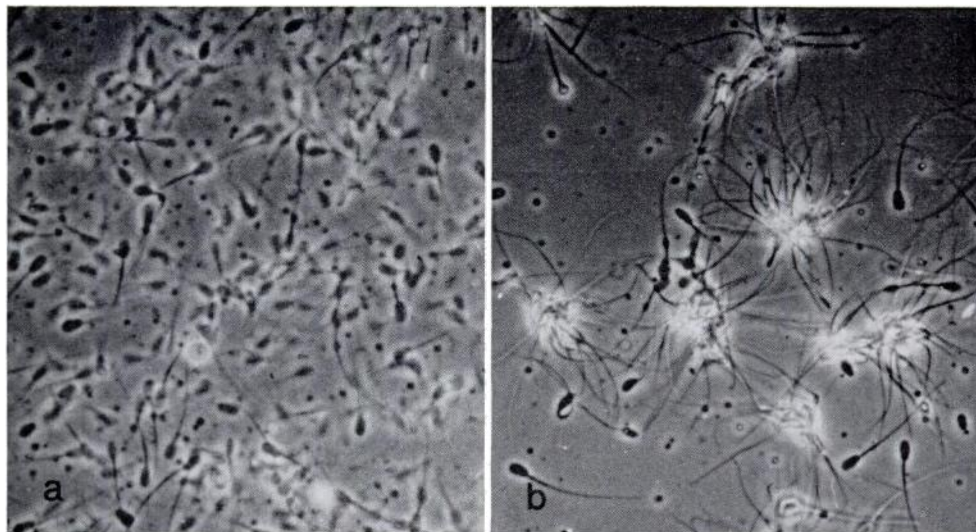

FIG. 1. (a) Washed ejaculated sperm incubated with preimmunization serum. (b) Washed ejaculated sperm with guinea pig antiserum (1/50) against rabbit seminal plasma.

Uterine fluid was collected from superovulated New Zealand White does near the time of ovulation. Mature virgin does were caged individually 3 weeks before use. Each doe was injected intramuscularly with 150 IU of PMSG ("Gestyl", Organon) followed 72–84 h later by an intravenous injection of 75 IU of HCG ("A.P.L.," Ayerst). The does were killed by cervical dislocation 12 h after the HCG treatment and uterine fluid aspirated from the uterine horns. Protein concentrations of the uterine fluids were estimated by optical density determination at 280 nm. Sperm incubations *in vitro* with uterine fluid were carried out in dialysis tubing, which was bathed in 50 ml of synthetic medium without BSA at 37°C under a moist atmosphere of 5% CO_2–95% air.

Partially purified decapacitation factor (DF) was obtained by ultracentrifugation of rabbit seminal plasma by the method of Bedford and Chang (1962).

Insemination *in vitro* with capacitated sperm, recovered at varying intervals after mating, was carried out using ova flushed from rabbit oviducts by the procedure outlined by Brackett (1969). Ova were observed for cleavage 24 h after insemination. At this time, 4-cell stage embryos were most commonly seen. Cleavage resulting in two to four symmetrical blastomeres along with sperm cells stuck on the zona pellucida and with supplementary sperm cells frequently seen within the perivitelline space of individual ova were taken as evidence that fertilization had occurred. Additional support for the occurrence of fertilization in these experiments included the absence of cleavage of tubal ova incubated under the same conditions but without exposure to sperm, and the direct observation of the entire fertilization process following *in vitro* insemination of tubal ova with capacitated sperm using this procedure (Brackett, 1970).

RESULTS

Figure 1 shows the effects of control and immune sera on washed ejaculated rabbit sperm. Head-to-head agglutination indicated the effect of the antiserum produced by the guinea pig. The antiserum appeared to be equally effective in agglutination over a broad pH range (6–9). The time required for maximal agglutination of the sperm was 45 min, and all further agglutination assays were incubated for at least 45 min. Under these conditions, it was found that strong agglutination occurred when concentrations of 125×10^5 to 1×10^5 sperm were used. Agglutination occurred with fewer spermatozoa, but was considered diminished. The sperm agglutination titer for antisera obtained from different guinea pigs ranged from 1:32 to 1:128.

A serial dilution of antiserum in a double diffusion plate showed two strong precipi-

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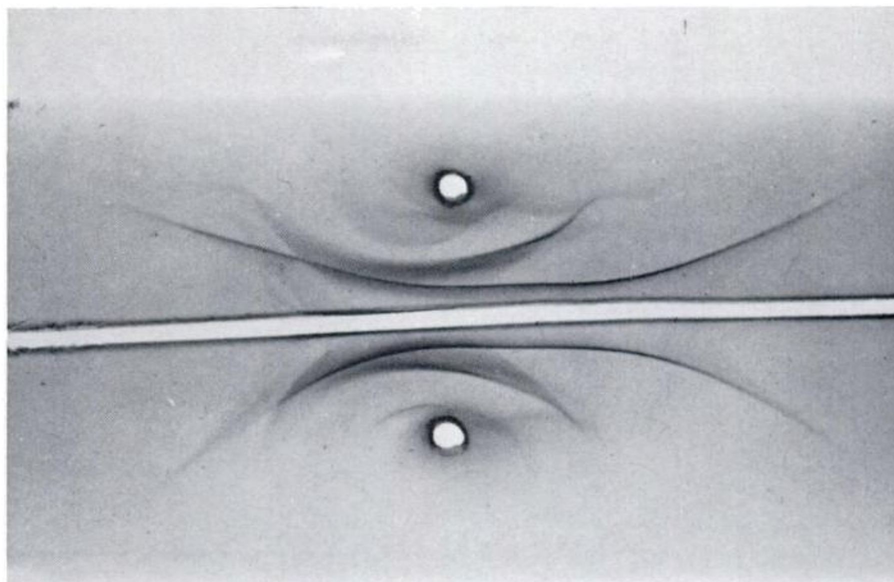

Fig. 2. Immunoelectrophoresis of rabbit seminal plasma. Fresh seminal plasma was utilized to fill wells, after electrophoresis guinea pig antiserum against rabbit seminal plasma was used to fill trough.

tin bands and one minor band, indicating the presence of antibodies against rabbit seminal plasma antigens. Experiments of this sort with preimmunization serum gave no precipitin bands. Further, rabbit serum when diffused against guinea pig antiserum to rabbit seminal plasma produced no precipitin bands. The antiserum had antibodies to at least seven seminal plasma antigens as shown by immunoelectrophoresis (Fig. 2). Agglutination of rabbit sperm was a function of the antibodies to seminal plasma components since agglutination was completely inhibited by a 1-h preincubation of antiserum with an equal volume of fresh seminal plasma. Agglutination was not inhibited by preincubation of antiserum with BSA at a protein concentration equal to that of seminal plasma.

The agglutinating ability of rabbit spermatozoa when mixed with antiserum could be influenced experimentally (Table 1). Extensive periods of washing *in vitro* in synthetic medium for up to 18 h did

TABLE 1
Agglutination and Cleavage-Inducing Abilities of *In Utero* Incubated Rabbit Spermatozoa

Sperm *in utero* incubation time (h)	Agglutination after treatment with antibodies to seminal plasma	Ovum cleavage after *in vitro* insemination	
		% Cleavage	Ova cleaved/ ova inseminated
0	3+	0	0/95
3	2+	9.4	3/32
6	+	44.4	8/18
12	−	50.1	180/359
18	−	74.1	40/54
6 (pseudopregnant)	+	—	—
18 (pseudopregnant)	+	—	—

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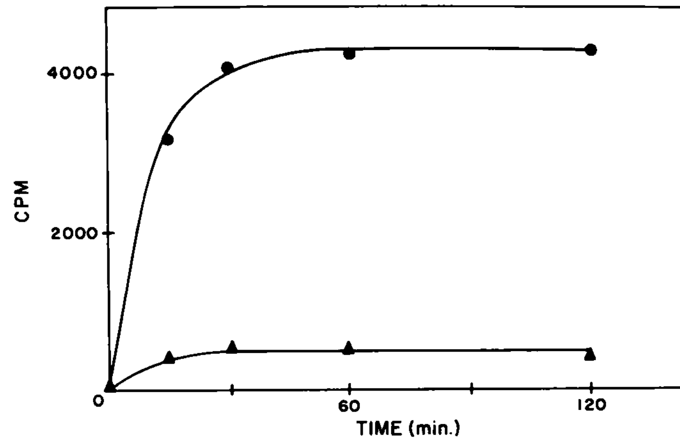

FIG. 3. Sperm-antiserum incubation time. Uptake of ^{14}C-antiserum 1/20 (—●—) and ^{14}C-preimmunization serum 1/20 (—▲—) by 15×10^6 sperm. Each point is the average of duplicate determinations. (Data corrected for 20% nonspecific adsorption.)

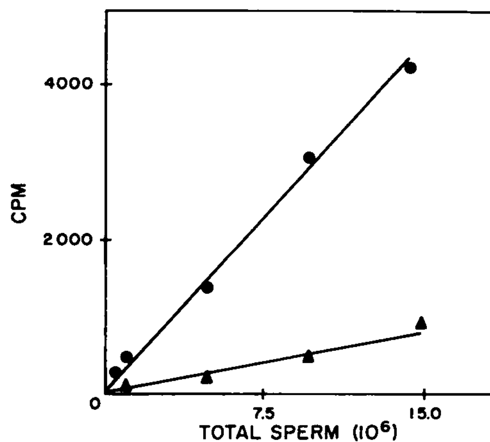

FIG. 4. Sperm concentration effect on uptake of ^{14}C-antiserum 1/20 (—●—) and ^{14}C-preimmunization serum 1/20 (—▲—). Each point is the average of duplicate determinations.

not diminish the agglutination reaction. However, following incubation of sperm in the female reproductive tract, the agglutination of sperm cells induced by the antiserum to seminal plasma diminished with time *in utero*. Additional data indicated that higher percentages of cleaved ova resulted following insemination of rabbit ova *in vitro* with uterine spermatozoa recovered at increasing intervals after coitus (Table 1). When uterine sperm agglutination with antiserum could occur, ovum cleavage rates (ova cleaved/ova inseminated) resulting from insemination with uterine sperm of comparable ages were low.

Further, it was found that epididymal sperm were readily agglutinated by antiserum to seminal plasma components, indicating that at least some of the antigens present in the seminal plasma are either also present or cross react with antigens on the sperm surface in the epididymis. The pseudopregnant rabbit uterus appears also to be effective in removal or alteration of the seminal plasma antigens at both 6 and 18 h after surgical introduction of sperm into the uterus.

The ^{14}C γG fractions of the preimmunization serum and the antiserum had activities of 6.2×10^5 and 8.6×10^5 cpm/mg, respectively. The ^{14}C-antiserum γG was found to readily agglutinate ejaculated sperm and had a titer of 1:256, which was equal to that before reductive alkylation. The preimmunization serum γG did not agglutinate ejaculated sperm before or after reductive alkylation.

When ^{14}C-antibodies and ^{14}C-preimmunization γG's were incubated with sperm for varying time intervals results shown in Fig. 3 were obtained. The reaction appears complete by 45 min; however, to insure

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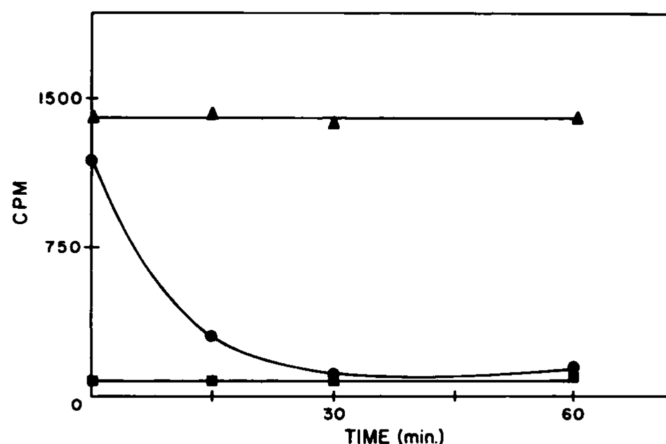

FIG. 5. Uptake of ^{14}C-labeled sera by sperm after: 1, pretreatment of ^{14}C-antiserum for various lengths of time with BSA (20 mg/ml) (—▲—) and seminal plasma (20 mg/ml) (—●—); 2, pretreatment of ^{14}C-preimmunization serum with seminal plasma (20 mg/ml) (—■—). Each point is the average of duplicate determinations.

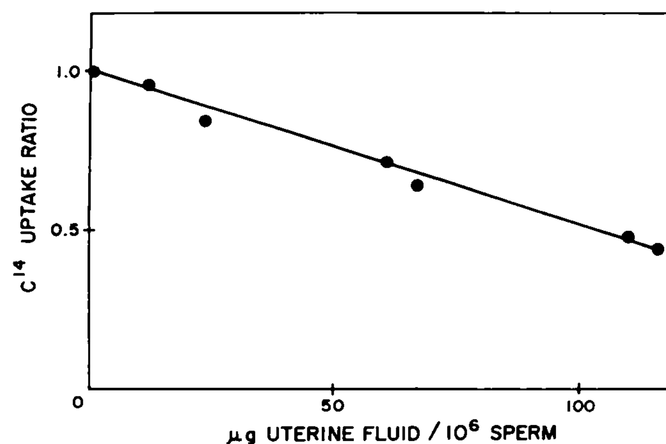

FIG. 6. Effect of sperm treatment with increasing amounts of uterine fluid protein on ^{14}C-antiserum uptake by treated sperm when 2-h incubations were used. ^{14}C-uptake ratio = uptake by treated sperm/uptake by untreated sperm. Each point is the average of duplicate determination.

complete reaction, all further assays were incubated for 60 min. Figure 4 indicates that the amount of counts picked up is a function of the sperm concentration, and that the ^{14}C-antiserum γG fraction is much more readily picked up than the ^{14}C-preimmunization γG fraction. Figure 5 shows the uptake by the sperm of ^{14}C-antibodies and ^{14}C-preimmunization serum when the serum fractions have been pretreated for varying time with seminal plasma or BSA of equal protein concentration to seminal plasma. It can thus be concluded that most of the ^{14}C-counts bound to the sperm are due to specific interaction between the ^{14}C-antibodies to seminal plasma and the seminal plasma bound on the sperm surface. The effect of pretreatment of sperm for 2 h in varying concentrations of uterine fluid is indicated in Fig. 6. The data are reported as the ratio of ^{14}C-labeled antibody uptake between ejaculated sperm

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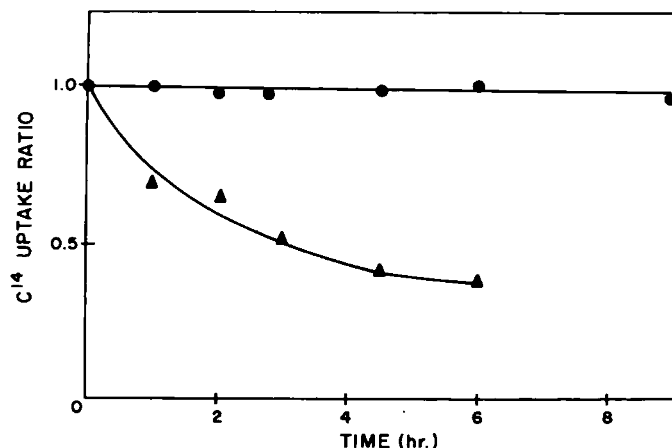

FIG. 7. Uptake of ^{14}C-antiserum by sperm after treatment with synthetic medium (—●—) and uterine fluid 0.07 mg protein per 10^6 sperm (—▲—, data shown in Table 2). ^{14}C-uptake ratio = uptake by untreated sperm/uptake by treated sperm.

treated with uterine fluid and that not treated. The values are reported as ^{14}C uptake ratios in order to relate results obtained with different ejaculates, since uptake of ^{14}C-antiserum from ejaculate to ejaculate ranged from approximately 3500 to 6000 cpm per 15×10^6 sperm. Treatment with uterine fluid appears to cause removal or alteration of the seminal plasma components bound on the sperm surface. When ejaculated sperm are incubated (washed) for up to 24 h, the ratio between the uptake of ^{14}C-antibodies by fresh ejaculated sperm and those incubated for 24 h remains constant. However, as shown in Fig. 7, when the ejaculated sperm are incubated in uterine fluid in a fixed ratio of 0.07 mg protein per 10^6 sperm, the uptake of ^{14}C-antibodies by the treated sperm rapidly falls off with increasing time of incubation (Table 2).

When uterine fluid heated at 100°C for 15 min was used for incubation with sperm, there was no decrease in the

TABLE 2
ALTERATION OF SEMINAL PLASMA ANTIGENS ON SPERM SURFACE BY UTERINE FLUID

Incubation time (h)	CPM[a] taken up by treated sperm / CPM[a] taken up by untreated sperm (ratio)				Avg. ratio
1	$\frac{3920}{5700}$ (0.69)	$\frac{3580}{5280}$ (0.68)	$\frac{3465}{5280}$ (0.66)		0.68
2	$\frac{3250}{5110}$ (0.63)	$\frac{3060}{5110}$ (0.60)			0.62
3	$\frac{3150}{5700}$ (0.55)	$\frac{3070}{5700}$ (0.54)	$\frac{2240}{3625}$ (0.61)	$\frac{2240}{3625}$ (0.61)	0.57
4.5	$\frac{1600}{3625}$ (0.44)	$\frac{1710}{3625}$ (0.47)			0.46
6.0	$\frac{2800}{5280}$ (0.53)	$\frac{2660}{5280}$ (0.51)	$\frac{1810}{5110}$ (0.35)	$\frac{1630}{5110}$ (0.32)	0.43

[a] ^{14}C-labeled antiserum to seminal plasma. Sperm treatment consisted of incubation of 15×10^6 sperm with 1.05 mg uterine fluid protein for indicated intervals.

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amount of ^{14}C-antibodies taken up by the treated sperm (Table 3). However, when uterine fluid was dialyzed prior to using it for treatment of sperm, there was no effect on its ability to remove the sperm-bound seminal plasma antigens, as indicated by the decrease of ^{14}C taken up by the treated sperm.

Once the bound seminal plasma components were removed by uterine fluid treatment, the affinity of the treated sperm for the ^{14}C-labeled antibody was restored by a 20-min incubation with 1.0 mg of partially purified decapacitation factor per 10^5 sperm (Table 4).

TABLE 3

EFFECT OF ALTERATION OF UTERINE FLUID[a]

Experimental treatment	CPM (antibody uptake)	Ratio[b]
Washed ejaculated sperm	4860 (4920, 4800)	1.0
Uterine fluid-treated sperm	3200 (3230, 3170)	0.66
Heat-treated uterine fluid treated sperm	4940 (4930, 4950)	1.03
Dialyzed uterine fluid-treated sperm	3170 (3220, 3120)	0.66

[a] Average of two experiments (values for individual experiments in parentheses).

[b] $\frac{^{14}\text{C uptake by treated sperm}}{^{14}\text{C uptake by untreated (washed ejaculated) sperm}}$

TABLE 4

DECAPACITATION OF SPERM[a]

Experimental treatment	CPM (antibody uptake)	Ratio
Washed ejaculated sperm	2450 (2360, 2540)	1.0
Uterine fluid-treated sperm	1735 (1735, 1735)	0.71
Uterine fluid-treated sperm plus incubation with decapacitation factor	2690 (2680, 2700)	1.09

[a] Average of two experiments (values for individual experiments in parentheses).

DISCUSSION

Chang (1957) discovered that capacitated sperm could be decapacitated by incubation in seminal plasma. A decapacitation factor (DF) was subsequently isolated from seminal plasma (Bedford and Chang, 1962), and the biochemical nature of decapacitation factor (Williams *et al.*, 1969) has been studied. The process of capacitation has been considered to include the alteration or removal of seminal plasma component(s) from the sperm. The technique described in this work provides a rapid method for following the removal or alteration of seminal plasma antigens normally present on the surface of epididymal and ejaculated spermatozoa.

The agglutination in these studies on rabbit sperm appeared totally as a head-to-head agglutination. This is in contrast to the agglutination of rabbit sperm observed by Weil and Finkler (1968) and Smith (1950), who observed essentially a "tail" type of agglutination, but is similar to the agglutination of rabbit sperm by guinea pig antiserum observed by Menge (1971). Agglutination was reported by Smith (1950) to be dependent on prior washing of the ejaculated sperm cells. However, Weil and Finkler (1958) indicated that under their conditions prewashing of the ejaculated sperm was not necessary to allow antibody-induced sperm agglutination. This is probably due to a higher antibody titer in the studies of Weil and Finkler, since with lower titers, excess soluble seminal plasma antigens successfully compete for the antibody with the sperm-bound seminal plasma antigens. This was shown in the present study by pretreatment of the antiserum with seminal plasma, which resulted in loss of agglutinating capability of the treated antiserum. Further, this indicates that the sperm agglutination is solely a function of the interaction between seminal plasma components bound to the sperm surface and the antibodies produced to seminal plasma. In addition, this is not simply a nonspecific effect of added protein, since addition of

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bovine serum albumin in concentration equal to the protein concentration of seminal plasma had no effect on the agglutinating activity of the antiserum.

The double diffusion experiments produced two major bands and one minor band close to the antigen well, which is similar to the results of Weil and Finkler (1958), and the antiserum showed no cross reactivity with rabbit serum. At least some of these antigens are present on the sperm in the epididymis since epididymal sperm strongly agglutinate when treated with antiserum. Antibodies to epididymal components might be expected since epididymal fluid is a normal component of seminal plasma (Mann, 1964). These results are in contrast to those of Weil and Rodenberg (1962), who suggest that sperm-coating antigens originate from the seminal vesicles. However, this work is in accord with the results of Weil and Finkler (1958) and Menge and Protzman (1967) and is most likely of significance in the process of sperm capacitation, since the decapacitation factor has been shown to be present in epididymal fluid (Weinman and Williams, 1964).

These antigens are tightly bound to the sperm since viable sperm strongly agglutinate even after 24 h of washing *in vitro*. This was also indicated by Weil and Finkler (1958), who found that sperm refrigerated for 1 to 8 weeks still were reactive to complement fixation. This tight binding of the seminal plasma components would then allow for virtually permanent decapacitation of the sperm until they underwent a specific change, which normally occurs in the female reproductive tract. This change is indicated in this study by the decreased agglutination of sperm incubated in the uterus for increasing intervals of time. Further, this removal of seminal plasma antigens is also indicated by Menge (1971), who found no complement fixation with sperm that has been incubated in the uterus, and by Johnson and Hunter (1972), who found that flourescent antibodies to seminal plasma no longer bound to sperm, which had incubated for 3 h *in utero*. These results are in contrast to those of Weil and Stefanovic (1969), who reported that fluorescent antibodies to seminal plasma would still bind to sperm recovered from the rabbit uterus up to 24 h after mating.

The decrease of agglutination of sperm incubated *in utero* indicates that by 3 h *in utero* much of the seminal plasma components have been removed or altered and such sperm induced cleavage in only 9% of ova inseminated. By 6 h *in utero*, most of the seminal plasma components detectable by this method have been removed and the sperm are capable of inducing cleavage of 44% of the ova. The increased proportions of ovum cleavage with uterine sperm recovered 12 and 18 h after mating may be due to a further release of the decapacitating substances, which is undetectable by the agglutination procedures described here or to the progressive occurrence of a second step in the capacitation process. The possibility of a second step is supported since the pseudopregnant rabbit uterus also removes the bound seminal plasma antigens from the sperm surface, but is known not to be able to fully capacitate sperm (Chang, 1958). The removal of seminal plasma components may have been the basis for Ericsson's (1967) tetracycline assay for sperm capacitation, assuming that one or more of the sperm-bound seminal plasma components binds tetracycline. However, the technique reported here provides a method for assessing the extent of removal of the seminal plasma antigens bound to the sperm surface, without having to consider any change in binding of tetracycline to the antigens.

Further, this paper shows that the seminal plasma antigens bound to the sperm are also removed by uterine fluid, and most importantly this removal can be quantitated using ^{14}C-labeled antibodies against seminal plasma.

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Previously, radioactively labeled antibodies were used to observe the interaction between seminal plasma and sperm by Noyes (1969), who used ^{131}I to label a patient's serum to show an interaction with sperm, and by Erickson (1971), who used ^{125}I-labeled antimouse globulin to quantitate the amount of antigen bound to mouse sperm.

In the present experiments, the antiserum was labeled once, and the same ^{14}C-antiserum was used for all experiments. The reaction between the ^{14}C-antiserum and the seminal plasma components occurred very rapidly; by 30 min it was virtually complete. However, to insure that minor variations were not a function of the reaction not reaching completeness, all reactions were incubated for 1 h. When the uptake of ^{14}C-antibodies and ^{14}C-preimmunization γG were compared, it was found that about 20% of the counts could be attributed to nonspecific adsorption on the sperm surface. Consequently, all subsequent experiments were corrected for this nonspecific adsorption. The specificity of the reaction between sperm and antibodies was indicated by its virtually complete inhibition following preincubation of the antibodies with seminal plasma. The latter reaction took just about the same time as that for antibodies to interact with the sperm. Further, the specificity of the inhibition is indicated by the constant uptake when BSA is used for preincubation with the antiserum at a protein concentration equal to that in seminal plasma.

The concentration dependence of the removal of seminal plasma components from the sperm surface by uterine fluid correlated well with previous results (Brackett *et al.*, 1972), which showed an increase in cleavage of ova following insemination *in vitro* with sperm samples that were treated with increasing volumes of uterine fluid per 10^6 sperm. The fact that uterine fluid does not cause complete loss of sites for antibody binding may be in part the result of antibody binding to semen particles difficult to separate from sperm and which possess antigens in common with sperm and soluble seminal plasma components (Metz *et al.*, 1968). Extended washing of ejaculated sperm does not decrease the amount of ^{14}C-antibodies taken up by the sperm. This is in accord with the finding that 24 h of washing the sperm did not decrease the ability of antibodies to seminal plasma to agglutinate the sperm.

Since dialyzed uterine fluid is still effective, the agent acting on the seminal plasma components is macromolecular. The heat inactivation of the uterine fluid indicates the alteration or removal of the sperm-bound seminal plasma components may be enzymatic. This was previously indicated by Noyes *et al.* (1958) who found that sperm could not be capacitated *in utero* if they were also enclosed in a dialysis bag, and by Hamner and Sojka (1968), who carried out similar experiments but with sperm enclosed in Millipore filters.

Further, the fact that partially purified decapacitation factor recombines with sperm that have had their bound seminal plasma components removed by uterine fluid is in accord with the work of Chang and Bedford (1962) and Weinman and Williams (1964), who demonstrated that capacitated sperm could be decapacitated by incubation with DF.

In conclusion, this technique provides a rapid method for evaluating whether sperm have undergone changes which occur during the process of capacitation, and it also provides a rapid, quantitative assay for the changes that occur to sperm experimentally treated with uterine fluid. This assay may, therefore, be useful in further studies carried out to elucidate the mechanism of sperm capacitation.

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