PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 240-243 (1976)

Influence of Serum Albumin on the Fertilizing Ability *In Vitro* of Rat Spermatozoa (39182)

B. K. DAVIS

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

The use of albumin in media employed for fertilization of mammalian eggs was described by Yanagimachi (1), who found that the protein could partly replace heat detoxified serum in the capacitation of hamster spermatozoa. Chemically defined media containing albumin have since been described that capacitate sperm cells from rats, mice, and guinea pigs (2-4). Albumin has also been used in place of whole serum in mammalian cell cultures (5), where it is a source of essential fatty acids (6). The significance of serum albumin in promoting sperm fertilizing ability has not yet been established. As small amounts of cholesterol evidently bind to the protein during sperm capacitation (7), lipid exchange between the sperm plasma membrane and albumin is also possibly implicated in this process. The objective of the present investigation was to clarify the role of albumin in the capacitation of rat spermatozoa by determining (a) if these sperm cells are capacitated in the absence of albumin, (b) if other macromolecules can replace this protein in the medium employed, and (c) whether or not changes in albumin lipid content influence its ability to induce capacitation. This paper describes the effects of these treatments on fertilization rate and sperm motility.

Materials and methods. Sprague-Dawley rats, CD strain, were purchased from Charles River Breeding Laboratory for these experiments. Cauda epididymal spermatozoa from mature animals (body weight > 500 g) and eggs from superovulated 24- to 28-day females were employed in this work. The procedures adopted to achieve *in vitro* fertilization have been given previously (8), with the exception that the Krebs Ringer bicarbonate medium contained 3.4 m*M* Ca^{2+} and incubation lasted for 13 to 14 hr. After staining with 0.25% lacmoid, eggs undergoing fertilization revealed pronucleus formation, the tail of the fertilizing spermatozoa, and two polar bodies under microscopic examination (× 250).

Effects on the vigor of sperm motility were estimated from the degree of turbulence within suspensions of cauda epididymal spermatozoa. The suspensions contained approximately 3×10^6 sperm cells in 0.1 ml of medium, which was covered with a layer of paraffin oil during incubation at 37° in a humidified atmosphere of 5% CO_2 and 95% air. Following examination under a dissecting microscope (× 80), the number of turbulence swirls formed at the suspension edge was recorded. Turbulence appeared to yield a reproducible, quantitative index of sperm motility that conformed with subjective estimations.

Macromolecules added to the medium included crystalline bovine serum albumin (Sigma), cytochrome *c* from horse heart (Sigma), rabbit β-globulin (Nutritional Biochemicals), rabbit γ-globulin (Nutritional Biochemicals), beef blood hemoglobin (Sigma), porcine pancreatic ribonuclease (Sigma), crystalline egg white lysozyme (Sigma), crystalline ovalbumin (Sigma), fibrinogen from rabbit serum (Nutritional Biochemicals), and polyvinylpyrrolidone (PVP) with an average molecular weight of 40,000 (Sigma). Concentrated solutions of the macromolecules were dialyzed against Krebs Ringer bicarbonate buffer, pH 7.0, before use. Cholesterol saturated albumin was prepared by the method of Avigan (9). Albumin essentially depleted of fatty acids was purchased from Sigma, or prepared as described by Chen (10).

Results. Among 140 (70 + 70) eggs examined from immature, superovulated rats, a total of 114 (65 + 49) eggs (81%) were fertilized *in vitro* by epididymal spermatozoa in medium with 10 mg/ml of albumin (Table I). By contrast, none of 53 (11 + 42) eggs became fertilized in medium without albumin. When the medium contained 1

TABLE I. FERTILIZATION *In Vitro* OF RAT EGGS IN MEDIUM CONTAINING VARIOUS MACROMOLECULES.

Experiment Number	Macromolecule	Concentration (mg/ml)	Number of eggs examined	Eggs	
				Fertilized Number (%)	Polyspermic %
1, 2	Nil		11	0	0
	Albumin	10	70	65 (93)	5
	Ovalbumin	1	29	2 (7)	0
	Cytochrome *c*	10	21	0	0
	βG	10	23	0	0
	γG	10	27	0	0
	Hemoglobin	10	4	0	0
	Lysozyme	10	38	0	0
	PVP	10	22	0	0
	RNAase	5	9	0	0
3, 4	Nil		42	0	0
	Albumin	10	70	49 (70)	4
	Albumin + cholesterol	10	39	0	0
	Albumin − fatty acids	10	38	33 (87)	12

mg/ml of ovalbumin only a 7% (2/29) fertilization rate was obtained. Eggs that had been incubated with 10 mg/ml of ovalbumin fragmented and disintegrated during transfer from the medium. No fertilization occurred in the presence of 10 mg/ml of cytochrome *c*, β-globulin, γ-globulin, lysozyme, hemoglobin, and PVP, and 5 mg/ml of ribonuclease. Fatty acid free albumin was associated with a higher rate of fertilization than unmodified serum albumin in two experiments (87 vs 70%), and this difference approached statistical significance ($\chi_1^2 = 2.91$, $0.10 > P > 0.05$), whereas the use of albumin presaturated with cholesterol resulted in no fertilization (0/39).

Figure 1 indicates that high motility was maintained for over 2.5 hr by epididymal spermatozoa in suspensions (3×10^6 cells/0.1 ml) containing 10 mg/ml of cytochrome *c*, β-globulin, γ-globulin, and albumin. Motility appeared lower with hemoglobin and there were few turbulence swirls formed in sperm suspensions having ribonuclease, lysozyme, PVP, ovalbumin, and fibrinogen, all at a concentration of 10 mg/ml. While high motility was sustained in a suspension having no added macromolecule, addition of albumin, or cytochrome *c*, appeared necessary for vigorous motility when the spermatozoa concentration was lowered to between 0.5 and 1.0×10^6 cells/ml. Sperm concentrations of this level were employed for *in vitro* fertilization. Sperm motility was

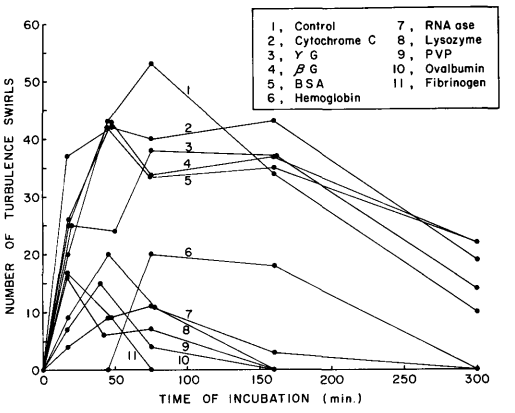

FIG. 1. Effect of various macromolecules on high sperm motility. The number of turbulence swirls created by motile sperm at the edge of these suspensions is shown for indicated times during incubation. γG, γ-globulin; βG, β-globulin; BSA, bovine serum albumin; RNAase, ribonuclease; and PVP, polyvinylpyrrolidone.

diminished in the presence of 30 μg of cholesterol/ml. Much longer periods of high motility than shown in Fig. 1 were observed after Ca^{2+} was reduced from 3.4 to 1.7 m*M* in the medium. However, lower fertilization rates were obtained at the lower Ca^{2+} concentration. Although turbulence decreased after 5 hr. (Fig. 1), spermatozoa in these suspensions showed good motility for at least 12 hr. Fertilization takes place after epididymal rat spermatozoa have been incubated in Krebs Ringer bicarbonate medium

for about 5 hr, which is after the period of highest sperm motility between 1 to 3 hr.

Discussion. High sperm motility and fertilization rates were observed only with medium containing albumin in these experiments using rat gametes. Some macromolecules (cytochrome *c*, β-globulin, γ-globulin) were associated with high sperm motility, but failed to allow fertilization. The results suggest that the action of albumin in enhancing sperm capacitation involves more than a nonspecific macromolecular effect. Since somewhat higher fertilization rates were achieved with defatted albumin than with unmodified albumin, it seems clear that the protein is not acting as a fatty acid source.

In medium containing 1 mg/ml of ovalbumin, the fertilization rate was 7% (2/29). By comparison, 4.3 to 37.6% mouse eggs were fertilized by epididymal spermatozoa using Krebs Ringer bicarbonate medium with 1 mg/ml of albumin (11). At this concentration, both proteins appear to be equally effective for capacitation of these rodent spermatozoa. Ovalbumin and albumin have comparably hydrophobic amino acid constitutions (12). This could be significant if protein interactions with the sperm cell plasma membrane play a role in capacitation. Apolar bond formation occurs during the interaction of albumin with membrane lipid (13, 14). Changes in the lipid phase of the plasma membrane could affect cell permeability, membrane-associated enzyme activity, and membrane fluidity (15). During capacitation, sperm cells apparently undergo modifications in these respects. On the other hand, cytochrome *c*, hemoglobin, and lysozyme also penetrate the membrane lipid bilayer (15), but no fertilization was obtained in media containing these proteins.

The absence of fertilization among eggs that had been incubated in medium having albumin presaturated with cholesterol indicates that albumin-bound lipid can influence the process of sperm capacitation. This result may be considered as evidence that albumin enhanced sperm-fertilizing capacity by removing lipid (cholesterol) from the cell membrane (7). Replacement of depleted lipid could account for the inhibition of fertilization by seminal plasma membrane vesicles (8, 16, 17). It would seem interesting to directly determine the possible changes in sperm cell plasma membrane produced by the conditions studied in the present experiments and by the membrane vesicles attributed with decapacitation activity.

Summary. Under defined conditions, in the presence of 10 mg/ml of bovine serum albumin, cauda epididymal rat spermatozoa displayed vigorous motility, and a high proportion (81%) of eggs were fertilized. In contrast, no fertilization was observed after omission of albumin, or replacement of the protein by 10 mg/ml of cytochrome *c*, β-globulin, γ-globulin, hemoglobin, lysozyme, and polyvinylpyrrolidone, and 5 mg/ml of ribonuclease. However, high motility occurred in suspensions containing 3×10^6 spermatozoa/0.1 ml of medium with cytochrome *c*, β-globulin, or γ-globulin. In medium with 1 mg/ml of ovalbumin, 7% (2/29) eggs were fertilized. Use of defatted albumin resulted in a higher rate of fertilization than unmodified albumin (87 vs 70%), and this difference approached statistical significance. No fertilization was obtained in the presence of albumin presaturated with cholesterol. These results suggest that: (a) rat sperm cells failed to capacitate in the absence of albumin; (b) the protein exerted more than a nonspecific macromolecular effect; and (c) lipids associated with albumin may modify its ability to promote sperm capacitation.

This work was financially supported by N.I.H. Contract No. 1 HD-3-2781.

1. Yanagimachi, R., Biol. Reprod. **3,** 147 (1970).
2. Toyoda, Y., and Chang, M. C., J. Reprod. Fert. **36,** 9 (1974).
3. Toyoda, Y., Yokoyama, M., and Hosi, T., Jap. J. Anim. Reprod. **16,** 147 (1971).
4. Yanagimachi, R., and Usui, N., Exp. Cell Res. **89,** 161 (1974).
5. Fisher, H. W., Puck, T. T., and Sato, G., J. Exp. Med. **109,** 649 (1959).
6. Yamane, I., Murakami, O., and Kato, M., Proc. Soc. Exp. Biol. Med. **149,** 439 (1975).
7. Davis, B. K., Seventh Ann. Meeting Soc. Study Reprod., Abs. 62, p. 74 (1974).
8. Davis, B. K., and Niwa, K., Proc. Soc. Exp. Biol. Med. **146,** 11 (1974).
9. Avigan, J., J. Biol. Chem. **234,** 787 (1959).
10. Chen, R. F., J. Biol. Chem. **242,** 173 (1967).
11. Miyamoto, H., and Chang, M. C., J. Reprod. Fert.

32, 193 (1973).
12. Bigelow, C. C., J. Theoret. Biol. **16,** 187 (1967).
13. Sweet, C., and Zull, J. E., Biochim. Biophys. Acta **173,** 94 (1969).
14. Dark, A., Finer, E. G., Flook, A. G., and Phillips, M. C., J. Mol. Biol. **63,** 265 (1972).
15. Papahadjopoulos, D., and Kimelberg, H. K., Prog. Surface Sci. **4,** 141 (1973).
16. Davis, B. K., Experientia **29,** 1484 (1973).
17. Davis, B. K., J. Reprod. Fert. **41,** 241 (1974).

Received March 15, 1975. P.S.E.B.M. 1976, Vol. 151.