

## FRACTIONATION OF HAMSTER SPERM-CAPACITATING COMPONENTS FROM HUMAN SERUM BY GEL FILTRATION

D. B. MORTON AND B. D. BAVISTER\*

*Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN, and  
Physiological Laboratory, Downing Street, Cambridge CB2 3EG*

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Efficient capacitation of hamster spermatozoa can be induced by follicular fluid (Barros & Austin, 1967; Yanagimachi, 1969a, b) and blood sera of several species (Barros & Garavagno, 1970; Yanagimachi, 1970a; Talbot, Franklin & Fussell, 1974). Two components are involved: one is dialysable, heat-stable and stimulates sperm motility, while the other is non-dialysable, heat-labile and induces the acrosome reaction (Yanagimachi, 1969a). Our understanding of capacitation and the acrosome reaction might be enhanced if the nature and mode of action of these factors were known. Blood serum is an easily available source of both factors and this preliminary report describes the recovery of the sperm motility-stimulating activity from human serum.

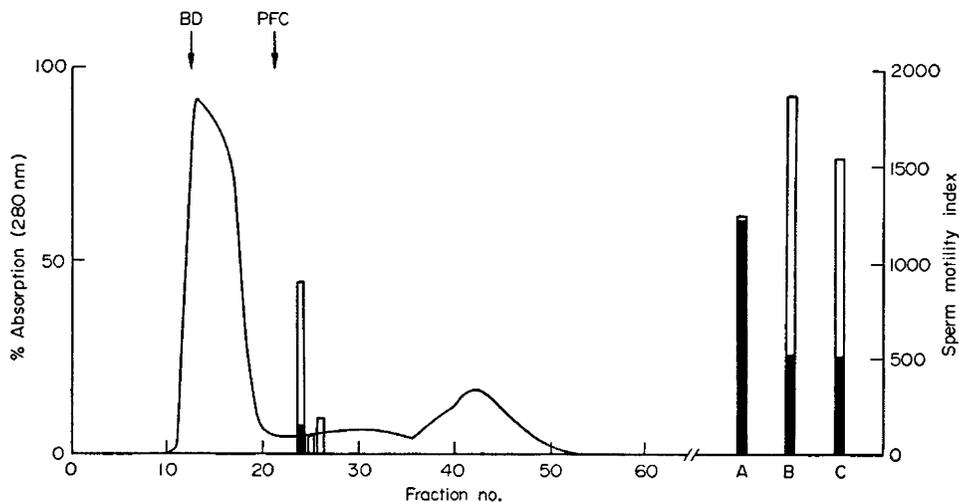
Fresh human serum was heated to 56°C for 30 min to destroy unidentified toxic factor(s) (Yanagimachi, 1970a). The serum was chromatographed on Sephadex G-25 (medium grade) and equilibrated in Tyrode's solution supplemented with 0.33 mM-sodium pyruvate, 0.01 mg phenol red/ml, 100 i.u. penicillin/ml and 10 mg Dextran T70 (Pharmacia)/ml. Equilibration in culture medium avoided further dilution of the fractions in the test system. Protein elution was monitored with a L.K.B. Uvicord II measuring absorption at 280 nm and columns were pumped under positive pressure at a flow rate of about 50 ml/cm<sup>2</sup>/hr. Column calibration was carried out with Blue Dextran (Pharmacia) and potassium ferricyanide. The fraction size was approximately 5% bed volume.

In order to test the fractions, 200- $\mu$ l aliquots were placed under paraffin oil in plastic tissue culture dishes (Falcon Plastics) and equilibrated with 5% CO<sub>2</sub> in air at 37°C. Hamster epididymal spermatozoa were added to the drops so that the final sperm concentration was about  $1 \times 10^6$ /ml, and the drops were then reincubated. At intervals between 1 and 6 hr, the percentage of motile spermatozoa and the degree of sperm motility in each drop were estimated, and a 'sperm motility index' was calculated as previously described (Bavister, 1974). The proportion of motile spermatozoa showing the acrosome reaction was also noted at 3 and 6 hr.

The 'sperm motility-stimulating activity' was constantly found to elute

\* Present address: Department of Anatomy, University of Hawaii School of Medicine, 1960 East West Road, Honolulu, Hawaii 96822, U.S.A.

between 110 and 115% (Fraction 24) of the elution volume of potassium ferricyanide (see Text-fig. 1). The range of strongly motile spermatozoa after incubation in this fraction for 3 and 6 hr was 60 to 70% and 10 to 20%, respectively; less than 3% of the motile spermatozoa showed an acrosome reaction. After incubation for 3 hr, approximately 25% of the motile spermatozoa showed the vigorous 'whiplash' motility (activation) that is characteristic of capacitated hamster spermatozoa (Yanagimachi, 1970b). In the other column fractions, neither motility-stimulating nor acrosome-reaction-inducing activities were found (up to 6 hr incubation).



TEXT-FIG. 1. Fractionation of sperm motility-stimulating activity in human serum on Sephadex G-25. Absorption at 280 nm is shown by the Uvicord trace. Peak fractions of Blue Dextran (BD) and potassium ferricyanide (PFC) from a separate calibration run are arrowed. The sperm motility index for spermatozoa incubated with unfractionated serum diluted 1/10 in culture medium (A), with Fractions 13 and 24 combined (B) and with Fractions 14 and 24 combined (C), are shown. The vertical bars represent the SMI for spermatozoa incubated for 3 hr (open bars) and 6 hr (solid bars). All the fractions from 1 to 60 were tested; absence of a vertical bar means zero activity.

A second experiment was conducted to examine the effect of recombining the separated serum proteins with the fraction containing motility-stimulating activity (Fraction 24). An aliquot from either Fraction 13 or 14 (both protein-rich) was mixed with an equal volume of Fraction 24 and spermatozoa were added as previously described. The presence of serum proteins increased the sperm motility-stimulating activity of Fraction 24 approximately twofold after 3 hr and fivefold after 6 hr (Text-fig. 1, B and C). After incubation for 3 hr, 70 to 80% of the spermatozoa were strongly motile; between 50 and 100% of these were showing the typical 'activation' type of motility. In addition, 50% of the motile spermatozoa examined after 3 hr showed an acrosome reaction and this proportion increased to 90% after 6 hr (50 to 60% motile). Comparable values were obtained using diluted unfractionated serum (Text-fig. 1, A).

To check the heat stability of the motility-stimulating activity, an aliquot of Fraction 24 was immersed in boiling water for 15 min, cooled, and then tested as before. There was no reduction in the sperm motility index.

The partly purified motility-stimulating activity obtained from human serum by gel filtration was considerably reduced compared with unfractionated serum. This may be due in part to the inevitable dilution incurred during chromatography, but other serum factors may also contribute to sperm motility-stimulating activity. This activity was greatly enhanced by the protein-rich peak and in addition, the acrosome reaction was induced effectively only in the presence of this peak. The motility-stimulating activity recovered by gel filtration of serum is heat-stable, and its position on the Sephadex column indicates that the active factor should be dialysable. These observations are compatible with those of Yanagimachi (1969a), who studied the properties of the motility-stimulating activity obtained from bovine follicular fluid by dialysis; similar components are probably involved. The retardation of the motility-stimulating activity on the column compared with the low molecular weight marker is interesting and suggests that the active factor might be a molecule with aromatic groupings or a high isoelectric point.

Our results indicate that the gel filtration fractions containing sperm motility-stimulating activity, and the protein-rich fraction, together contain the components of human serum that are involved in sperm capacitation. Gel filtration appears to be a satisfactory method for isolating and partly purifying the sperm motility factor(s): it has the advantages of being a simple reproducible procedure and large quantities of serum may be processed if desired.

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