

Clinical and Immunological Study of an Exceptional Case of Reaginic Type Sensitization to Human Seminal Fluid

B. N. HALPERN, T. KY AND B. ROBERT

Institut d'Immuno-Biologie, Hôpital Broussais, Paris XIV

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Summary. An exceptionally rare case of spontaneous allergic sensitization to a glycoprotein rich in sialic acid and present in human seminal fluid is described in a woman. An anaphylactic reaction, consisting of giant urticaria, oedema, asthma, uterine pain and cardiovascular collapse followed within minutes of coitus. A high degree of atopic hypersensitivity was shown by skin reactions to human seminal fluid, but not to that of animal origin. Passive transfer of the hypersensitivity to man and monkey was successful and the responsible antibody was shown to be reagin-like and associated with the group of γ A-globulins.

INTRODUCTION

Although autoimmune reactions to isologous human proteins are known, instances in which such sensitization occurs as a reaginic type allergy are exceptional. As far as we know the case described here has no precedent.

We report on the spontaneous sensitization of a female subject to protein constituents of human seminal fluid. Evidence is presented to show that an atopic hypersensitivity reaction is involved, the antibody being reaginic γ A-globulin and the antigen a glycoprotein of human seminal fluid.

MATERIALS AND METHODS

The patient

Mrs P., an apparently normal well-developed Caucasian female, aged 29, was referred to the Allergy Clinic of this hospital in September 1962 because of violent immediate type (atopic) allergic reactions following coitus. She stated that she had recently married and had had no previous sexual intercourse. Most of the symptoms recurred after every coitus. Corticosteroids and antihistaminic drugs had little effect. The patient came from an 'atopic' family, her mother having a long history of asthma and atopic eczema; a twin brother suffering from contact dermatitis and a younger sister from atopic dermatitis. The patient herself had no other allergic symptoms or complaints. The circumstances of the onset of the patient's clinical manifestations suggested that they were of the atopic type and that the sensitizing substance came from the semen.

Symptoms immediately after first coitus with her husband were: large erythematous, papular and pruritic plaques covering all parts of her body; oedematous swelling of the lips, eyelids and of the tongue and pharynx which caused difficulty in breathing; severe asthmatic dyspnoea accompanied by intense congestion of the mucous membranes; violent pain in the pelvis with contractions of the uterus and general malaise culminating in loss of consciousness. The manifestations reached a maximum 15–30 minutes after

coitus and then began to subside, tumefaction of the face and vulva, pain in the uterus, urticarial plaques and general weakness persisting over the next 24 hours.

Sera and antisera

Blood from the patient was obtained by venepuncture and allowed to clot at room temperature. After centrifugation a few hours later the resulting sterile serum was used either fresh or after storage at -20° .

Antisera to human seminal fluid were prepared in rabbits by giving once a week for 4 weeks a subcutaneous injection of 2.5–5 mg of seminal proteins in complete Freund's adjuvant. After a week of rest, the animals were bled from the carotid artery. Serum was collected, sterilized by filtration through a millipore membrane and kept frozen until used.

Horse anti-human γ M-globulin serum was obtained commercially from Behringwerke (Marburg/Lahn, Germany).

Rabbit anti-human γ A-globulin sera were obtained through the courtesy of Dr Burtin (Institut du Cancer, Villejuif, France) and were further purified by absorption with lyophilized human cord serum (5 mg/ml of antisera) which proved not to contain γ A-globulin. Immunoelectrophoresis (Fig. 1) showed complete removal of γ G-specific antibodies after absorption with the cord serum.

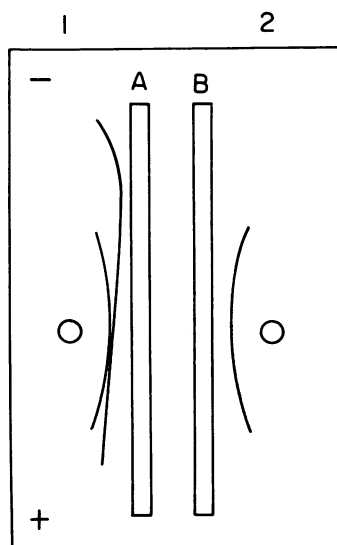


FIG. 1. Immunoelectrophoresis of Mrs P.'s serum run with rabbit anti-human γ A antiserum. Row A, unabsorbed anti- γ A serum: two precipitin lines corresponding to γ G- and γ A-globulins. Row B, anti- γ A serum absorbed with cord serum: a single precipitin line corresponding to γ A-globulin.

Antigens

Human semen was obtained from individual donors as well as from the patient's husband. The samples were used individually or pooled when needed in larger quantities. The semen was centrifuged immediately after collection to separate the spermatozoa from the seminal fluid which was kept frozen at -20° . However, slowly progressive deterioration of the skin reaction-eliciting activity occurred even under these conditions of storage and tests were therefore performed with fresh seminal fluid only.

Semen from bulls and horses were obtained from the Veterinary High School (Maisons-Alfort, France) and semen from rabbits and guinea-pigs in the laboratory. Bovine submaxillary mucin was prepared in the laboratory by the method of Tsuiki, Hashimoto and Pigman (1961). The bovine testicular hyaluronidase was a commercial preparation (Wydase) used in a concentration of 150 units/ml.

Zone electrophoreses

Paper electrophoresis was done in veronal buffer, 0.05 M, pH 8.6 at 120 V for 16 hours. The paper strip was cut lengthwise; half of it was used for the localization of the proteins by staining with amido black; the other half was cut into small strips according to the location of the protein bands. These strips were applied wet to small scarifications on the back of the patient.

Cellulose acetate electrophoresis was done on cellulose acetate strips, 2 cm wide. Migration was allowed to proceed for 4 hours in a Veronal buffer, 0.05 M, pH 8.6 at 120 V (Kohn, 1957).

Starch gel electrophoresis was done according to Smithies (1955) in a borate buffer, 0.03 M, pH 8.6 at 120 V. After 16 hours staining was done with amido black. A thick layer of gel was prepared (preparative electrophoresis) and only the upper slice was used for the staining of the fractions. The rest of the gel was sliced transversely with the staining reactions as a guide. The individual blocks were eluted with isotonic saline, dialysed, concentrated and applied to the scarified skin of the patient.

Immunoelectrophoresis

This was done according to Grabar and Williams (1955) (see also Grabar and Burtin, 1960) using Kodak photographic plates of 180×130 mm (migration 2 hours, veronal-HCl buffer, 0.05 M, pH 8.2). After development of the precipitin lines, the plates were washed with saline and stained with azocarmine.

Ion exchange chromatography

Separation of the protein antigens of the seminal fluid on a preparative scale was achieved by chromatography on a column of DEAE-cellulose according to a modification (Fahey and Horbett, 1959) of the method of Sober and Peterson (1958).

Skin tests

Skin scratch and intradermal tests were done in the conventional manner (Cooke, 1947) on the volar surface of the arms or on the back. Fresh seminal fluid was used for the reactions. In other skin tests substances were applied either in dry form or in solution, in concentrations indicated in the appropriate tables. For intradermal tests the antigen solutions were sterilized by addition of phenol (0.5 per cent, w/v) and injected adequately diluted into the skin of the back, spaced at least 2 cm apart.

Prausnitz-Kuestner transfers

These were done according to Prausnitz and Kuestner (1921).

Passive cutaneous anaphylaxis in guinea-pigs and primates

These were done as described by Ovary (1958) for guinea-pigs and by Layton, Lee and Yamanaka (1962) for monkeys.

Schultz-Dale reactions with sensitized guinea-pig ileum

The technique used was as described by Halpern, Liacopoulos-Briot, Binaghi and van Neer (1959).

Passive haemagglutination

A modification (Halpern, Jacob, Binaghi and Parlebas, 1961) of the bis-diazotized benzidine technique (Stavitsky and Arquilla, 1955) was used.

Haematological tests

The usual blood grouping test and differential blood counts were done.

Agglutination tests

Various isoagglutinins in the patient's serum such as agglutinins against human (husband) spermatozoa (Rumke, 1959), human normal red cells (direct Coombs test) (Coombs, Mourant and Race, 1945), enzyme treated heterologous red cells (Dausset, 1956), cold and hot leucoagglutinins and thromboagglutinins (Dausset, Colombani and Okochi, 1964) were investigated by Dr Dausset.

Complement fixation

The 50 per cent haemolysis complement fixation was determined according to the method described by Roulier (1950).

Chemical estimations

Proteins were determined by the Biuret method of Gornall, Bordawill and David (1949) and sialic acid according to Svennerholm (1957).

RESULTS

SKIN SCRATCH TESTS

Positive results (Fig. 2) were produced in the patient (Mrs P.) with whole human semen (obtained either from the husband or from human donors) and with human seminal fluid devoid of spermatozoa. Washed human spermatozoa did not elicit any reaction. Negative results were also obtained with the serum of the husband, with semen from the rabbit, guinea-pig, horse and bull, with bovine submaxillary mucin and bovine testicular hyaluronidase. This shows that the responsible allergens were species-specific, and present in seminal fluid, but apparently absent from the spermatozoa.

INTRADERMAL SKIN TESTS

Intradermal injections of seminal fluid diluted with saline gave very strong reactions in Mrs P., dilutions of 1 : 1,000,000 still producing a positive reaction. Reactions with the same fluid in normal subjects were negative.

PASSIVE SERUM TRANSFER TO NORMAL HUMAN SKIN (PRAUSNITZ-KUESTNER REACTION)

Five healthy young female subjects, without known history of allergy, were used as recipients. Millipore filtered serum of the patient was injected intradermally into the skin of the back of the recipients in dilutions of 1 : 1, 1 : 5, 1 : 10, 1 : 50, 1 : 100 and 1 : 200, about 2 cm apart, isotonic saline containing 0.5 per cent (w/v) of phenol being used as the

Reaginic Type Sensitization to Human Seminal Fluid



FIG. 2. Cutaneous skin tests done in Mrs P. with human semen and other constituents: ST, whole human semen (+ + +); L, human spermatic fluid (+ + +); S, spermatozoa (-); H, bull sperm (-); M, mucin (-); T, control.

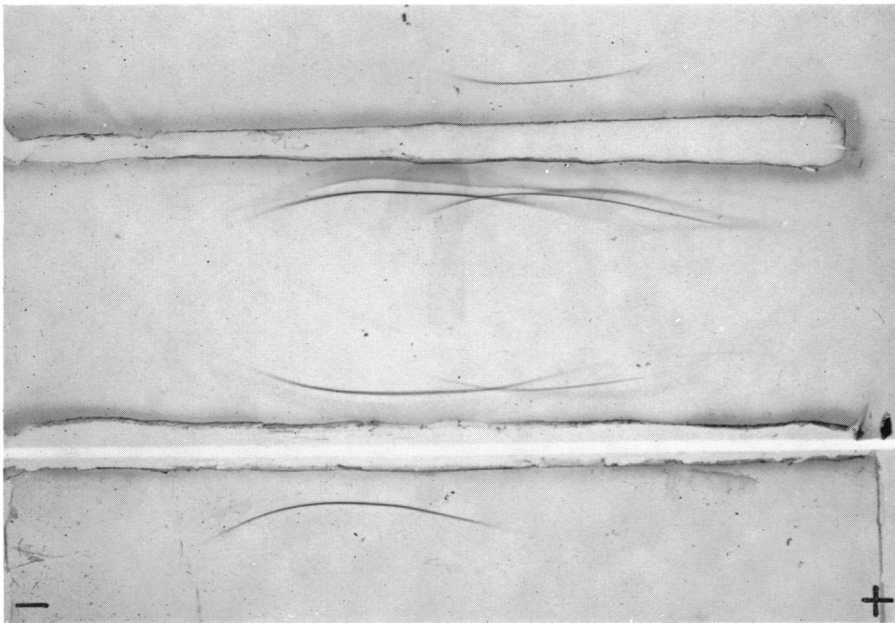


FIG. 4. Immunoelectrophoretic patterns of human seminal fluid obtained with a rabbit anti-human seminal fluid antiserum. In the middle area, the well-defined precipitation lines obtained with whole spermatic fluid. Upper area a single precipitation line obtained with fraction 3; lower area precipitation line obtained with fraction 2.

diluent. Twenty-four hours later, intracutaneous tests were made with the allergen solution diluted in the same solvent (Table 1) at the prepared sites. Control tests were made on a non-sensitized site with the diluent. Readings were taken within 30 minutes after the injection.

TABLE 1
PASSIVE TRANSFER WITH THE SERUM OF MRS P. (PRAUSNITZ-KUESTNER REACTION)

Injected serum	Oedema: mean diameter (mm)	Erythema: mean diameter (mm)	Intensity of reaction
Mrs P.'s serum			
Undiluted	32 ± 6	64 ± 17	+++
Diluted 1:10	20 ± 3	36 ± 5	++
Diluted 1:20	17 ± 3	25 ± 5	+
Diluted 1:100	8 ± 2	15 ± 3	±
Diluted 1:200	0	0	0
Control serum			
M. X undiluted	0	0	0

The transfer was performed in five non-allergic female individuals.

Positive reactions were obtained with seminal fluid up to serum dilutions of 1:20 to 1:100. However, when serum heated for 4 hours at 56° was injected, negative results were obtained, even with undiluted serum.

PASSIVE TRANSFERS TO EXPERIMENTAL ANIMALS

Hartley strain guinea-pigs were injected intradermally with 0.1 ml of the patient's serum (undiluted). For comparison additional injections were made of normal fresh human serum, rabbit antiserum to human serum albumin (630 µg antibody N/ml, diluted 1:20) and isotonic saline. Four hours later, one group of animals received an intravenous injection of a mixture containing 0.5 ml of fresh human seminal fluid diluted in 1 ml of pre-warmed saline and added with 0.5 ml of a 0.5 per cent (w/v) solution of Evans Blue dye while the animals of the other group received 1 ml of a 1:200 (w/v) solution of human serum albumin to which was added 0.5 ml of Evans Blue solution. The animals were killed 20 minutes later by carotid section and the results read on the inside of the reversed skin.

No evidence of passive cutaneous sensitization could be obtained in this way nor by passive *in vitro* sensitization of guinea-pig ileum.

Passive transfers were successful in monkeys. Two young monkeys (*Macacus cynomolgus*) were injected intradermally on the anterior surface of the arm with 0.1 ml of the patient's undiluted serum. Control injection was made with 0.1 ml of serum from a patient suffering from hay-fever. Thirty hours later, a mixture containing 1 ml of fresh human seminal fluid diluted in 4 ml of prewarmed saline together with 2 ml of a 0.5 per cent (w/v) solution of Evans Blue was injected intravenously and the sites examined. One hour later, the site previously sensitized with the serum from the pollen sensitive patient was tested by the injection *in situ* of a grass pollen solution and the results noted.

In both the monkeys used a well-delineated blue spot was obtained at the site sensitized with the patient's serum 15 minutes after the intravenous challenge with human seminal

fluid. No blue spots were observed at sites injected with other sera or with saline. The site previously sensitized with the serum of the pollen sensitive patient also showed rapid blueing. The same pollen solution injected into an unprepared site did not provoke any reaction.

PASSIVE HAEMAGGLUTINATION

Proteins of human seminal fluid were fixed to the surface of rabbit red cells by means of the bis-diazotized benzidine technique and titrated with various dilutions of the patient's serum. The highest serum dilution producing visible agglutination of the red cells was noted. Dilutions lower than 1:16 to 1:32 were considered non-significant. With one exception, all the results were negative.

HAEMATOLOGICAL FINDINGS

The following unremarkable findings were obtained: blood group A, Cc, Dec, Kk; red blood cells 4,280,900/mm³; white blood cells, 5000/cm³; polymorphonuclear neutrophils, 52 per cent; eosinophils, 2 per cent; lymphocytes, 15 per cent; large lymphocytes, 24 per cent; monocytes, 6 per cent.

ATTEMPT TO DETECT OTHER AGGLUTININS

No agglutinins to human spermatozoa could be detected in the patient's serum nor could any lysis of spermatozoa in the presence of the serum be observed, i.e. isoantibodies could not be detected.

The Coombs test and agglutination of heterologous enzyme-treated red cells in the presence of albumin also gave negative results. No cold agglutinins or leucoagglutinins (with leucocytes of the husband) or thromboagglutinins ('cold' or 'hot') could be detected.

COMPLEMENT FIXATION

Serum dilutions from 1:1 to 1:1000 were used, both with human semen and with bull semen. All results were negative.

ATTEMPTS TO IDENTIFY THE SENSITIZING ANTIGEN IN HUMAN SEMINAL FLUID BY DEAE ION EXCHANGE CHROMATOGRAPHY

A 3.5×50 cm column was filled with DEAE-cellulose (Serva Entwicklungslab., Switzerland) and equilibrated with a Tris-phosphate buffer 0.03 M, pH 8.2. A 70-ml sample of a pooled human seminal fluid containing 880 mg of proteins was passed through the column and eluted with a gradient of Tris-phosphate from 0.03 M to 0.5 M, pH 8.2 in a refrigerated fraction collector at 4°. Seventy-five fractions of 20 ml each were collected and their absorption followed at 280 mμ in a Beckman 'DU' spectrophotometer. The contents of the tubes were mixed (according to the scheme shown in Fig. 3) and the pooled fractions were dialysed and freeze-dried. The fractions were then analysed by electrophoresis on cellulose acetate strips to identify the proteins according to their electrophoretic mobility. Immunochemical identification of the proteins was done by immunoelectrophoresis using the rabbit anti-human seminal fluid.

Three main fractions were obtained after elution with the Tris-phosphate buffer.

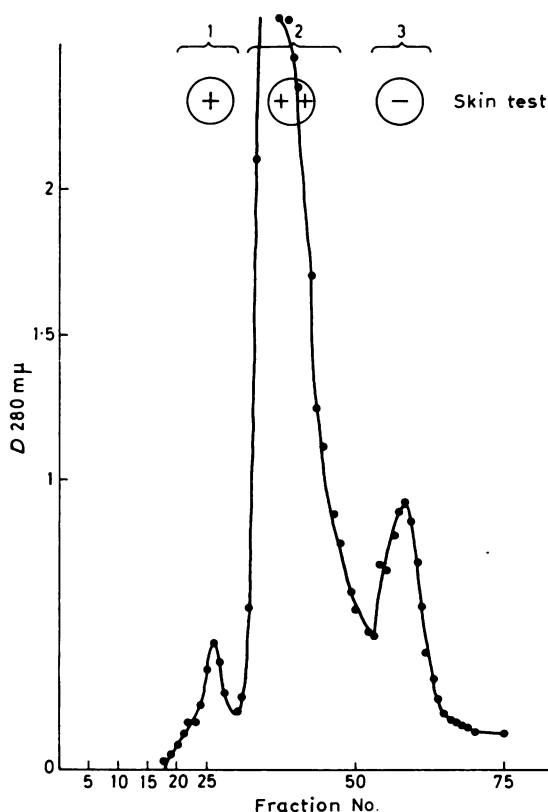


FIG. 3. Elution patterns of human seminal proteins separated by chromatography on DEAE-cellulose. The fractions were identified according to their electrophoretic mobility and classified in three main families. Fraction 2 migrating as serum β -globulin and staining intensely with PAS-Schiff reagent, contains the bulk of the antigenic constituents. The intensity of the skin reactions obtained with the various fractions is indicated in the circles.

TABLE 2
SIALIC ACID CONTENT OF THE SEMINAL
FLUID AND OF THE DEAE FRACTIONS

Fraction No.	Sialic acid (mg/100 mg protein)
Seminal fluid	0.87
1	0.74
2	1.66
3	0.13

Protein and sialic acid were determined in each fraction and are shown in Table 2. Fraction 2 was the richest in sialic acid. The immunoelectrophoretic pattern of the separate fractions is given in Fig. 4. While seminal fluid gave six well defined precipitation lines, Fraction 2 produced with the same antiserum a single line in the region corresponding to the slow β -globulins. When the same fractions were then tested on the patient's skin, a very strong reaction was obtained with Fraction 2.

Zone electrophoresis

Zone electrophoresis on different supporting materials (paper, cellulose acetate, starch gel) were used to separate the protein constituents of the seminal fluid, in order to localize

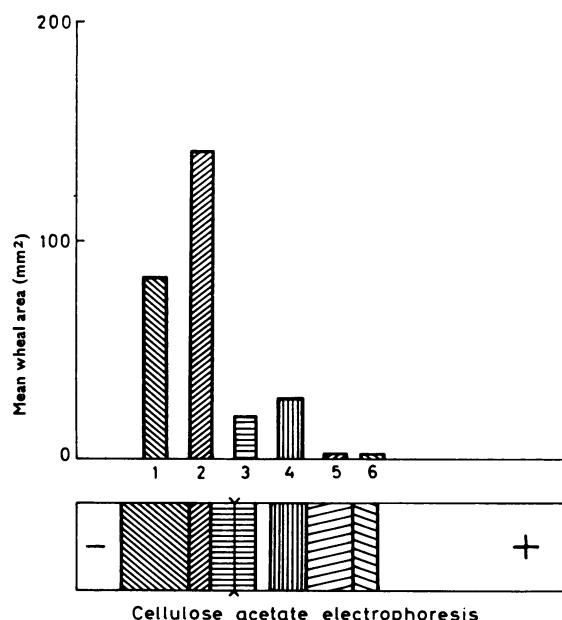


FIG. 5. Evaluation of the wheal reactions produced by the various fractions of human seminal fluid separated by electrophoresis on paper strip. The paper strip was cut into small strips according to the location of the protein bands, and applied to scarifications on the back of the patient. Positive reactions were observed with the two most basic fractions migrating to the cathode.

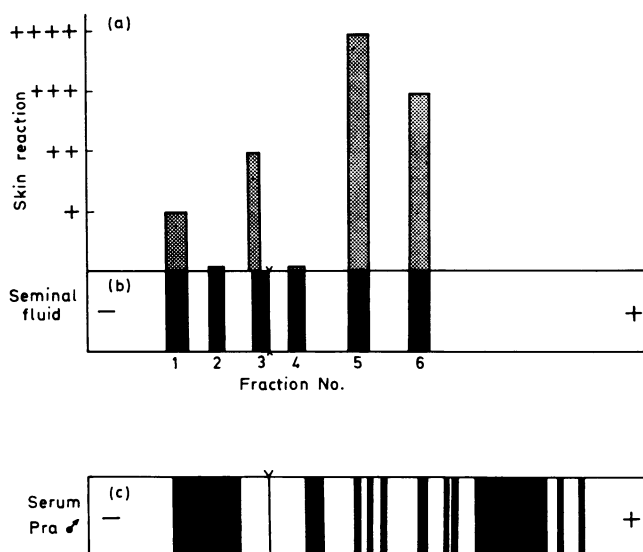


FIG. 6. Starch gel electrophoresis. Diagrammatic representation of the human seminal fluid fractions in relation to their skin reactive properties in Mrs P. From top to bottom: (a) Intensity of the skin reactions (intradermal injections). (b) Electrophoretic fractions obtained from pooled human semen. (c) Electrophoretic fractions obtained from human serum (the active fractions of the semen were located in the electrophoretic regions corresponding to the serum globulins).

and identify the protein(s) responsible for the allergic phenomenon. The patient's high sensitivity to the seminal protein made it possible to detect it with minimal amounts by applying small strips containing the electrophoretically separated semen fractions directly to the scarified skin of the patient. With the paper strips a strong reaction with urticarial wheal formation, reaching its height 10–20 minutes later, was obtained with the most basic fractions, migrating to the cathode (Fractions 1 and 2). The other fractions gave reactions identical with those of the saline control (Fig. 5).

On cellulose acetate electrophoresis, five to six bands were observed after staining with nigrosin, depending on the source of the seminal fluid. Here again, the two most basic protein fractions gave the strongest results in the cutaneous tests performed as described previously.

The results obtained with the protein isolated by starch gel electrophoresis were less well defined than those obtained with the other methods, as the eluates not containing proteins produced some non-specific skin irritation. It could nevertheless be seen that Fractions 5 and 6 gave consistently stronger reactions than the other eluates. These fractions migrated between serum albumin and the fast α_2 -globulin bands (Fig. 6).

IDENTIFICATION OF THE ANTIBODY IN THE SERUM OF THE PATIENT. PRECIPITATION WITH SPECIFIC ANTISERA

These investigations were performed with two different antisera, one directed against γ M-globulin and the other against γ A-globulin components of the immunoglobulins. The absorbed serum of the patient which lacked the γ M-globulin component (as tested by immunoelectrophoresis) was as effective as her whole serum in transferring the sensitivity to human receptors. The serum devoid of the γ A-globulin did not, however, transmit the sensitivity to any of the six receptors used. The control transfer made with the patient's whole serum in the same receptor gave positive results (Table 3).

TABLE 3
PASSIVE TRANSFER WITH THE SERUM OF MRS P. BEFORE AND AFTER ABSORPTION WITH RABBIT ANTISERUM FOR HUMAN IgA

Nature of serum	Results	Nature of serum	Results
Mrs P. serum Undiluted	4+, 4+, 4+, 3+, 3+, 3+	Serum of Mrs P. undiluted after precipitation with rabbit antiserum for IgA	$\pm$, $\pm$, 0, 0, 0, 0
Diluted 1:10	2+, 3+, 2+, 1+, 2+, 2+	Control serum undiluted	$\pm$, 0, 0, 0, 0, 0
Diluted 1:100	$\pm$, 1+, $\pm$, 1+, $\pm$, 0	Saline	$\pm$, 0, 0, 0, 0, 0

The sera were tested on six different recipients.

FRACTIONATION OF MRS P.'S SERUM BY DEAE CHROMATOGRAPHY

This was done on a DEAE-cellulose column 4×75 cm equilibrated with a Tris-phosphate buffer, 0.03 M, pH 8.2. Thirty millilitres of serum dialysed against this same buffer were applied to the column; elution was done with a gradient from 0.03 to 0.3 M Tris-phosphate buffer, pH 8.2 at 4°.

One hundred and fifty fractions of 10 ml were collected and the protein peaks were localized by their absorption at 280 m μ , pooled as shown in Fig. 7, dialysed against 0.9

per cent w/v NaCl and concentrated by dialysing against a 25 per cent (w/v) solution of Ficoll (Pharmacia Uppsala, Uppsala, Sweden.)

Fig. 7 shows the elution pattern obtained, as well as the pooled fractions prepared by mixing the contents of the appropriate tubes. Fig. 7 also shows the electrophoretic patterns of these pooled fractions obtained on cellulose acetate strips.

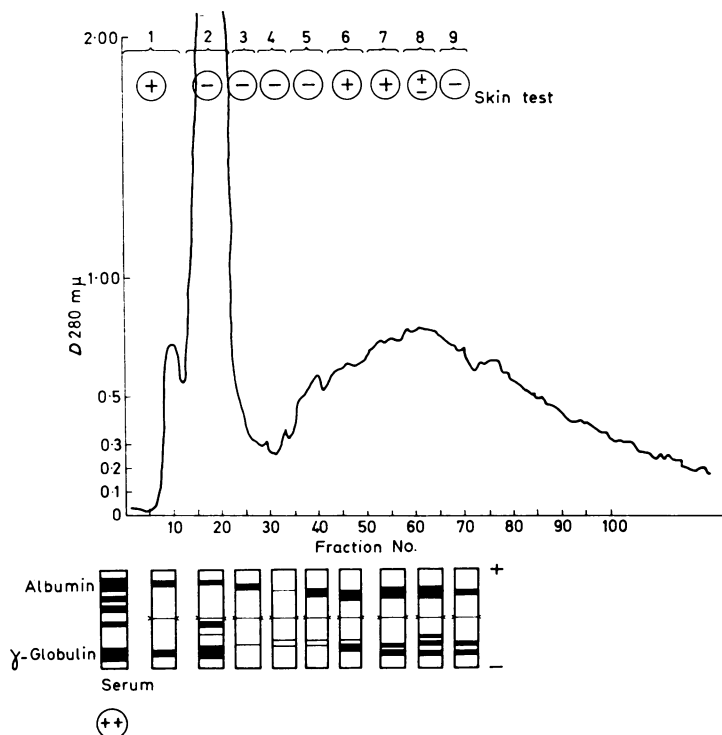


Fig. 7. Protein fractions of Mrs P.'s serum obtained by DEAE column chromatography. The electrophoretic patterns of the fractions obtained on cellulose strips and the location of the serum fraction containing the antibody identified by passive transfer.

Positive reactions in passive transfer were observed with fractions 6 and 7 and a weak reaction was obtained with fraction 1.

Passive transfer was done with all the protein fractions. Fraction 1 gave a weakly positive result and strongly positive results were obtained with fractions 6 and 7. All the other fractions gave negative results. Fraction 1 was eluted from the column in front of the main peak of the 7S γ-globulins (γG) and fractions 6 and 7 contained among other globulins the bulk of γA-globulin (to be described elsewhere).

HYPOSENSITIZATION

For the past 2 years Mrs P. has been submitted to a 'hyposensitization' treatment. She has been receiving injections of total human seminal fluid in an attempt to induce the formation of 'blocking antibodies' which are credited with the attenuation of clinical symptoms in other reaginic allergies (Loveless, 1940).

In spite of the treatment, the tolerance of the patient to the seminal fluid remained unchanged as judged by the systemic reaction occurring regularly with a defined dose

and by the intensity of the symptoms after normal sexual intercourse. The serum antibody level measured by passive haemagglutination remained insignificant.

DISCUSSION

The case of Mrs P. is unprecedented in that it seems to represent a spontaneous hypersensitization to a constituent of human origin. The allergy presented by Mrs P. combines all the main symptoms of the immediate type of hypersensitivity (Gell and Coombs, 1963). Beside the rapid onset of the symptoms, all the classical manifestations of anaphylactic shock were present: generalized urticaria and oedema; congestion and swelling of the mucous membranes with asthmatic symptoms; cardiovascular collapse and loss of consciousness. Mrs P. has a strong atopic family history although she herself had not had clinical evidence of other atopic conditions.

According to the patient, symptoms occurred after the very first sexual intercourse and we believe that she had indeed not had previous sexual experience. This makes it difficult to perceive how the initial antigenic stimulation can have occurred. There was evidence of gestation with a twin brother. One is left with the possibility of sensitization due to some unknown substance cross-reacting with human seminal protein.

Our studies show that the sensitizing antigen was associated with a glycoprotein of seminal fluid which was absent from serum. The spermatozoa themselves were found not to carry the antigen. We have as yet not been able to ascertain the exact origin of this glycoprotein; it may be derived from the prostate, the seminal vesicles, the epididymis or the spermatic cords. The seminal antigen was found to be specific for man. It was labile, even when kept at 0° as judged by the skin tests. It could be stored somewhat longer at -20°.

It has to be admitted that the purified antigen was less active in eliciting skin reactions than the original unfractionated seminal fluid, possibly due to some denaturation during isolation; another possibility is that the tested fraction represented only one of several active antigens. However, no additional active fraction could be isolated. The reaginic nature of the antibody involved in the reactions described here was established by the following criteria: (1) it was found to be thermo-labile at 56°, and (2) it had the type of tissue affinity characteristic of reagins previously described by Augustin and co-workers (Augustin, 1961; Augustin, Connolly and Lloyd, 1964); i.e. the active antibodies could be passively transferred to human and primates, but not to other animal species.

In contrast to this we were able to show in recent experiments (Kabat, Liacopoulos, Halpern and Relyveld, 1963) that γ_2 (= γ G-globulin) human antibodies (such as anti-diphtheria toxin or antidextran antibodies) can be transferred to the skin of guinea-pigs. These antibodies also sensitized *in vitro* the ileum of guinea-pigs. Our failure to sensitize guinea-pig tissues *in vivo* and *in vitro* with the antibodies of Mrs P. therefore supports the claim that they are not of the γ G-globulin type, but are similar to reaginic antibodies. Our negative results obtained with the passive haemagglutination technique are also in agreement with this conclusion. Again, similar negative results were obtained by others with antibodies of a reaginic nature (Augustin, 1961; Augustin *et al.*, 1964). The fact that the sensitizing antibodies of Mrs P. were found to be present mainly, if not exclusively, in a serum fraction containing the γ A-globulins further confirms this conclusion since this same immunoglobulin fraction was also found by others to contain the reaginic antibodies (Augustin and Hayward, 1960; Heremans and Vaerman, 1962; Augustin *et al.*, 1964; Fireman, Vannier and Goodman, 1963).

The apparent inability of our patient to produce blocking antibodies for the same allergen contrasts with the formation of such antibodies in spontaneous allergies and may be due to the fact that an homologous antigen was involved in this exceptional case.

REFERENCES

- AUGUSTIN, R. (1961). 'Precipitins, reagins and blocking antibodies.' *Acta allerg. (Kbh.)*, **16**, 473.
- AUGUSTIN, R. and HAYWARD, B. J. (1960). 'Human reagin to grass pollens and moulds: their purification and physico-chemical characterization.' *Immunology*, **3**, 45.
- AUGUSTIN, R., CONNOLLY, R. C. and LLOYD, G. M. (1964). 'Atopic reagins as a prototype of cytophilic antibodies.' *Proc. 11th Coll. on Protides Biol. Fluids, Bruges*, 1963, p. 56. Elsevier, Amsterdam.
- COOKE, R. A. (1947). *Allergy in Theory and Practice*. Saunders, Philadelphia.
- COOMBS, R. R. A., MOURANT, A. F. and RACE, R. R. (1945). 'A new test for the detection of weak and incomplete Rh agglutinins.' *Brit. J. exp. Path.*, **26**, 255.
- DAUSSET, J. (1956). *Immunohématologie Biologique et Clinique*, p. 431. Editions Médicales Flammarion, Paris.
- DAUSSET, J., COLOMBANI, J. and OKOCHI, K. (1964) *Immunological Methods* (Ed. by J. F. Ackroyd), pp. 575-595. Blackwell Scientific Publications, Oxford.
- FAHEY, J. L. and HORBETT, A. P. (1959). 'Human γ globulin fractionation on anion exchange cellulose columns.' *J. biol. Chem.*, **234**, 2645.
- FIREMAN, P., VANNIER, W. E. and GOODMAN, H. C. (1963). 'The association of skin-sensitizing antibody with the β_2 A-globulins in sera from ragweed-sensitive patients.' *J. exp. Med.*, **117**, 603.
- GELL, P. G. H. and COOMBS, R. R. A. (1963). *Clinical Aspects of Immunology*, p. 317. Blackwell Scientific Publications, Oxford.
- GORNALL, A. G., BORDAWILL, C. J. and DAVID, M. M. (1949). 'Determination of serum proteins by means of the biuret reaction.' *J. biol. Chem.*, **197**, 757.
- GRABAR, P. and WILLIAMS, C. A. (1955). 'Immuno-electrophoretic analysis of mixtures of antigenic substances.' *Biochim. biophys. Acta (Amst.)*, **7**, 61.
- GRABAR, P. and BURTIN, P. (1960). *L'Analyse Immuno-electrophorétique*. Masson, Paris.
- HALPERN, B. N., LIACOPOULOS, P., LIACOPOULOS-BRIOT, M., BINAGHI, R. and VAN NEER, F. (1959). 'Patterns of "in vitro" sensitization of isolated smooth muscle tissues with precipitating antibody.' *Immunology*, **2**, 351.
- HALPERN, B. N., JACOB, M., BINAGHI, R. and PARLEBAS, J. (1961). 'Mise en évidence et dosage des anticorps allergiques par hémagglutination "in vitro" dans les syndromes allergiques et expérimentaux.' *Rev. franç. Allerg.*, **1**, 201.
- HEREMANS, J. F. and VAERMAN, J. P. (1962). ' β_2 A-globulin as a possible carrier of allergic reaginic activity.' *Nature (Lond.)*, **193**, 1019.
- KABAT, E. A., LIACOPOULOS, P., LIACOPOULOS-BRIOT M., HALPERN, B. N. and RELYVELD, E. H. (1963). 'Studies on the sensitizing properties of human antisera and purified antibodies.' *J. Immunol.*, **90**, 810.
- KOHN, J. (1957). 'A cellulose acetate supporting medium for zone electrophoresis.' *Clin. Chim. Acta*, **2**, 297.
- LAYTON, L. L., LEE, S. and YAMANAKA, E. (1962). 'Allergen testing on monkeys passively sensitized by hay fever and asthma reagins of human sera.' *Nature (Lond.)*, **193**, 988.
- LOVELESS, M. H. (1940). 'Immunological studies of pollinosis. I. The presence of two antibodies related to the same pollen-antigen in the serum of treated hay fever patients.' *J. Immunol.*, **38**, 25.
- OVARY, Z. (1958). 'Immediate reactions in the skin of experimental animals provoked by antibody-antigen interaction.' *Progr. Allergy*, **5**, 459.
- PRAUSNITZ, C. and KUESTNER, J. (1921). 'Studien über Überempfindlichkeit.' *Zbl. Bakt. II Abt.*, **86**, 160.
- ROULIER, P. (1950). 'Sur une réaction quantitative de fixation du complément titré à 50% d'hémolyse.' Thèse de Pharmacie présentée à Montpellier.
- RUMKE, P. (1959). 'The presence of sperm antibodies in serum of two patients with oligozoospermia.' *Vox Sang. (Basel)*, **4**, 135.
- SMITHIES, O. (1955). 'Zone electrophoresis in starch gels. Group variation in the serum proteins of normal human adults.' *Biochem. J.*, **61**, 269.
- SOBER, H. A. and PETERSON, F. A. (1958). 'Protein chromatography on ion-exchange cellulose.' *Fed. Proc.*, **17**, 1116.
- STAVITSKY, A. B. and ARQUILLA, E. R. (1955). 'Micromethods for the study of proteins and antibodies. III. Procedure and applications of haemagglutination and haemagglutination inhibition reactions with bis-diazotized benzidine and protein conjugated red blood cells.' *J. Immunol.*, **74**, 306.
- SVENNERHOLM, L. (1957). 'Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method.' *Biochim. biophys. Acta (Amst.)*, **24**, 604.
- TSUKI, S., HASHIMOTO, J. and PIGMAN, W. (1961). 'Comparison of procedures for the isolation of bovine submaxillary mucin.' *J. biol. Chem.*, **236**, 2172.