Sperm Agglutinins in Human Semen and Blood. (20982)

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Antibodies against spermatozoa have been known to impair sperm functions(1-3). Such antibodies, however, have not previously been found under natural conditions and, particularly, not in man.* This is a report of 2 men whose spermatozoa agglutinate in the ejaculate. Antibodies were found in their seminal plasma and blood serum. These cases were encountered by the writer in the past year and a half during the routine clinical investigation of approximately 100 sterile couples.

Clinical observations. Case 1. White male, age 30. American Jewish descent. Family and personal medical history negative. A brother has one child. Blood group *AB*, $Rh_0(D)$ positive; no history of blood transfusions or injections. Married 10 years. Wife unable to conceive although in good health, her sterility investigation negative and no contraception used. Her blood group is *B*, $Rh_0(D)$ positive. She promptly became pregnant following artificial insemination of a donor's semen and is now in her last trimester. *Case 2.* White male, age 36, American Jewish descent. Family and personal medical history negative. A brother has 2 children and a sister has one child. Blood group *B*, $Rh_0(D)$ negative, *anti-*$Rh_0(D)$ negative. No history of blood transfusions or injections. Married 3 years. Wife unable to conceive although in good health, her sterility investigation negative and no contraception used. Her blood group is *O*, $Rh_0(D)$ positive. In both cases the semen was examined many times and consistently showed normal values for the ejaculate volume, sperm count, viscosity, initial motility, morphology and non-spermatozoal cellular content. However, these specimens always showed marked clumping of the spermatozoa and early loss of motility. It was therefore decided to study this phenomenon in detail.

Methods. All semen specimens were obtained by masturbation after a period of sexual abstinence of not less than 48 hours. The ejaculates were collected directly into clean, dry glass containers and, on several occasions, in 2 separate fractions (split-ejaculate). Sperm agglutination was detected by microscopic examination of a wet film prepared by laying a cover-slip over a drop of semen on a clean glass slide. The presence of agglutinins in the blood serum and seminal plasma was determined by mixing a drop of the respective fluid with a drop of fresh normal semen and examining the wet film microscopically for sperm clumping. As soon as the initial microscopic observation of sperm motility and agglutination was completed, the edges of the cover-slip were sealed with melted vaseline. The slides were re-examined at intervals up to 24 hours or until all spermatozoa were immobilized. The rapidity, degree and type of agglutination (head, tail or mixed), the influence of temperature (4°, 20°, and 37°C) and the relation between agglutination and sperm motility were noted. The latter was studied by a) observing the fresh ejaculate microscopically during liquefaction (while the spermatozoa were becoming motile), b) observing spermatozoa during post-coital and Kurzrok-Miller cervical mucus invasion tests, and c) comparing the behavior of normal motile and heat-immobilized spermatozoa (56°C for 30 minutes) in the presence of

*Kibrick *et al. (Fertil. and Steril.* 1952, v3, 435) reported agglutination of human spermatozoa by some normal sera of rabbits, guinea pigs and man in low dilutions. They used a macroscopic tube agglutination technic with gelatin. No significance was attributed to this observation which was considered non-specific. It was rare in dilutions over 1:4 in contrast to the titers up to 1:256000 obtained with potent anti-human-sperm rabbit's sera. Because of the different technics employed, it is not possible to compare the findings of these workers with the observations reported here. Spermatozoa were not agglutinated by normal human sera with the method used in this study.

blood and seminal agglutinins. Serial dilutions of the blood sera were made with isotonic saline. Complement was inactivated by heating serum and seminal plasma in a 56°C waterbath for one hour.

Absorption tests were performed as follows: equal amounts of dilute serum were placed in 2 small test tubes; fresh normal semen of high sperm count and active motility was added to one tube and an equal volume of isotonic saline to the other; after heating in a 37°C waterbath for one hour, the tubes were centrifuged and the supernates were tested for sperm agglutinins as described above.

Results. Agglutination of the spermatozoa of normal subjects was produced by the seminal plasma and blood serum of the 2 men who are the subject of this report. The microscopic appearance of the sperm clumps was identical with that seen in their ejaculates. Agglutination was not obtained with the seminal plasma or serum of 8 fertile men or the serum of 8 pregnant women.

The speed of agglutination varied from one minute to one hour depending upon the agglutinin titer, temperature and quality of sperm motility. The clumps continued to grow as long as sperm motility was preserved. However, even after 24 hours, a few motile spermatozoa remained unclumped.

As observed under the microscope, agglutination began in one of 3 ways: 1. *"Head" agglutination*: motile spermatozoa formed into groups of 2, 3 or 4, joined side by side with their heads in apposition and their tails separate. The tails soon became entangled with the tails or heads of other groups, thereby forming larger and larger clumps. Individual motile and immotile spermatozoa, as well as cellular and other debris, similarly became enmeshed in the clumps and contributed to their growth. 2. *"Tail" agglutination*: the tails of several motile spermatozoa became intertwined while their heads remained separate at the periphery forming a rosette-like cluster. Other spermatozoa swam into or were caught in the network of tails and thus the size of the clump increased. 3. *"Mixed" agglutination*: the tail of one spermatozoon became attached to the head of another. Soon other spermatozoa were entangled and a clump formed.

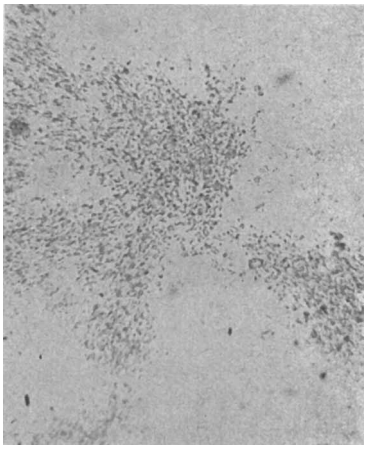

FIG. 1. Sperm agglutination in ejaculate of Case 1. Unstained wet film preparation (50 ×).

Regardless of the original type of agglutination, within a short time all the larger clumps had a similar appearance. This was predominantly that of "tail" agglutination with free sperm heads visible at the periphery. Occasionally a particularly active spermatozoon was seen detaching itself from the edge of a clump by its own effort. The agglutinated spermatozoa evidently were not firmly bound to each other (at least during the first hour) since the clumps were partially disrupted by removing the cover-slip from the slide. The clumps were completely dispersed by bubbling air through the ejaculate but reagglutination took place in a few minutes. The new clumps, however, did not attain their previous size probably because some of the spermatozoa, in the meantime, had lost their motility. The appearance of sperm agglutination at 50 × and 215 × magnification is shown in Fig. 1 and 3. For comparison, nonagglutinated spermatozoa are shown in Fig. 2 and 4.

Sperm motility. Agglutination occurred only in the presence of motile spermatozoa and none was seen in the fresh ejaculate until liquefaction began and active sperm movement developed (5 to 15 minutes). Within a short time, however, the agglutinated spermatozoa became sluggish and finally lost their motility. This was true of the majority of the spermatozoa and, particularly, those deep inside the clumps. Nevertheless, a small number on the periphery and some of those

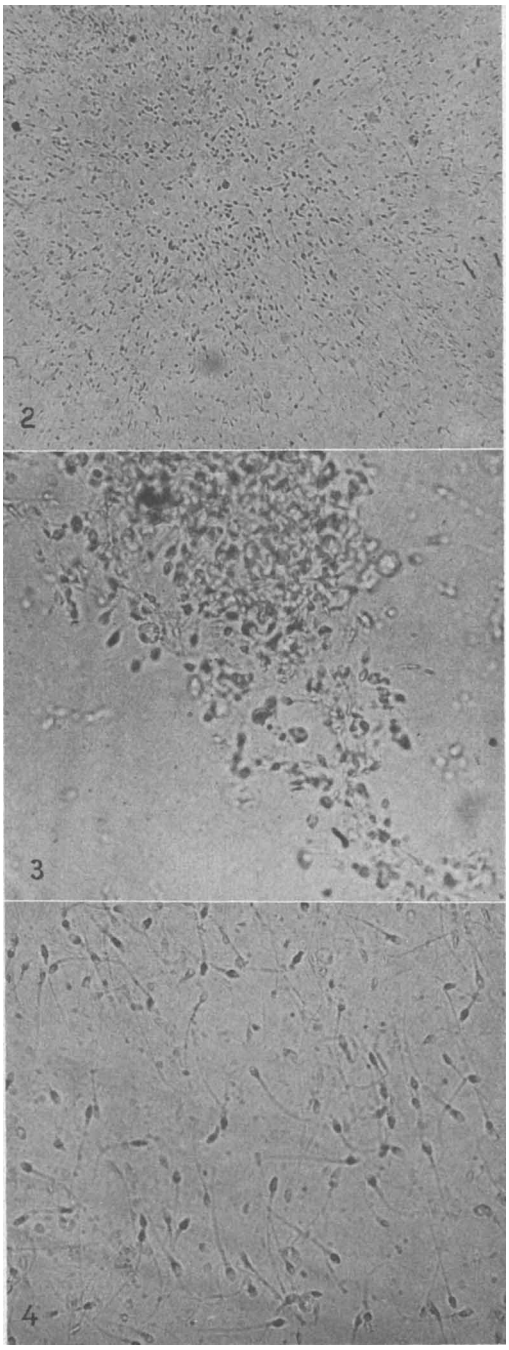

FIG. 2. Normal semen showing absence of sperm agglutination (50 ×).

FIG. 3. Higher magnification of Fig. 1 (215 ×). The dominant appearance is of "tail" agglutination with sperm heads free at periphery of clump.

FIG. 4. Higher magnification of Fig. 2 (215 ×).

remaining free continued to exhibit fairly active movement for as long as 24 hours if the preparation was sealed with vaseline to prevent drying and was kept at a temperature between 4° and 20°C.

Temperature. Agglutination occurred at room temperature (20°C) but was more rapid at 37°C due to the increased sperm motility at the higher temperature. Low temperatures retarded clumping by reducing sperm motility while temperatures appreciably above 37°C immobilized the spermatozoa and halted agglutination.

Post-coital and cervical mucus invasion tests. Post-coital examination of the cervical mucus, performed during the pre-ovulatory phase of the menstrual cycle and 3 to 48 hours after intercourse or artificial insemination, demonstrated the presence of unusually few spermatozoa and the motility of these was sluggish or absent. Agglutinated spermatozoa were not seen in the cervical mucus probably because penetration was prevented by agglutination and impaired motility. Cervical mucus invasion tests gave similar results: spermatozoa passed through the interface but quickly lost their motility. Sperm clumps were present in the seminal portion but not in the mucus.

The split-ejaculate. The first portion of the ejaculate contained many more spermatozoa than the second portion and agglutination was seen in both.

Immunologic data. Clumping took place in dilutions of the blood serum up to 1:80 in *Case 1* and 1:20 in *Case 2*. Agglutination was not affected by inactivating the complement in the serum and seminal plasma. Sera kept at 4°C for 3 months did not lose their capacity to clump spermatozoa. The agglutinins in the blood serum were removed by absorption. Agglutination did not occur when normal spermatozoa and normal seminal plasma (or blood serum) of subjects with different ABO and $Rh_0(D)$ blood groups were mixed.

Discussion. No satisfactory explanation for the presence of sperm agglutinins in the blood and semen in these 2 cases can be given at present. Clumping has been observed in infected ejaculates(4) and has been produced

experimentally in human or animal semen by acids, alkalis, carbon dioxide, salts of heavy metals, dyes, extracts of eggs, bacteria and antisera developed by injecting spermatozoa of the same or different species(5). None of these agents appears to be involved in the cases reported here.

Summary. The presence of sperm agglutinins in the seminal plasma and blood serum of 2 sterile men whose spermatozoa agglutinate in the ejaculate is reported. The sera have antibody titers of 1:80 and 1:20, respectively. Head, tail, and mixed varieties of agglutination are seen in the early stages, but tail agglutination predominates in the larger clumps. Agglutination occurs only in the presence of motile spermatozoa and none is seen in the fresh ejaculate until liquefaction begins and active sperm movement develops. Temperature influences agglutination by its effect on sperm motility. The motility of the clumped spermatozoa is impaired with consequent loss of the ability to penetrate cervical mucus. The clumps can be completely dispersed by mechanical agitation after which the spermatozoa reagglutinate although not quite to the original extent. Complement inactivation does not influence agglutination. The antibody can be removed by absorption.

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Combined Effects of X-Irradiation and Testosterone Propionate on Accessory Sex Organs. (20983)

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A temporary inhibition of cell division appears to be a general action of radiation, having been demonstrated in a great variety of cells. If the cells are prevented from dividing, but continue to grow at the normal rate, then the size of the cells will increase instead of their number. When division is delayed by irradiation it is of interest to determine at which stage or stages the retardation occurs. The answer to these questions can most readily be obtained in the case of materials in which the cells develop synchronously, so that all the cells can be irradiated at the same stage. It is obvious, that this experimental condition cannot as a rule be fulfilled in growing tissues as they are composed of cells at different stages of cell division, so that it is not possible to choose a single stage to be irradiated(1). Experiments in which a single stage was irradiated were performed by Henshaw(2), who treated unfertilized eggs of the sea urchin, *Arbacia punctulata*, with x-rays and studied the delay of first cleavage following subsequent fertilization. In these experiments all cells were in the resting stage during irradiation and cell division was induced subsequently by fertilization.

It occurred to us that irradiation of the secondary sex organs (seminal vesicles and prostate) of the castrate male rat and subsequent stimulation by an androgen offers a unique opportunity for an analogous study on the effect of radiation on mammalian cells.

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