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Adsorption of Albumin on Rabbit Sperm Membranes

M. Blank, L. Soo, and J.S. Britten

Department of Physiology, Columbia University,
630 W. 168 Street, New York, New York 10032

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Summary. When mammalian sperm cells are exposed to solutions of albumin there are changes in the membranes of some species that resemble those that normally occur in the uterus prior to fertilization. We have shown that albumin molecules adsorb on to the membranes of ejaculated rabbit sperm cells, and that the equilibrium binding constant, K, (1) varies inversely with the albumin concentration, (2) is independent of the sperm cell concentration in the range 10^6–10^7 per ml, (3) is independent of the time of exposure of the sperm cells to the albumin solution, and (4) decreases in the presence of Ca^{++} and Mg^{++} ions. An unusual aspect of the adsorption is that if the albumin concentration is given the symbol $[A]$, $K[A]$ is a constant in our measurements. This means that for virtually the entire range of $[A]$ studied, the sperm cells bind albumin so that half of the available surface is coated and half remains uncoated. This situation is rather remarkable and it suggests a role that adsorption could play in the physical processes preceding fertilization. In purely physical systems, the optimum for the bridging and flocculation of particles that are coated with adsorbed macromolecular films occurs when half of the available surface is covered. The sperm cell appears to provide the optimal situation for interacting with itself or with another surface.

We have been studying changes in the properties of the membranes of rabbit spermatozoa related to fertilization. By analyzing the noise that occurs when suspended sperm cells contact an electrode, we were able to gain information about adsorption on to sperm cells (Blank, Soo & Britten, 1974*a*), and by direct titration, we were also able to estimate the surface charge of the sperm cell (Blank, Soo & Britten, 1974*b*). The results of those measurements indicate clear differences between ejaculated and immature (caudal epididymal) sperm cells of rabbit, with regard to the ability to adsorb surface active material in competition with a charged electrode surface, and the ability to bind Zn^{++} ions as a measure of the membrane surface charge. Ejaculated sperm cells are less able to adsorb surface active material in comparison to the epididymal cells, and the adsorption is more easily stopped by divalent

cations. Titration experiments with divalent Zn^{++} show that ejaculated cells have a much greater cation binding ability. In all cases, control experiments interchanging cells and suspending media, e.g., using epididymal cells in seminal fluid, indicate that the measured differences relate to the cell membranes and not the suspending media.

Of the many changes in the plasma membranes of sperm cells at various stages of their development, those that occur in the female tract immediately prior to fertilization, i.e., capacitation and the subsequent acrosome reaction, lead to the relatively radical changes in the structure of parts of the membrane. For this reason, an investigation of the molecular events under the conditions of capacitation should enable us to gain insight into the factors that cause membrane instability. Sperm capacitation, first described by Chang (1951) for the rabbit, and by Austin (1952) for the rat, has been demonstrated in several other species, for epididymal as well as ejaculated sperm, and for both *in vitro* and *in vivo* conditions. *In vitro* capacitation has been carried out in chemically defined media containing blood serum or follicular fluid, but resently it was shown by Miyamoto and Chang (1973) that capacitation can be achieved in a chemically defined medium containing bovine serum albumin (BSA). These findings now make it possible to study the membrane changes occurring during capacitation under well-defined conditions.

Materials and Methods

Ejaculated sperm cells were collected from New Zealand rabbits with the aid of an artificial vagina, and centrifuged at $500 \times g$ for 2 min. The pellet was diluted with a saline solution containing 135 mM NaCl, 5 mM KCl and low concentrations (i.e., in the micromolar range) albumin at pH 7.1, (solution I). In some cases the cells were suspended in a solution containing four cations, i.e., (in mM) 130 NaCl, 5 KCl, 3 CaCl and 1 $MgCl_2$, at the same pH and protein concentration. The concentrations of the cell suspensions were determined by the standard cell count method using a Levy-Hausser Counting Chamber,

The cell suspensions were placed in a rapidly dropping mercury electrode apparatus, the Metrohm Polarecord Model E261, thermostatted at 25 °C. The dissolved O_2 was removed by bubbling purified N_2 through the solution and continuing flow of N_2 above the solution during the measurement. The binding of Zn^{++} ions with the anionic groups on the sperm cells was then measured polarographically (Blank *et al.*, 1974*b*). Microliter quantities of the Zn^{++} containing solutions were added to the sperm cells and the polarographic current measured after the addition.

In studying the reaction of albumin [*A*] with sperm cells [*S*] to form a complex [*A*:*S*] where anionic groups are no longer accessible, we have assumed the simplest type of reaction,

$$A + S = A:S,$$

and have defined an equilibrium constant,

$$K = \frac{[A:S]}{[S][A]}. \tag{2}$$

The value of K is determined for any set of conditions by measuring:

1. the Zn^{++} bound to $[A]$ when present alone
2. the Zn^{++} bound to $[S]$ when present alone
3. the Zn^{++} bound to the reacted $A+S$.

The amount of $[A:S]$ formed is determined from the difference between 3 and (2+1), and K can then be calculated. The value of K was studied as a function of the sperm concentration, the concentration of $[A]$ in solution after binding equilibrium was reached and the time after mixing.

Results

The calculated equilibrium binding constant of albumin on to ejaculated sperm cells is shown in Fig. 1 as a function of the albumin concen-

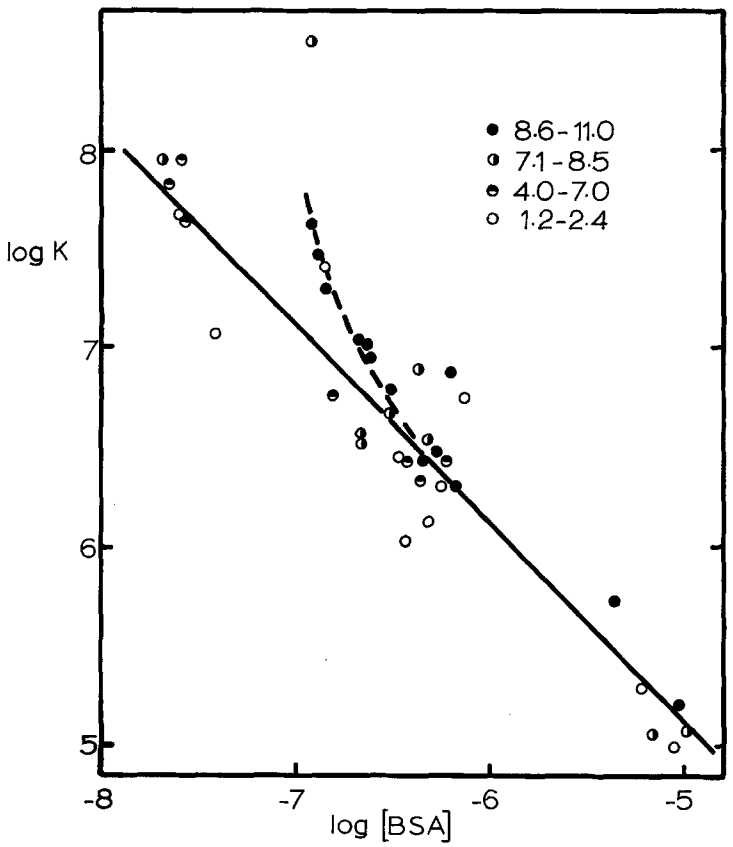

Fig. 1. The logarithm of the equilibrium binding constant of BSA to sperm cells as a function of the logarithm of the BSA concentration. The different symbols, which correspond to different concentrations of cells, are defined on the diagram in terms of millions of cells/ml. The solid line has a slope $\equiv -1$ and the dashed curve shows the deviations from this line at high cell concentrations and low BSA concentrations

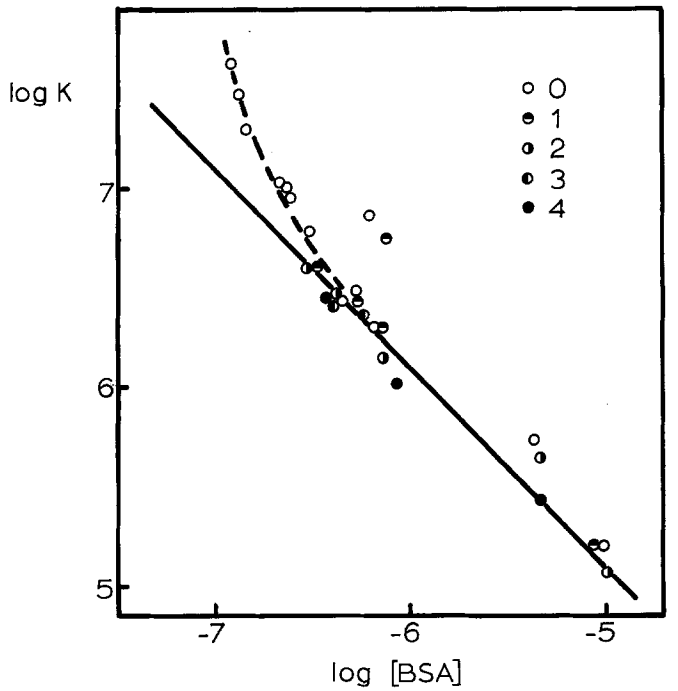

Fig. 2. The logarithm of the equilibrium binding constant of BSA to sperm cells as a function of the logarithm of the BSA concentration. The different symbols correspond to experiments done at various times after mixing and are given on the diagram in units of hours. The experiments involved cell concentrations of about 10^7 cells/ml (range 0.8–1.1×10^7 cells/ml) and the solid and dashed lines are the same as in Fig. 1

tration. The results cluster around the line of slope $= -1$, indicating that the value of K varies inversely with the albumin concentration. (The deviations from the line at high cell concentration will be discussed below.) The data of Fig. 1 also show that the results are independent of the sperm cell concentration which range between 10^6–10^7 per ml.

The binding constant is also independent of the time of exposure of the sperm cells to the albumin solution. Fig. 2 shows the variation of the value of K (for the densest cell suspensions) at different times, and all of the points appear to be members of the same population. Similar results were obtained for the other ranges of cell suspensions shown in Fig. 1.

The above (BSA) binding experiments were carried out in an isotonic medium that contained only Na^+ and K^+ as the cations. When binding was studied in a medium that contained four cations (Ca^{++} and Mg^{++} in addition), the results were different. Fig. 3 shows that for the same

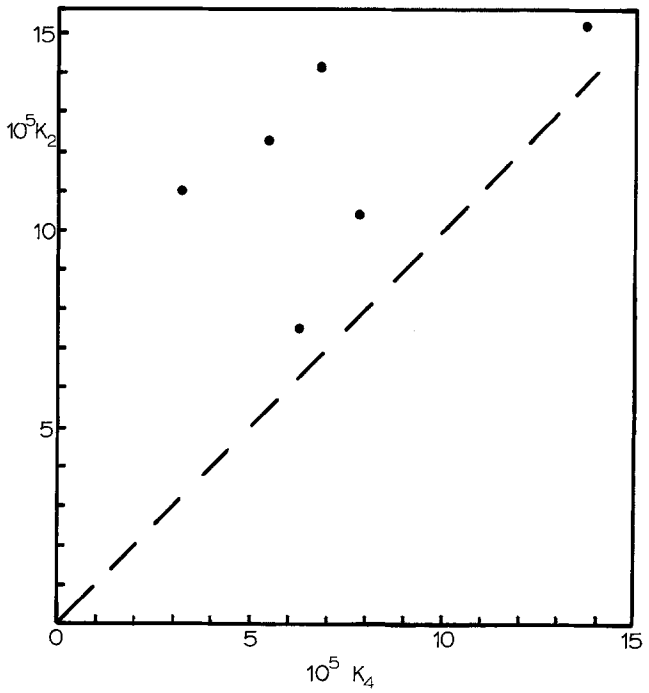

Fig. 3. K_2, the equilibrium binding constant of BSA to sperm cells determined in the presence of the two major cations Na^+ and K^+, plotted *vs.* K_4, the same constant determined in the presence of the four cations Na^+, K^+, Ca^{++} and Mg^{++}. Each point corresponds to two experiments using the same sperm sample, and the dashed line represents the ideal correlation if K_2 were equal to K_4. All of the points are above the line and the actual ratio of the means of K_2 and K_4 is 1.6

cell sample and the same amount of BSA, the binding constant in the presence of two cations, K_2, was greater than the binding constant in the presence of the four, K_4. The averages, $K_2 = 11.8 \times 10^5\ M^{-1}$ and $K_4 = 7.2 \times 10^5\ M^{-1}$ are in the ratio of 1.6. If one calculates the ratio for each of the points in Fig. 3, the mean ratio is 1.9. It is clear that small amounts of Ca^{++} and Mg^{++} interfere with the binding of BSA to the sperm cells.

Discussion

The Adsorption Isotherm

The simple adsorption isotherm used to summarize our data, Eq. (2), appears to be quite adequate for describing what must be a very complicated process. The sperm cell surface is undoubtedly inhomogen-

eous both morphologically and chemically, so it is somewhat surprising that the simple expression holds for a wide range of cell concentrations and over several orders of magnitude BSA concentration.

Even the deviations of the K values from the solid line at lower values of albumin concentration, seen in Fig. 1 and 2, can be understood in terms of the simple adsorption isotherm. The deviation, which only occurred at the lowest BSA concentrations and for the densest cell suspensions studied, was undoubtedly due to the serious depletion of albumin in the aqueous solution as a result of binding. If we estimate the membrane area of a sperm cell as 200 μ^2 (assuming a smooth surface having the dimensions of the cell), a close packed albumin monolayer occupying 1 m^2/mg (Blank, Lee & Britten, 1973) on 10^7 cells/ml, would require all of the protein in a solution of 10^{-8} M. The actual membrane area is undoubtedly greater than the assumed value because of "roughness" at the molecular level. Also, the binding of the albumin to the membrane must involve attachment at some areas with the rest of the molecule remaining in solution. For these reasons, it appears that the departure of the dashed line in Figs. 1 and 2 from the correlation line occurs at a physically reasonable point and in line with our simple picture of the adsorption process.

The effects of small concentrations of Ca^{++} and Mg^{++} ions on the adsorption isotherm are in line with our expectations regarding the removal of adsorption sites seen in our electrode noise measurements (Blank *et al.*, 1974*b*). Ca^{++} was shown to be more effective than Mg^{++}, but both were effective at concentrations below 1 mM.

Our use of Eq. (2) for calculating the equilibrium binding constant was based mainly on the attempt to find the simplest possible expression to summarize the data. From our results, it appears that this choice is adequate for both unifying the results and demonstrating internal consistency. However, the convention of expressing binding in terms of a binding constant, formulated as in Eq. (2), may not always be the best way to present such data. In this case, particularly, the use of K obscures a rather dramatic conclusion that may help to explain some of the properties of the sperm cell membranes after they are coated with albumin. All the results of the binding studies, shown in Figs. 1 and 2, indicate that $K[A]$ is a constant (except for the range of low $[A]$ and high sperm concentration which has already been discussed). According to Eq. (2), this means that

$$\frac{[A:S]}{[S]}=1 \tag{3}$$

for virtually the entire range of $[A]$ concentrations studied. The sperm cells bind albumin so that half of the available surface is coated and half remains uncoated. It should be remembered that the effective albumin concentration for *in vitro* capacitation is about 10^{-4} M (Miyamoto & Chang, 1973), well above the concentration range of our experiments.

This situation is rather remarkable and it may indicate the important role that adsorption plays in the physical processes preceding fertilization. In purely physical systems, the optimal condition for the strong interaction between two particles that are coated with adsorbed macromolecular films occurs when half of the available surface is covered. This means that the molecules on the covered half of one particle can interact with the free spaces on the uncovered half of another particle and give rise to bridging and flocculation phenomena (LaMer & Healy, 1963). The sperm appears to provide the optimal situation for interacting with itself (which may be inhibited because of the presence of a large negative charge on the membrane) or with another surface.

The Rate of Adsorption

The experimental results summarized in Fig. 2 show that the adsorption of BSA at zero time after exposure to BSA (which is actually closer to 10–15 min because of the way in which the experiment is done) is as complete as after several hours. The adsorption rate is therefore fast in terms of these measurements and also in terms of the time scale of capacitation, which can require several hours. Our earlier study analyzing electrode noise indicated that adsorption on the sperm cell occurred upon contact with the electrode surface over a period of less than a second (Blank *et al.*, 1974*a*). The adsorption rate is therefore very rapid.

Our studies of the rate of adsorption of albumin at an air/water interface (Blank, Lee & Britten, 1975) show that the adsorption rate is greatest at the isoelectric point, and decreases when the molecule is charged. The rate also increases with the ionic strength. These results suggest that the adsorption rate of albumin at an interface, and probably on sperm cell membranes as well, is affected primarily by an electrical barrier. (This may explain why the adsorption of albumin on epididymal cells appears to be greater than on the more highly charged ejaculated cells, and why it is possible to capacitate epididymal cells *in vitro* quite readily.)

Physical Changes Following Adsorption

Our results indicate that BSA will bind to sperm cell membranes rapidly and cover about half the surface under conditions that approximate those of *in vitro* capacitation. The processes that follow adsorption and cause the changes that are characteristic of the acrosome reaction, may involve specific biochemical reactions. However, this is unlikely in view of the nonspecific binding of BSA to the sperm cells of several species. It would appear that physical mechanisms are more likely. Among these possibilities, we know that adsorption can lead to changes in:

1. The membrane permeability – altering the thickness, the surface charge or the partition relations of substances between the membrane and the aqueous phase. A change in permeability would cause changes in composition and volume of the sperm cell.

2. The mechanical properties of the membrane – by altering the elasticity and the yield strength of the structure. The binding of a macromolecule like the albumin to a membrane probably leads to the formation of intermolecular links (Blank, 1969) and changes in the mechanical properties of membranes (Blank, Lucassen & van den Tempel, 1970).

3. The reactivity of the membrane surfaces leading to adhesion or agglutination – by altering the surface layers so that half the surface is covered and half remains uncovered.

Our results suggest that the third factor may be particularly important. However, all of these changes are bound to occur to some extent and probably all contribute to the characteristic changes in membrane properties following capacitation.

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