Absence of a Sperm Coating Protein after Epididymovasostomy

Report of One Case

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Studies in animals indicate that epididymal sperm maturation requires the secretion of an epididymal protein that binds to the sperm surface. The possibility that this also occurs in man has been explored in the present study of one patient who was treated for bilateral obstruction of the cauda epididymis by epididymovasostomy. The patient's ejaculates obtained up to 18 months after surgery contained immotile sperm. Analysis of proteins in the seminal plasma revealed that the semen was deficient in a 38,000 dalton protein found in seminal plasma of normal fertile men and in epididymal cytosol of patients with carcinoma of the prostate. This 38,000 dalton protein also was found in sperm of normal men, but was absent in sperm of the patient. This observation supports the possibility that an epididymal protein coats the sperm surface during epididymal transit in man as well as in numerous other species.

Key words: human epididymis, specific proteins, sperm motility, epididymovasostomy

Testicular spermatozoa are immature and immotile, and it is during transit through the epididymis that they acquire motility and the capacity for fertilization (Bedford, 1975). This phenomenon is androgen dependent and is mediated by factors secreted by the epididymis (Orgebin-Crist et al, 1975). Micropuncture studies have revealed a high concentration of carnitine, glycerophosphocholine, sialic acid, and inositol in epididymal fluid (Hinton, 1980). As yet, however, the definite roles of these organic compounds in sperm maturation have not been established.

Recently, work in several species has provided evidence that the epididymis secretes specific proteins into the lumen of the duct (see discussion). As spermatozoa pass through the epididymis they acquire these proteins on their surface (Oliphant and Singhas, 1979; Olson and Hamilton, 1978; Wong et al, 1981). This alters their surface membrane properties (Bedford, 1963) and eventually leads to maturation and development of motility. Although there is evidence that sperm maturation in the human also takes place in the epididymis, there is a paucity of information on the secretion of specific proteins by the human epididymis. If epididymovasostomy is performed with the vas sutured to the proximal 7 mm of the epididymis, the ejaculated sperm are immotile. However, if the anastomosis is distal to the initial 8 mm of the epididymis, the ejaculated sperm are both motile and fertile (Turner and Howards, 1976). Bedford et al (1973) found that the capacity for progressive motility increases as sperm pass through the human epididymis. Furthermore, Hinrichsen and Blaquier (1980) found that, in the human, only sperm removed from the cauda epididymidis are capable of penetrating zona-free hamster oocytes. These observations support the concept of epididymal maturation of human sperm. In this article we present evidence that a seminal plasma protein present on the surface of normal sperm was absent from the sperm surface in a patient studied 18 months after epididymovasostomy.

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Methods

Patient

Unilateral epididymovasostomy was performed in a 23-year-old man with bilateral obstruction of the vas

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deferens near its junction with the cauda epididymidis. The bilateral obstruction demonstrated by vasogram during surgery was probably caused by previous disease. Prior to surgery, seminal fluid analysis repeatedly demonstrated azoospermia. During surgery, the right vas was anastomosed to the initial 8 to 10 mm of the caput epididymidis. Three months after the operation, immotile sperm appeared in the patient's ejaculates. The following study was undertaken 18 months after surgery.

SDS Gel Electrophoresis of Seminal Plasma

Four samples of seminal plasma obtained from the patient over a period of eight months and 2 samples from each of three healthy men with proven fertility were subjected to SDS-gel electrophoresis. Aliquots of seminal plasma, each containing 50 μg protein, were mixed with equal volumes of sample buffer (final concentrations: 5mM Tris HCl, *p*H 8.0; 0.5 mM EDTA; 0.5% SDS; 1% β-mercaptoethanol; 0.0025% bromophenol, and 10% sucrose), and were heated at 100 C for 10 minutes. Electrophoresis was performed in 0.1% SDS polyacrylamide gel slabs (2 mm thick with 7.5 cm length of 10% separating gel and 1 cm length of 4% stacking gel) (Laemmli, 1970). Immediately after electophoresis, the gels were fixed-stained overnight with 0.25% Coomassie Blue in 50% methanol and 7% acetic acid and destained with the same solvent.

Radioiodination of Sperm Surface Proteins

The surface proteins of spermatozoa from each semen sample were labeled with radioactive iodine using the lactoperoxidase technique (Philips and Morrison, 1971). This technique has been used successfully for iodination of surface proteins of ram (Voglmayr et al, 1980), rabbit (Oliphant and Singhas, 1979) and rat spermatozoa (Wong et al, 1981). Spermatozoa were washed three times with buffer containing (mM): NaCl (61.9), KCl (4.82), $CaCl_2$ (2.25), $MgSO_4$ (1.17), KH_2PO_4 (1.19), Tris (50; *p*H = 7.2), and glucose (5.55) by centrifugation at 800 × g for 2 minutes. Washed sperm were adjusted to a concentration of 2.75×10^6 per ml. The reaction mixture contained 1.1×10^6 sperm (0.4 ml), 10^{-5}M-KI (20 μl), 5 μCi-Na ^{125}I (10 μl) and 1 μg-lactoperoxidase (20 μl). The reaction was initiated by the addition of 50 μM-H_2O_2 (20 μl) at 5 minutes and 10 minutes. At 15 minutes, the reaction mixture was centrifuged at 6000 × g for 30 seconds. Spermatozoa were washed two times by centrifugation and resuspended in 1.5 ml buffer to remove free iodine. The ^{125}I-labeled spermatozoa were solubilized in 1% SDS, 1% mercaptoethanol and subjected to SDS-gel electrophoresis. At the end of the electrophoresis, gels were sliced into 1 mm sections and the radioactivity of the slices was determined using a gamma counter (LKB Rack Gamma).

Preparation of Human Epididymal Cytosol

Epididymides were obtained during orchidectomy in two patients with carcinoma of the prostate. They were dissected free of fat and connective tissues. Only the caput epididymides were homogenized in 10 ml saline. Cytosol was obtained following centrifugation for 20 minutes at 12,000 × g using Sorvall RC 5 centrifuge.

Binding of Protein A to the Patient's Sperm

Spermatozoa from four different samples of the patient's ejaculate were washed three times in phosphate buffered saline (PBS; *p*H 7.2) by centrifugation at 800 × g. The washed sperm were then incubated in seminal plasma from normal men for 30 minutes at 33 C. Previous work in the rat has shown that binding of an epididymal protein to rat epididymal sperm reaches saturation after 30 minutes (Wong and Tsang, unpublished observations). The sperm concentration was adjusted to 2.75×10^6/ml. At the end of the incubation period, spermatozoa were washed three times by centrifugation in PBS and then immediately subjected to surface protein labeling by the method described above.

Results

Eighteen months after surgery, the patient's sperm count was $66 \pm 47 \times 10^6$/ml (mean ± SE, n = 20; range 11 to 170×10^6/ml). The percentage of oval forms was 60 ± 6% (means ± SE, n = 20; range 50 to 75), but the sperm were immotile.

The protein patterns of one specimen of seminal plasma from the patient and a specimen from one of the normal controls are shown in Figs. 1A and 1B. Twenty bands were identified in the seminal plasma of fertile men (Fig. 1A, the results for all three normal men were similar) and 19 bands in the seminal plasma of the patient (Fig. 1B, similar results were obtained from the four different samples of seminal plasma from the patient). Band A (MW 38,000) was absent from the ejaculates of the patient. This difference in the number of bands may be attributed to the absence of epididymal contribution to the seminal proteins in the patient's ejaculate. In other experiments, histologically normal epididymides (obtained during orchidectomy of two patients with carcinoma of the prostate) were homogenized and the protein pattern studied as described. It was found that the normal epididymal tissue contained a protein that corresponded in electrophoretic mobility to band A in the seminal plasma of normal men (Fig. 1C). Comparison of the seminal plasmas of the patient and of normal men suggested additional differences between these samples. For example, band B (MW 45,000) appeared to be more intense in the seminal plasma of the patient, while the 20,000 dalton and the 94,000 dalton proteins were reduced or absent (Fig. 1).

To determine whether the epididymal protein (band A, Fig. 1A) interacted with spermatozoa, the surface proteins of *immotile* sperm from four different samples of the patient's ejaculate and motile sperm from three normal men were labeled with radioactive iodine, solubilized in SDS, and then subjected to SDS gel electrophoresis. The

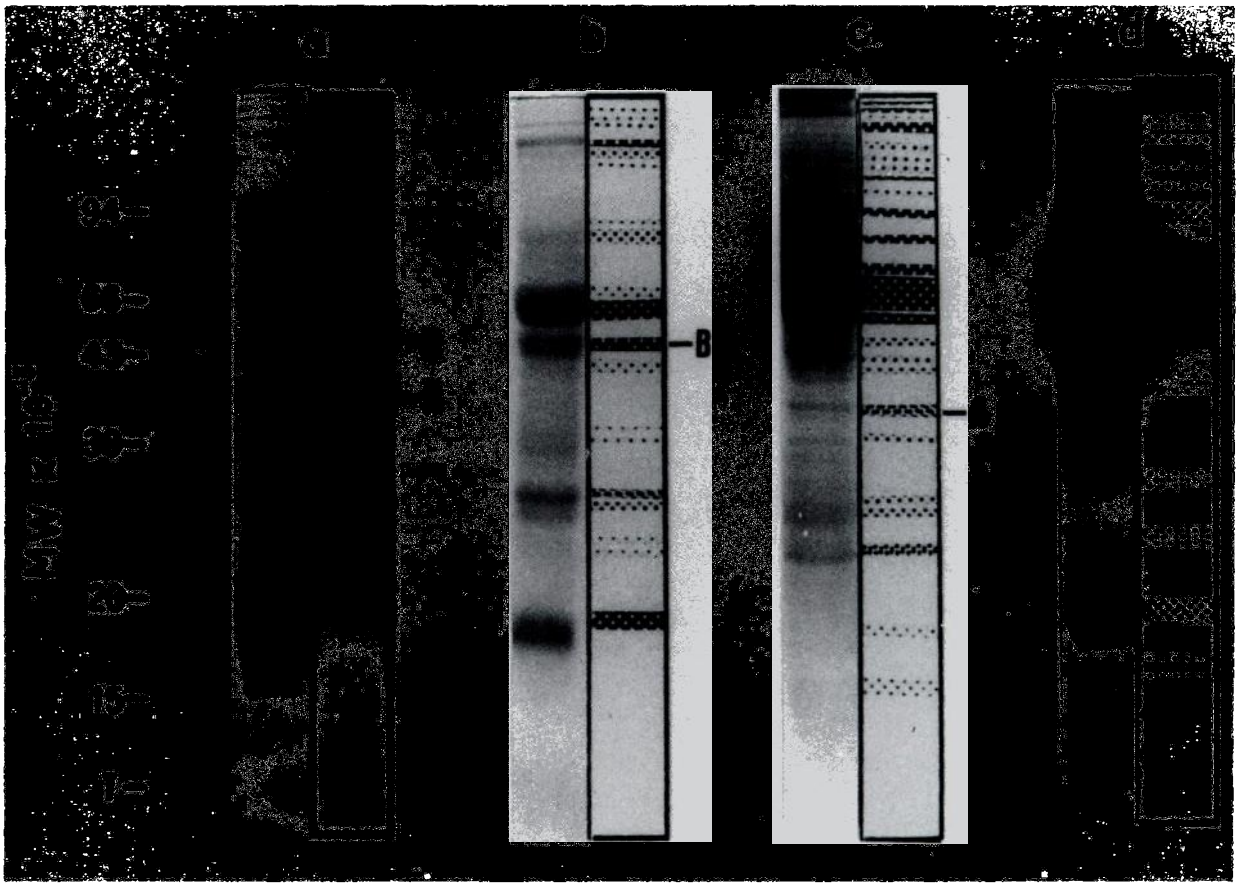

Fig. 1. SDS polyacrylamide gel electrophoresis of (a) seminal plasma from a fertile man (results from two other men were similar and not shown), (b) one of the four samples of seminal plasma of a patient who had a right epididymovasostomy done 18 months previously (results from the other three samples of ejaculates were similar and not shown), (c) homogenates of human caput epididymidis (obtained from two men with prostatic carcinoma), and (d) blood sera of fertile men. The diagrammatic presentation of the protein bands is also shown. Each gel contained about 50 μg protein.

normal spermatozoa contained 12 radioactive peaks (Fig. 2A). Peak 7 comigrated with band A in the seminal plasmas of normal men (Fig. 1A). However, this peak was found to be absent (or markedly reduced) from the surface of immotile spermatozoa of the patient (Fig. 2B). When the patient's spermatozoa were incubated for 30 minutes in the seminal plasma from normal men and then subjected to surface protein labeling, peak 7 was found to be partially restored (Fig. 2C). In experiments on four different semen samples from the patient, the activities of peak 7 before and after incubation with normal seminal plasma were 70 ± 21 cpm and 150 ± 20.6 cmp, respectively (means ± SE, $P < 0.05$; Students t test).

Discussion

The results of the present study indicate that normal seminal plasma contains a protein (MW 38,000) which is also present on the surface of the ejaculated motile spermatozoa. This protein was found in the cytosol of the caput epididymis but not in blood serum, indicating that it may originate from the epididymis and that it coats the sperm surface during epididymal transit. However, this protein was absent on ejaculated sperm and in seminal plasma from a patient examined 18 months after epididymovasostomy. This presumably was due to a lack of epididymal contribution to the ejaculate following this procedure. Although the patient's immotile spermatozoa were devoid of this surface protein because they had not passed through the epididymis and encountered epididymal secretion, they could bind this protein when exposed to seminal plasma of normal men (Fig. 2C).

Although the present study was based on studies from a single patient, the results are consistent with reports on the rat (Lea et al, 1978; Wong et al, 1981; Tsang et al, 1981), the rabbit (Oliphant and Singhas, 1979; Moore, 1980), the bull (Acott and Hoskins, 1981), the ram (Voglmayr

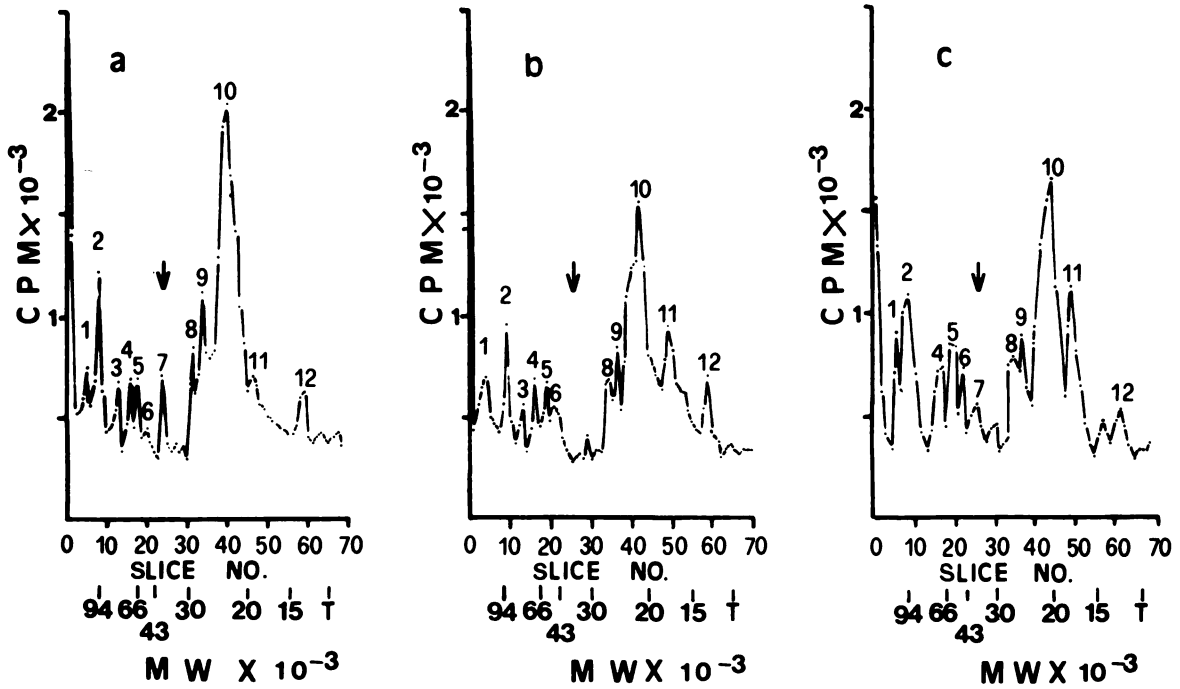

Fig. 2. SDS polyacrylamide gels showing the pattern of radioactivity in ^{125}I-labeled (a) ejaculated (motile) sperm from a fertile man (results from the other two normal men were similar and not shown), (b) ejaculated (immotile) sperm from the patient (results from the three other samples of patient's sperm were similar and not shown), and (c) ejaculated sperm from the patient after incubation in the seminal plasma of normal man for 30 minutes (results from three other experiments were similar and not shown). The arrow indicates the position of peak 7.

et al, 1980), and the hamster (Moore, 1980) that show that the caput epididymidis secretes specific proteins (MW 30,000 to 40,000) into the lumen of the epididymis. Incubation of the patient's spermatozoa with normal seminal plasma suggested that the sperm could bind this protein.

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