

In vitro studies of human seminal plasma allergy

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A 23-yr-old woman experienced generalized urticaria, angioedema, and respiratory obstruction after intercourse. Reactions increased in frequency and severity over a 2-yr period; sexual exposures were limited to her husband. Fresh, centrifuged seminal plasma samples from four donors, including her husband, evoked positive immediate puncture skin-test reactions in dilutions of 1:100 or 1:1,000; no reactions were seen in normal control males. A borderline elevation in serum IgE antibodies to seminal plasma was noted by the radioallergosorbent test (RAST). However, the patient had elevated IgE antibodies to a partially purified seminal plasma fraction (IV) obtained by Sephadex G-200 gel filtration. Seminal plasma from all four donors showed similar allergenic activity when tested in fraction IV RAST inhibition experiments. Further in vitro studies have characterized the allergenic components in fraction IV. Allergenic components (pool III) (1) are distinct from acid phosphatase; (2) have an apparent molecular weight range from 20,000 to 30,000 daltons; (3) by isoelectrofocusing produced multiple bands with pI of 6.6, 7.0, and 7.5; and (4) produced multiple bands in polyacrylamide gel electrophoresis, indicating a heterogeneous group of antigens. Comparison of pool III with seminal vesicle secretions and prostatic homogenate via thin-layer isoelectrofocusing revealed protein bands which appeared to be common to all three materials. Thus, it remains uncertain as to whether allergenic proteins are derived from seminal vesicle or prostatic secretions. Condom usage by the patient's husband has essentially prevented subsequent allergic reactions. However, serum IgE antibodies to fraction IV remained consistently elevated during a 28-mo follow-up period.

The first description of a female with systemic allergic sensitivity to male semen was published in the Dutch literature by Specken in 1958,¹ followed by a case report in English by Halpern et al. in 1967.² Subsequently, several articles have described similar patients with various allergic responses during or after intercourse, ranging from local pruritus to systemic anaphylaxis.³⁻¹⁰ We report a 23-yr-old woman with IgE-mediated seminal plasma allergy, as confirmed by in vivo and in vitro analyses. Further in vitro in-

vestigations were performed to characterize the allergenic components of male seminal plasma.

CASE REPORT

A 23-yr-old married woman was initially seen in October, 1976, after experiencing an episode of generalized urticaria and laryngeal edema during intercourse with her husband. During the two previous years, she had developed progressively severe allergic nasal and eye symptoms during or soon after intercourse. She also complained of angioedema of the lips and eyelids with occasional generalized urticaria. During this 2-yr interval, all symptoms became more frequent and severe and occurred only upon sexual intercourse with her husband. The performance of oral-genital sex was denied. The last episode prior to coming to our clinic required emergency treatment with epinephrine because of respiratory obstruction. Previous medical consultants had attributed her symptoms to a number of factors, including cosmetic allergy, oral contraceptive hypersensitivity, or emotional responses. She had been married for 4 yr and sexual exposures were limited to her husband. She had no personal history of allergy. Physical examination was normal; there was no evidence of dermatographism.

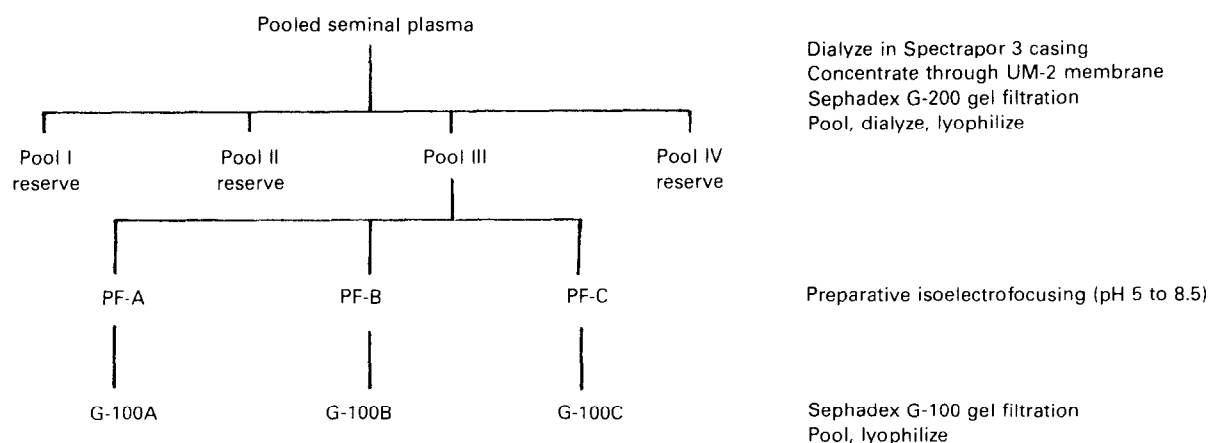
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**FIG. 1.** Seminal plasma fractionation scheme.

Routine laboratory studies, including quantitative serum immunoglobulin levels and determinations of C3, C4, and C1 esterase levels, were normal. Intradermal skin tests to common inhalant antigens were negative.

Condom usage by her husband since October, 1976, has essentially eliminated subsequent allergic reactions. Nevertheless, occasional condom leakage has resulted in mild anaphylactic symptoms.

MATERIALS AND METHODS

Skin tests

Masturbated semen specimens were obtained from four healthy adult donors, including the patient's husband. The samples were promptly centrifuged at 3,000 rpm for 15 min at 4° C to remove spermatozoa. The supernatant, termed seminal plasma, was removed, sterilized by Millipore filtration through a 0.2- μ m filter, and diluted in phosphate-buffered saline (pH 7.4). Dilutions of 1:1, 1:10, 1:100, 1:1,000, and 1:10,000 were frozen at -20° C until further use.

Serum IgE antibody measurements

Serum IgE antibodies to seminal plasma constituents were measured by the radioallergosorbent test (RAST)¹¹ with solid-phase RAST reagents prepared as described previously.⁷ As a result of previous studies on a similar patient, seminal plasma had been fractionated into six pools by Sephadex G-200 gel filtration. Skin-test reactivity in this previous patient was maximal to fraction IV, containing materials with an apparent molecular weight range of 12,000 to 25,000 daltons. This fraction, as well as unfractionated seminal plasma, had been coupled to microcrystalline cellulose particles and stored at 4° C for 2 yr.

In the performance of the test, 0.5 ml of either crude seminal plasma-cellulose or seminal plasma fraction IV-cellulose (1 mg/ml) was incubated with 0.05 ml of either patient or normal test serum. After overnight incubation, the polymer was centrifuged and washed twice, then incubated overnight with affinity chromatograph-purified radiolabeled anti-IgE (20 ng/tube). After a second overnight incubation and two additional washing procedures, the radioactivity

TABLE I. Measurement of serum IgE antibodies to crude seminal plasma and fraction IV

Serum	RAST (% total counts bound)	
	Crude seminal plasma	Fraction IV
Negative control	1.04	3.10
Patient		
12/1/76	1.34	5.22
9/13/77	1.35	5.56
1/23/78	1.39	5.55
9/19/78	1.22	4.96
11/21/78	1.20	4.07
12/7/78	1.20	4.69
3/4/79	1.27	4.90

All serums were tested in a single RAST assay; results listed are the means of duplicate determinations.

associated with the solid-phase material was determined by counting in a gamma spectrometer. Additional RAST experiments were performed using filter paper disks to which had been coupled freshly prepared seminal plasma constituents obtained by fractionation of crude seminal plasma. RAST results were expressed in terms of total radioactive counts bound to the polymer.

RAST inhibition studies

RAST inhibition experiments¹¹ were performed with the patient's serum, utilizing varying quantities of the four crude seminal plasma samples as the inhibitors and fraction IV as the solid-phase allergen. Dose-response inhibition curves could be constructed which, when plotted in a log-linear fashion, approximated straight lines with measurable slopes, thus permitting the qualitative allergenic comparison of the various seminal plasma samples.

Seminal plasma fractionation

The flow diagram for fractionation of seminal plasma is shown in Fig. 1. Seminal plasma was obtained from 12 random semen samples submitted for sperm counts. After

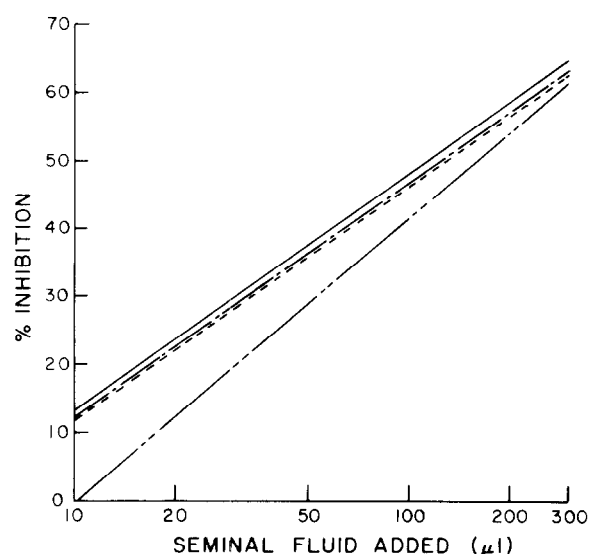


FIG. 2. RAST inhibition measurements utilizing the patient's serum with varying quantities of four crude seminal plasma samples as the inhibitors. Fraction IV is the solid-phase allergen.

centrifugation of whole semen at 20,000 rpm for 20 min, the supernatants (seminal plasma) were removed, frozen at -20°C , and subsequently thawed, pooled, and dialyzed for 3 days against 0.01 M phosphate–0.2 M NaCl (pH 7.45) using 3,500 mw cutoff casing (Spectrapor 3, Spectrum Medical Industries, Inc., Los Angeles, Calif.). The dialyzed seminal plasma was concentrated to 10.5 ml by pressure filtration through an Amicon UM-2 membrane (Amicon Corp., Lexington, Mass.) and then applied to a calibrated Sephadex G-200 column (3.2×200 cm) and gel filtered at 12 ml/hr with an eluting buffer of 0.01 M phosphate–0.2 M NaCl.

Acid phosphatase assay

Acid phosphatase activity was determined by a colorimetric assay using *p*-nitrophenyl phosphate as a substrate at pH 4.8 (Sigma Chemical Co., St. Louis, Mo.).

Isoelectrofocusing experiments

Pooled fractions from Sephadex G-200 gel filtration were tested by thin-layer isoelectrofocusing in 5% polyacrylamide gel slabs by the method of Awdeh et al.¹² using a 2% ampholyte concentration and pH gradients of either 3.5 to 9.5 or 5.0 to 8.5. Samples were applied in duplicate; after completion of the focusing run, circular segments of the unstained gel were punched out with a cork borer, eluted with distilled water, and coupled to CNBr-activated filter paper discs which were subsequently tested by RAST with the patient's serum. Preparative flat-bed isoelectrofocusing was performed by the method of Winter et al.¹³ using a Sephadex G-75 support with a 2% ampholyte concentration and a pH gradient of 5.0 to 8.5. Fractions were tested for pH and for allergenic activity using the RAST. Ampholytes were subsequently removed from pooled fractions by gel

filtration over a calibrated Sephadex G-100 column (2.5×50 cm) equilibrated and eluted with 0.2 M NH_4HCO_3 (pH 8.2).

Prostatic and seminal vesicle extracts

Seminal vesicle and prostatic secretions were obtained from a surgical specimen removed for a prostatic carcinoma. Seminal vesicle secretions were scraped from the gland into 0.1 M NH_4HCO_3 buffer, centrifuged, and the supernatant stored at -20°C . Similarly, nondiseased prostatic tissue was homogenized in the same buffer, centrifuged, and the supernatant frozen at -20°C . These extracts were thawed as required for use in direct RAST and analytical isoelectrofocusing experiments.

Analytical polyacrylamide gel electrophoresis (PAGE) studies

Polyacrylamide gel disc electrophoresis (disc-PAGE) was performed in 7% gels with alkaline buffers as described by Davis¹⁴ and also in 15% gels containing urea–acetic acid buffer (urea-PAGE).¹⁵ Molecular weight estimates were attempted by PAGE in 8% gels containing 1% sodium dodecyl sulfate (SDS) with and without the addition of 0.04 M dithiothreitol (DTT).¹⁶

Results

The patient was skin tested with seminal plasma from four donors, including her husband. Immediate wheal-and-flare responses were evoked by puncture tests using either 1:100 or 1:1,000 dilutions of seminal plasma from all four donors. No skin-test reactions were seen at these dilutions in normal control males.

IgE antibodies to crude seminal plasma and to fraction IV were measured in patient serums obtained at intervals over a 2.5-yr period. With the fraction IV RAST polymer a control serum from a healthy male bound 2.20% to 3.10% total radioactive counts, while serum from the patient bound 4.07% to 7.64% total counts in six separate assays done during this period. Results from a single RAST assay are shown in Table I. Although it was not possible to show significant elevations of IgE antibodies to crude seminal plasma, IgE antibodies to fraction IV were clearly elevated. No significant change in antibody level was seen over the observation period despite the supposed decrease in exposure to seminal plasma.

All four seminal plasma samples tested showed significant allergenic activity by RAST inhibition, as shown in Fig. 2. Three samples were practically indistinguishable, whereas one was slightly less potent in its inhibitory capacity. However, the slopes of the inhibition curves were not significantly different ($0.1 > p > 0.05$) as tested by analysis of covariance.¹⁷ These data indicated that our patient had

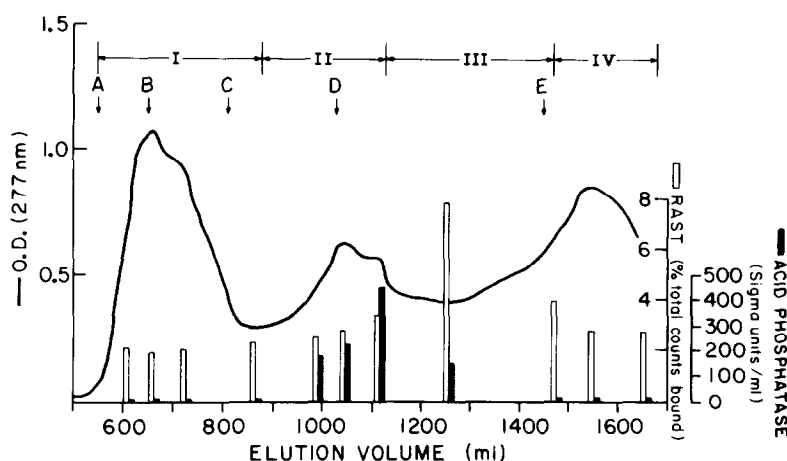


FIG. 3. Sephadex G-200 gel filtration of pooled seminal plasma. Selected fractions were tested for acid phosphatase activity as well as for allergenic activity by RAST. The RAST binding produced by a normal control serum ranged between 2% to 3% total counts. The elution volumes of various column markers (A-E) are indicated by arrows: A, blue dextran; B, ferritin; C, catalase; D, BSA; E, cytochrome C. Fractions were pooled as indicated (I-IV), with pool III containing peak allergenic activity.

serum IgE antibodies to constituents present in all seminal samples tested. The fact that rather large quantities of seminal plasma were required to achieve 50% inhibition indicated that the allergens probably accounted for only a small percentage of the protein present in seminal plasma.

The Sephadex G-200 chromatogram is shown in Fig. 3. Selected fractions were tested for acid phosphatase activity and for allergenic activity. Peak acid phosphatase activity was observed at an elution volume of 1,040 ml, whereas peak allergenic activity was observed at an elution volume of 1,250 ml. Fractions were pooled as indicated in Fig. 3 with pool III containing the peak allergenic activity.

Preliminary analytical isoelectrofocusing experiments with pool III from Sephadex G-200 using a pH gradient of 3.5 to 9.5 revealed that allergenic activity was confined to the pH range of 6.0 to 8.0. Therefore, a subsequent isoelectrofocusing run was made with a more narrow gradient of 5.0 to 8.5; results are shown in Fig. 4. Pool III samples were tested in duplicate with aliquots of the right-hand sample removed for testing by RAST prior to staining. Allergenic activity was associated with punches Nos. 10 to 17 (as counted from the anode) and also with the heavy doublet band at punch No. 21. It was apparent that pool III contained a heterogeneous group of proteins, as evidenced by the multiplicities of bands and RAST reactivities. Accordingly, we fractionated pool III (50 mg) by preparative isoelectrofocusing using the same pH gradient; results are shown in Fig. 5. Three peaks of allergenic activity were observed at pI 6.6, 7.0, and 7.5. Fractions containing these three peaks,

termed PF-A, PF-B, and PF-C, respectively, were pooled, lyophilized, and then gel filtered separately over a calibrated Sephadex G-100 column to remove ampholytes. The elution profiles are shown in Fig. 6. In each case, peak allergenic activity was associated with a small 277-nm absorbance peak eluting between the elution volumes of the bovine serum albumin (BSA) and chymotrypsinogen column markers (molecular weight, 69,000 and 25,000 daltons, respectively). Fractions were pooled as indicated in Fig. 5 and lyophilized; insufficient quantities of G-100A, G-100B, and G-100C were recovered to permit accurate weighing for calculation of yields.

The three PF pools, as well as seminal vesicle secretions and prostatic tissue extract, were tested by thin-layer isoelectrofocusing using a pH gradient of 5.0 to 8.5. This showed that the preparative isoelectrofocusing step had produced fairly good resolution of pool III into three components. Proteins common to PF-A, PF-B, and PF-C appeared to be present in both seminal vesicle secretions and prostatic homogenate, as shown in Fig. 7. Disc-PAGE of G-100A, G-100B, and G-100C revealed a multitude of protein bands, as did urea-PAGE and SDS-PAGE (data not shown).

The seminal vesicle secretions and prostatic tissue homogenates were also coupled to filter paper disc for use in a RAST assay with the patient's serum. However, RAST binding was not significantly increased over that seen with a negative control serum. Similarly, the addition of up to 0.2 ml of uncoupled seminal vesicle secretions or prostatic homogenate did not significantly inhibit the fraction IV RAST binding

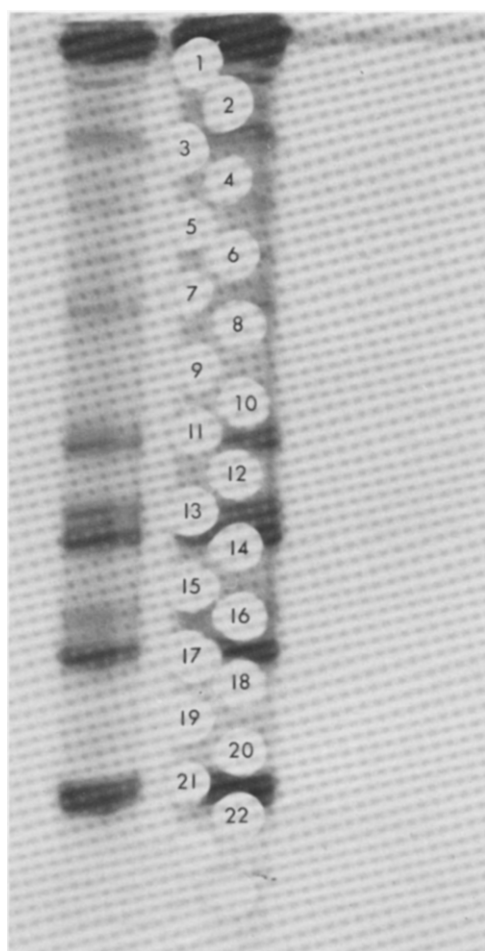


FIG. 4. Thin-layer polyacrylamide gel isoelectrofocusing (pH 5.0 to 8.5) of pool III; the anode is at the top. The sample was tested in duplicate, with aliquots of the right-hand sample removed for testing by RAST prior to staining. Allergenic activity was associated with punches Nos. 10 to 17 and No. 21.

produced by the patient's serum. The patient was not skin tested with the seminal vesicle secretions or the prostatic homogenate.

DISCUSSION

The literature contains a number of case reports of human seminal plasma allergy. As with our patient, most women have not had other evidence of allergic disease. There is at least one case report of a familial occurrence of local vaginal symptoms after semen exposure in four women within a family,⁸ although most women deny a family history of such events. There are some instances in which women have had allergic reactions with more than one male, indicating the presence of sensitizing components which may be common to all semen.⁷ Also, as with our patient, initial responses by the consulting physicians have mistakenly implicated other factors besides possible

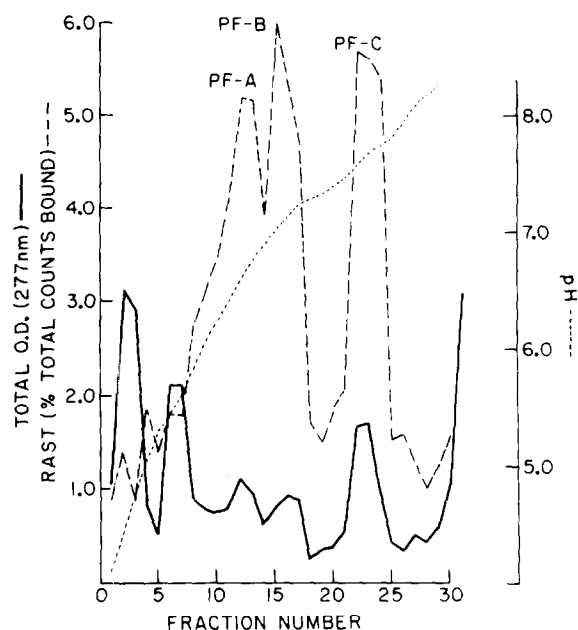


FIG. 5. Preparative isoelectrofocusing of seminal plasma pool III (pH 5.0 to 8.5). Selected samples were tested for pH, absorbance at 277 nm, and allergenic activity by RAST. Peak allergenic activity noted at pI 6.6, 7.0, and 7.5; these peaks were pooled separately as PF-A, PF-B, and PF-C, respectively.

seminal plasma allergy, such as food or contact allergies, or especially emotional responses.

IgE antibody appears to be responsible for mediating the allergic reactions. Positive wheal-and-flare skin-test reactions to low concentrations of seminal plasma can be evoked in allergic but not in nonallergic individuals.^{3, 7} Also, passive transfer of skin-test reactivity to nonsensitive recipients has been achieved.^{2, 6, 18} This transfer can be blocked by heating the patient's serum or by absorption with anti-IgE.

Previous attempts to demonstrate IgE antibodies to seminal plasma by RAST have been unsuccessful in our laboratory.⁷ Whether this has been due to low titers of specific IgE antibody in the patient's serum, technical difficulties in performance of the RAST technique, or heterogeneity of the antigens utilized as the solid-phase allergens is unclear. Nevertheless, our patient exhibited a clearly positive RAST to male seminal plasma constituents. RAST inhibition studies revealed a similar degree of inhibition with all samples tested, which agrees with skin-test reactivity findings and indicates that allergenic components are probably common to all seminal plasmas.

Taking advantage of our patient's positive RAST response, we were able to further characterize the allergenic components of seminal plasma through various *in vitro* analyses. As assessed by RAST reactivity, Sephadex G-200 column fractionation revealed

peak allergenic activity within pool III, within a 20,000 to 30,000 molecular weight range. These findings are consistent with those of others who utilized Sephadex G-200 fractionation and skin-test reactivity to determine that the allergenic components were in a similar molecular weight range.^{4, 7, 18} Analytical isoelectrofocusing of pool III produced three peaks of allergenic activity associated with pI 6.6, 7.0, and 7.5. This is in contrast to the report by Schulz et al.⁴ who noted a pI range of 8.4 to 8.6 for the allergenic components. Further analysis of these three peaks via thin-layer isoelectrofocusing, disc-PAGE, and urea-PAGE revealed a multiplicity of protein bands, thus indicating a heterogeneous group of antigens. Peak allergenic activity was not associated with acid phosphatase activity.

The source of the allergenic components in seminal plasma has not yet been determined. Previous investigators have demonstrated that allergenic proteins are restricted to the spermatozoa-free portion of the whole semen, namely the seminal plasma. Skin testing of allergic patients has been reported to produce positive immediate reactions to either whole semen or seminal plasma but not to spermatozoa. Also, skin tests have been positive to semen samples from vasectomized males, which are presumably devoid of spermatozoa.³ Moreover, these allergenic proteins appear common to all seminal plasmas as evidenced by the presence of skin-test reactivity in samples from all males tested in this previously reported patient. Our patient exhibited skin-test reactivity to three seminal plasma samples in addition to her husband's sample.

Utilizing prostatic tissue extracts, Siraganian et al.¹⁸ noted positive skin tests in an allergic female. We compared both seminal vesicle secretions as well as prostatic homogenate with pool III and its allergic components (PF-A, PF-B, and PF-C). Thin-layer isoelectrofocusing revealed protein bands which appeared to be common to all tissues studied. Unfortunately, attempts at RAST assay with seminal vesicle and prostatic samples revealed binding which was not significantly increased over that seen with negative controls. Likewise, uncoupled seminal vesicle secretions and prostatic homogenate produced no inhibition of the fraction IV RAST. Therefore, it remains uncertain as to whether allergenic proteins are derived from seminal vesicle or prostatic secretions.

The natural course of events in women with seminal plasma allergy has not been well documented. Most descriptions in the literature consist of case reports with little long-term follow-up. Therapy aimed at avoidance of semen exposure via condom usage has prevented symptoms in allergic women. Halpen et al. attempted hyposensitization using human seminal

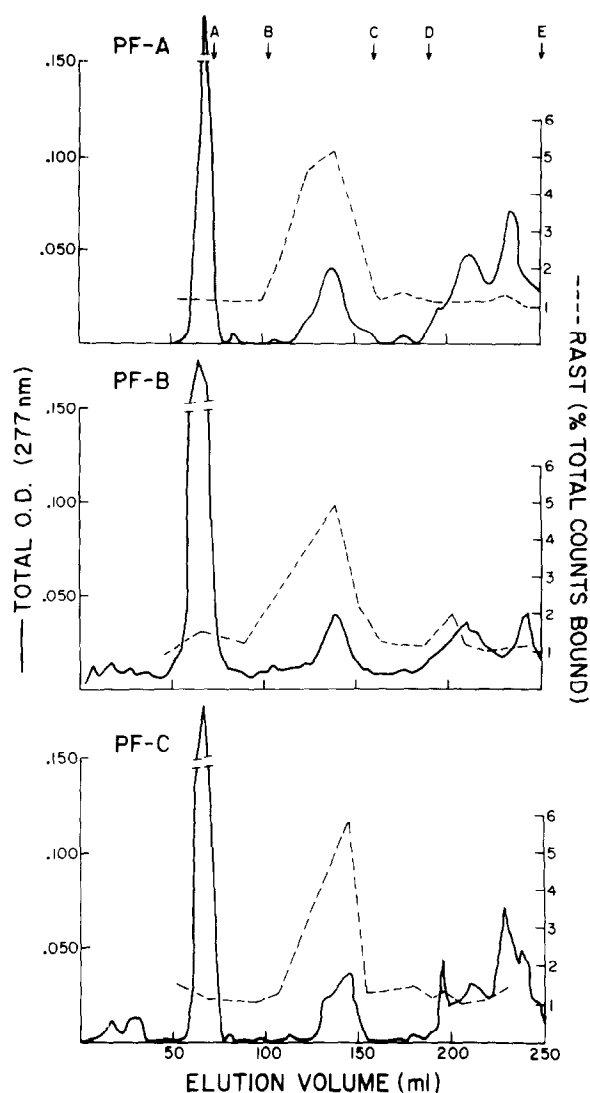


FIG. 6. Sephadex G-100 gel filtration of each preparative isoelectrofocusing pool. Markers: A, blue dextran; B, BSA; C, chymotrypsinogen; D, cytochrome C; E, ¹²⁵I.

plasma; however, his patient remained clinically allergic after 2 yr of treatment.² There is one recent report of successful immunotherapy in an allergic woman utilizing her husband's seminal plasma.¹⁹ Apparently, clinical allergy may wax and wane, and possibly some women lose their hypersensitivity with the passage of time. However, utilizing the RAST technique, our patient has clearly demonstrated continued IgE antibodies to seminal plasma constituents, even after 2 yr of condom usage by her husband. Furthermore, despite condom usage, she obviously remains clinically sensitive to seminal plasma as evidenced by the development of shortness of breath and hives during two episodes of condom leakage. Our patient and her husband have no children but now desire to begin a family. Because of her continuing

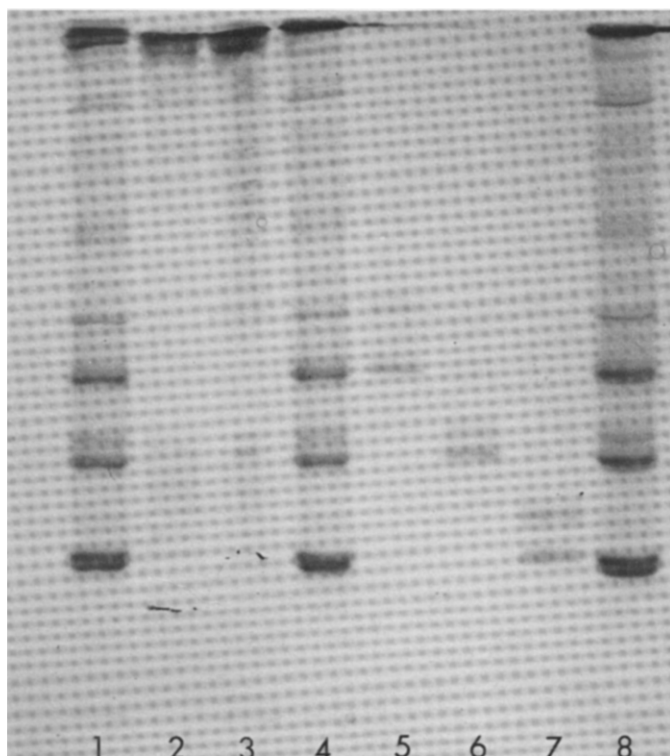


FIG. 7. Thin-layer isoelectrofocusing of various seminal plasma fractions and tissue extracts (pH 5.0 to 8.5). The anode is at the top. Samples included (1) Seminal plasma pool III; (2) seminal vesicle extracts; (3) prostatic tissue extract; (4) seminal plasma pool III; (5) pool III, PF-A; (6) pool III, PF-B; (7) pool III, PF-C; and (8) seminal plasma pool III.

hypersensitivity to semen, artificial insemination with washed spermatozoa is planned.

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