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Influence of seminal plasma proteins on motility of rabbit spermatozoa*

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Summary. Washed rabbit spermatozoa of freshly collected ejaculates were incubated in different fractions of seminal plasma proteins and examined for motility. Incubation media were protein fractions obtained by gel and ion-exchange chromatography. Motility was best maintained in the fractions which contained albumin, and rabbit serum albumin was more effective than bovine or human serum albumin. Concentrations of <4 mg rabbit serum albumin/ml resulted in markedly decreased motility.

Introduction

Seminal plasma is important for the motility and function of spermatozoa. Apart from some non-protein components, such as glucose, fructose and pyruvate (Murdoch & White, 1966), serum albumin is known to influence capacitation of mouse spermatozoa (Miyamoto & Chang, 1973), ion-binding systems of human spermatozoa (Lindholmer, 1974a, b; Linholmer & Eliasson, 1974) and adsorption on rabbit sperm membranes (Blank, Soo & Britten, 1976). It also appears to promote the survival of spermatozoa in man (Eliasson, 1971; Lindholmer, 1974a, b), in bull (Breddermann & Foote, 1971) and in some other species, including rabbit (Emmens & Swyer, 1948; Harrison, Dott & Foster, 1978). Albumin is known to be present in the seminal plasma of the rabbit (Kirchner & Schroer, 1976), but no studies have yet been performed to determine whether rabbit albumin also promotes sperm motility and, if so, whether other proteins with the same capabilities are also present.

In this study seminal plasma proteins were fractionated by gel and ion-exchange chromatography, and they and albumins from different species were examined for their effect on sperm motility.

Materials and Methods

Semen was collected repeatedly from 12 rabbits of the New Zealand White and Alaska strains by means of an artificial vagina. The samples were centrifuged at 12 000 *g* (4°C, 15 min) to remove all coarse particles and obtain seminal plasma. If the seminal plasma was not used immediately it was precipitated and stored in saturated ammonium sulphate solution. Osmotic values were determined with an osmometer at room temperature against a NaCl solution of 400 mosmol/kg. The fresh or stored seminal plasma was dialysed against 0·9% (w/v) NaCl solution and 1 ml was subjected to gel chromatography on Sephadex G-150 or G-75 SF (column size: 1·5 × 100 cm; flow rate: 8·0 ml/h; fraction size: 4·5 ml) with a 0·34 M-Tris–citric acid buffer (pH 7·1, 300–310 mosmol/kg) as the eluant. For ion-exchange chromatography the proteins of the second peak of the G-75 SF fractionation were concentrated in collodium bags (Sartorius, Membran Filter Gesellschaft, Göttingen) to a volume of 0·5 ml and passed through DEAE cellulose (Whatman DE-32) with a linear gradient from 0 to 0·5 M-NaCl in 0·1 M-Tris–HCl buffer, pH 8·0 (column size: 1 × 10 cm; flow rate: 8·0 ml/h; fraction size: 10 ml). All fractions were dialysed against the Tris–citric acid buffer.

For motility tests, rabbit spermatozoa were washed twice (White, 1953) as follows. Freshly

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collected ejaculates were diluted 1 : 1 with a washing medium containing 333 mM-Tris–citric acid buffer, pH 7·1, and 6·5 mM-glucose (300–310 mosmol/kg) and centrifuged at 37°C for 10 min at 280 *g*. The supernatant was discarded. The residual protein after washing was determined photometrically; extinction at 280 nm yielded contents of less than 0·002 mg protein/ml and we considered this to be sufficient removal of protein for our studies.

In the first series of experiments equal parts of washed spermatozoa were incubated under aerobic conditions at 37°C in equal volumes of solutions containing the protein fractions from the Sephadex G-150, G-75 SF and DEAE cellulose columns, whole seminal plasma or buffer only (no protein). The proteins in the fractions were each concentrated to a volume of 1 ml and thus the proteins in the fractions were represented at levels relative to their levels in seminal plasma. In the second series of experiments spermatozoa were incubated under the same conditions in a buffer solution containing bovine or human serum albumin (at 5 mg/ml). Rabbit serum albumin was isolated by fractionation of rabbit serum on Sephadex G-150 (first step) and ion-exchange chromatography of the third peak on DEAE cellulose (Whatman DE-32) with a linear gradient from 0 to 0·5 M-NaCl in 0·1 M-Tris–HCl buffer, pH 8·0 (second step) (Gelotte, Flodin & Killander, 1962). Bovine and human serum albumin were obtained from Behringwerke (Marburg) and were dated to the end of January 1977. In the third experimental series, rabbit spermatozoa were incubated in solutions containing different concentrations (0–30 mg/ml) of rabbit serum ablumin.

The motility of the spermatozoa (1–3 × 10^7 cells) in each incubation medium was examined over a period of 4 h, always by the same observer who was unaware of the identity of the incubation media in the first and the second experimental series. Thin smears of spermatozoa were inspected on a warmed microscope table (37°C) at a magnification of ×1000 and motile and non-motile cells were counted in at least 5 fields. The percentages of motile and non-motile spermatozoa were calculated every hour for each medium and are the average values from the 5 different fields. The significance of the differences was assessed by using Student's *t* test (Graf, Henning & Stange, 1966).

Gel electrophoresis (Davis, 1964) was conducted as described by Kirchner & Schroer (1967). Proteins were stained with Amido Black 10B. Immune electrophoresis was performed with 1·25% agar gel (Scheidegger, 1955). Fresh seminal plasma and its fractions from gel and ion-exchange chromatography were used as antigens. Sheep antiserum against whole seminal plasma (Kirchner & Schroer, 1976), goat antiserum against rabbit serum (Behringwerke, Marburg) and monospecific guinea-pig antiserum against rabbit serum albumin were used as sources of antibodies. Proteins were stained with Coomassie Brilliant Blue R 250. The total albumin concentration in rabbit seminal plasma was determined by electroimmunoassay (Laurell, 1972). Total protein concentrations were determined photometrically at 280 nm.

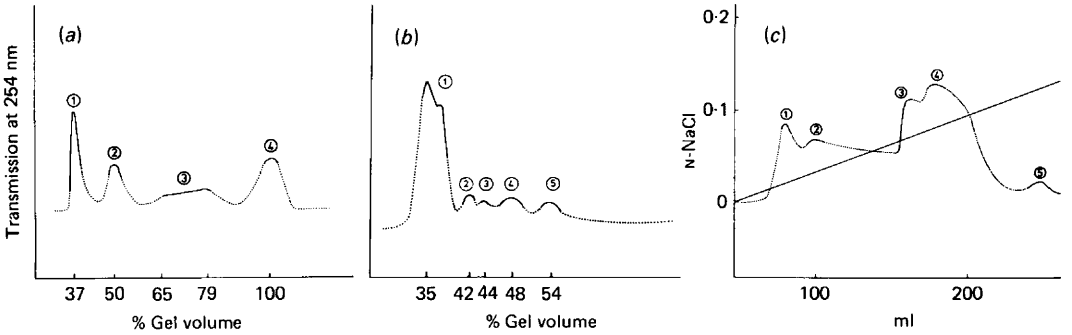
Results

Promotion of sperm motility by seminal plasma proteins

Rabbit seminal plasma ($n = 50$) had an average pH of 7·1 (range 6·9–8·2) and a mean osmolarity of 300 mosmol/kg (range 273–328 mosmol/kg).

Gel chromatography of fresh and ammonium sulphate-stored seminal plasma on Sephadex G-150 and G-75 SF yielded 4 and 5 peaks respectively. Rechromatography of the second peak from the G-75 SF chromatogram on DEAE cellulose yielded 5 peaks (Text-fig. 1a, 1b, 1c). The resulting fractions were used directly as incubation media because their pH and osmolarity were identical to those of fresh seminal plasma.

Incubations of washed spermatozoa in protein fractions containing these selected peaks, whole seminal plasma or buffer only showed that the most motile spermatozoa were those incubated in the third fraction of the G-150 chromatogram, the second fraction of the G-75 SF chromatogram and in the fourth fraction of the DEAE cellulose chromatogram (Table 1). Single protein fractions promoted motility better than the buffer alone but were worse than whole seminal plasma (see Table 1). Many of the differences between the protein fractions were significant ($P < 0·05$).



Text-fig. 1. Gel chromatogram of rabbit seminal plasma (a) on Sephadex G-150 and (b) Sephadex G-75 SF, and (c) an ion-exchange chromatogram on DEAE cellulose of the second peak of the G-75 SF fractionation. The diagonal line in (c) represents the linear gradient of NaCl/ml eluant.

Table 1. The percentage (mean ± s.d.) of motile spermatozoa (washed and from 5 rabbits) incubated in medium containing various protein fractions of seminal plasma, in whole seminal plasma and in buffer alone

Medium	Incubation time (h)				
	0	1	2	3	4
Whole seminal plasma	88·7 ± 3·8	87·3 ± 5·9	82·5 ± 4·9	80·2 ± 6·9	68·9 ± 9·1
Buffer alone	78·8 ± 5·9	41·3 ± 9·0	28·7 ± 9·3	24·2 ± 11·3	18·4 ± 9·9
G-150 Chromatogram					
Fraction 1	78·7 ± 5·8	77·5 ± 6·0*	69·0 ± 9·1*	42·1 ± 12·3*	25·9 ± 12·5*
2	82·3 ± 3·5	79·1 ± 6·9*	69·8 ± 11·3*	52·7 ± 7·1*	31·1 ± 9·9*
3†	86·5 ± 6·0	84·5 ± 6·0	73·5 ± 12·5	68·7 ± 8·0	52·0 ± 9·0
4	83·9 ± 5·9	80·2 ± 6·1	70·1 ± 10·9	37·9 ± 12·8*	29·4 ± 14·9*
G-75 SF Chromatogram					
Fraction 1	82·7 ± 4·9	77·8 ± 9·6*	71·7 ± 8·2*	58·0 ± 9·8*	42·5 ± 6·7*
2†	86·0 ± 5·5	82·2 ± 7·1	76·7 ± 7·3	72·0 ± 6·5	50·5 ± 10·0
3	83·6 ± 7·9	76·1 ± 9·1*	70·9 ± 6·8*	62·4 ± 7·9*	47·2 ± 9·3*
4	81·7 ± 5·6	73·1 ± 8·9*	68·1 ± 7·9*	52·5 ± 7·9*	40·9 ± 8·7*
5	80·7 ± 5·9	70·7 ± 9·9*	56·6 ± 9·8*	39·8 ± 9·8*	31·2 ± 9·6*
DEAE Chromatogram					
Fraction 1	84·9 ± 7·8	70·4 ± 3·6*	61·6 ± 8·9*	25·7 ± 10·0*	44·2 ± 7·8*
2	86·5 ± 7·0	49·4 ± 9·2*	27·9 ± 10·1*	21·3 ± 8·1*	14·7 ± 8·8*
3	82·0 ± 7·2	48·0 ± 9·7*	46·5 ± 9·8*	39·3 ± 6·7*	35·6 ± 9·1*
4†	88·0 ± 6·5	79·5 ± 8·0	71·7 ± 8·5	58·5 ± 9·5	53·0 ± 9·5
5	84·8 ± 8·2	64·2 ± 7·9*	54·0 ± 6·9*	44·7 ± 9·3*	32·6 ± 8·8*

* Values significantly different from those of the best (†) protein fraction of each fractionation at 1, 2, 3 and 4 h; $P < 0{\cdot}05$.

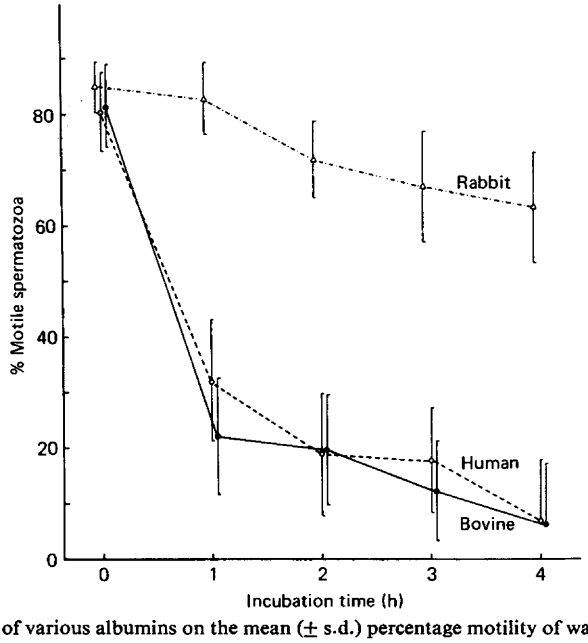
Disc and immune electrophoresis of the seminal plasma fractions showed that albumin was present in all the fractions in which sperm motility was best maintained. Fraction 4 from the ion-exchange chromatogram contained electrophoretically and immunologically pure albumin: a single and identical precipitate was obtained with the sheep antiserum against full seminal plasma and the goat antiserum against full rabbit serum and it was further identified with monospecific antiserum of guinea-pig against rabbit serum albumin. The migration value of rabbit seminal plasma albumin in gel electrophoresis was identical to that of rabbit serum albumin.

Species specificity of rabbit albumin for promotion of sperm motility and effects of different concentrations

Spermatozoa retained their motility better in a rabbit serum solution than in bovine and human serum albumin (Text-fig. 2). Incubations of spermatozoa with different concentrations of rabbit serum

albumin showed that media containing 4–30 mg/ml had no significant effect on sperm motility but there was a striking decrease in motility in media containing <4 mg serum albumin/ml (Table 2). Motility was lost rapidly in medium which contained no albumin (see also Table 1).

The albumin level in normal rabbit seminal plasma (N = 5) was 2·14 mg/ml (range 1·70–2·74 mg/ml), i.e. 6·1 % of the total protein per ml.



Text-fig. 2. Effects of various albumins on the mean (± s.d.) percentage motility of washed rabbit spermatozoa after incubation for 4 h. There were 5 samples at each point. Values with bovine and human serum albumin were significantly different from those with rabbit serum albumin at all times ($P < 0{\cdot}05$).

Table 2. The percentage (mean ± s.d.) of motile spermatozoa (washed and from 5 rabbits) incubated in medium containing various concentrations of rabbit serum albumin

Serum albumin conc. (mg/ml)	Incubation time (h)				
	0	1	2	3	4
0	79·5 ± 3·6	28·2 ± 10·5*	12·0 ± 5·4*	11·3 ± 6·3*	9·1 ± 7·4*
1	80·1 ± 4·0	57·9 ± 7·7*	54·4 ± 9·8*	41·0 ± 6·3*	37·1 ± 7·9*
2	80·7 ± 3·7	60·0 ± 7·2*	58·3 ± 8·1*	54·2 ± 5·4*	52·4 ± 10·8†
3	79·8 ± 3·9	69·1 ± 6·3*	65·1 ± 6·8*	60·7 ± 4·1*	55·3 ± 7·1*
4	82·1 ± 3·6	78·8 ± 1·4	77·1 ± 4·0	74·0 ± 1·0	78·6 ± 0·8
5	84·6 ± 4·0	79·4 ± 4·5	73·5 ± 5·7*	74·1 ± 8·3	80·1 ± 2·8
10	84·2 ± 4·1	71·7 ± 4·1*	69·8 ± 6·5*	78·4 ± 2·3	75·7 ± 12·8
15	82·6 ± 3·7	79·5 ± 8·4	77·5 ± 8·9	72·6 ± 3·0	80·6 ± 2·5
20	83·0 ± 3·0	81·4 ± 2·7	80·3 ± 3·1	78·8 ± 6·4	78·8 ± 0·1
25	85·9 ± 5·1	80·7 ± 6·6	82·3 ± 1·0	77·8 ± 4·9	82·9 ± 1·8
30	83·4 ± 4·3	79·0 ± 10·0	78·4 ± 7·8	79·6 ± 1·3	81·8 ± 4·5

* Values significantly different from those at 0 h, $P < 0{\cdot}05$.

Discussion

The results of gel chromatography of rabbit seminal plasma on Sephadex G-150 were similar to those obtained by Yantorno, Vides & Vottero-Cima (1972) on Sephadex G-200, and by Zappi, Smith & Shulman (1972), who compared the filtration capabilities of G-50, G-100 and G-200. Gel filtration on G-75 SF with redissolved ammonium sulphate precipitates of seminal plasma yielded a result identical to that of Kirchner & Schroer (1976) except for a final peak containing amino acids and small peptides. Examination of proteins from the G-75 SF column by DEAE cellulose ion-exchange chromatography has not been reported in the literature.

We performed the washing process only twice, because we considered this to be sufficient for removal of most of the protein and that additional washings and centrifugation, however gentle, would affect sperm motility adversely (White, 1953). By using a completely unphysiological washing medium, i.e. buffer, we tried to test only one physiological component, i.e. protein. The presence of glucose in the washing medium prevented a decrease in motility during the washing procedure because it is an energy source for rabbit spermatozoa (Murdoch & White, 1966). In contrast, the only natural component of seminal plasma present in the test medium was citric acid, thus ensuring that it was the influence of the proteins only which was tested. In all incubations one fraction was found to be the most effective in maintaining sperm motility and electrophoretic and immunological studies showed that albumin was the only component common to all fractions which maintained motility. The fact that it was the albumin itself which was having this effect was demonstrated by the results with the fourth fraction from the ion-exchange chromatogram, which consisted of pure albumin and was identical to rabbit serum albumin. The effects on sperm motility for this fraction were almost identical to those obtained with 2 mg rabbit serum albumin/ml (see Tables 1 and 2). Sperm density and motility can vary considerably in ejaculates collected, even from the same animals on succeeding days, and this could account for the higher standard deviation obtained in the present experiments (see also White, 1953; Harrison *et al.*, 1978).

Harrison *et al.* (1978) reported that there was no difference in the beneficial effects on rabbit spermatozoa of fatty acid-associated and defatted bovine and human serum albumin. Our results with pure bovine and human serum albumin from Behringwerke (Marburg) indicated no maintenance of sperm motility. Since the latest available bovine and human serum albumin samples (stored at 4°C) were 6 months older than the rabbit serum albumin before use, the storage of the human and bovine albumins could be one reason for their reduced effect (Pickart & Thaler, 1977). Moreover, we cannot exclude a species specificity of rabbit serum albumin which could be due to specific binding of the albumin to the sperm membrane.

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