THE ANTIGENS AND AUTOANTIGENS OF THE SEMINAL VESICLE

I. Immunochemical Studies on Guinea Pig Vesicular Fluid*

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The male reproductive system culminates its activities in the production of semen (seminal plasma + spermatozoa), and many studies have been made on the antigens of these constituents. Much of this work has been recently reviewed (1, 2). The seminal plasma receives many of its components from the several accessory glands of reproduction, especially the seminal vesicle and the prostate gland. Prostatic fluid and tissue have both been subjected to considerable antigenic and biochemical analysis. For example, human prostatic fluid has been shown to contain at least four tissue-specific antigens (3, 4). Furthermore, prostatic acid phosphatase has been demonstrated in several precipitation bands in gel diffusion precipitation of human prostatic fluid and prostate extract (5, 6). Ultracentrifugal analysis of human prostatic fluid revealed a small fast peak and a larger slowly sedimenting peak. These peaks had sedimentation coefficients of 15.6 S and 3.8 S, respectively (7).

It has been possible to produce autoantibodies to prostatic tissue by means of isoimmunization procedures in the rabbit (8, 9). The antigen involved, being absent from a large variety of rabbit tissues tested, can therefore be considered as an accessory glands-specific antigen. It was also absent from the prostatic tissue of a number of other species, indicating a high degree of species specificity.

Although these accessory reproductive tissues in the rabbit have been intensively studied in recent years, specific and significant tissue damage concomitant with immunologic mechanisms has not been observed. It was thought that this might involve differences in species susceptibility, because it is well documented that isoimmunization with other portions of the male reproductive system, namely, testes and spermatozoa, can readily induce aspermatogenesis in a limited number of species, especially the guinea pig, but not as readily in the rabbit (10). We therefore decided to study the most prominent accessory gland of the guinea pig, namely, the seminal vesicle, in an effort to explore the possibilities of establishing an experimental autoimmune disease of this gland. Preliminary work has been reported (11).

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Materials and Methods

Seminal Vesicle Structure.—The seminal vesicles are free in the peritoneal cavity except for their attachment at the urethral end. They are two delicate wormlike structures about 10 cm in length and 6 mm in diameter at their base; they taper somewhat from the urethral to the distal end. The outside is smooth and is covered by a fascial sheath which supports the blood vessels. The secretion within them consists of a clear, tenacious material which during life is fluid, but within a few minutes after death assumes the form of a gel, from which the fluid can be expressed.

Guinea Pig Seminal Vesicle (Vesicular) Fluid.—Mature male Hartley albino guinea pigs, usually 15 per preparation, were anesthetized with ether and then exsanguinated by cardiac puncture. The peritoneal cavity was opened and the seminal vesicles were tied off with suture material. These glands were then removed and the fluid inside them expressed onto a watch glass. The viscous fluid was weighed and diluted with saline solution (0.15 M NaCl) to a wt:v (g:ml) ratio of 1:3. The seminal vesicles of each animal usually contained approximately 1 g of fluid. This solution was centrifuged in a refrigerated Sorvall centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) at a speed of 10,000 rpm for 60 min. The supernatant fluid was divided into 4 ml portions and stored by freezing (−20°C). This is the material used in all further experiments; it will be called seminal vesicle (or vesicular) fluid (VF or GPVF).[1]

Protein concentration was determined on this and all other fluids, or extracts, using a Beckman model DU spectrophotometer at a wavelength setting of 540 mμ, according to the biuret method of Gornall, Bardawill, and David (12). An extinction coefficient of 2.8 was utilized, as determined in this laboratory for pooled human serum.

Organ Extracts.—Extracts were made of the guinea pig prostate and coagulating glands together, because these tissues were extremely difficult to distinguish from each other grossly. About 15 animals were used per preparation, as was the case for other organ extracts. 1 part (grams) of tissue was usually homogenized for 1 min with 1½ parts (milliliters) of saline solution, in a Sorvall Omnimixer. This homogenate was centrifuged in a refrigerated Sorvall centrifuge at a speed of 10,000 rpm for 60 min. The supernatant fluid was stored by freezing. Other tissues were treated in the same manner to obtain extracts. Protein concentration was determined as described above.

Adjuvant.—Complete Freund's adjuvant (CFA) was purchased from Difco Laboratories, Detroit, Mich. This adjuvant contained 0.5 mg/ml of *Mycobacterium butyricum*. Fortified adjuvant was prepared by adding 6 mg/ml of a dry powder of this bacterium to the complete adjuvant.

Isoantisera.—Eight groups of animals were included under this heading. These groups contained Hartley albino guinea pigs, which were immunized with either complete or fortified Freund's adjuvant. After all immunization schedules were completed, the animals in all these groups were exsanguinated by cardiac puncture. All of the immunization procedures and schedules are summarized in Table I. Control male guinea pigs were injected intradermally with another protein material, namely rabbit vesicular fluid, and with fortified complete Freund's adjuvant.

Heteroantisera.—Two groups of animals, New Zealand male rabbits, were included under this heading. All injections contained complete Freund's adjuvant mixed in equal parts with the protein solution. The volume of each injection was 1 ml and each was administered intradermally in the shaved back of the animal. If an animal received more than one injection, it was given at intervals of 3 wk.

[1] *Abbreviations used in this paper:* CFA, complete Freund's adjuvant; SP, seminal plasma; SV, seminal vesicle; HSA, human serum albumin; VF or GPVF, guinea pig vesicular fluid.

All rabbits were bled at weekly intervals from the central vein of the ear. The final bleedings of all animals were taken by cardiac puncture. All of the immunization procedures and schedules are summarized in Table I.

Passive Hemagglutination.—The tanned cell hemagglutination method was that of Boyden (13), as modified by Shulman et al. (8, 9). Human red blood cells of group O, preferably Rh negative, were used in this test. The antisera to be tested were diluted serially, using an apparatus manufactured by the Cooke Engineering Company (Alexandria, Va.) under the brand name Microtiter. Threefold dilutions were done with microdiluters in round-bottom microtiter plates.

The plates were shaken to insure an even distribution of cells and read after about two hr at room temperature. The sedimentation patterns were graded +++, ++, +, ±, or −.

TABLE I

Schedule of Injections in Test Animals

Group No.	Animal No.	Animal species and sex		No. of injections	Injected antigen		Injection intervals	Bleeding intervals
						(*mg*)	(*wk*)	(*wk*)
Isoimmunization								
1*	7, 9, 10	GP	M	4	GPVF	10	3	3
2*	11, 12, 13, 14	GP	M	4	GPVF	1	3	3
3	15, 16, 17, 18	GP	M	4	GPVF	10	3	3
4	19, 21, 22	GP	M	4	GPVF	1	3	3
5*	1, 3, 5, 3677, 3687, 3698, 3694	GP	M	4	GPVF	10	3	3
6	30–39	GP	F	3	GPVF	10	3	(1–3)
7§	26, 27, 28, 29	GP	F	3	GPVF	10	3	3
8§	76–85	GP	M	3	GPVF & SV	(0.1 g by wt.)	—	—
Heteroimmunization								
9	5, 6, 8	R	M	1	GPVF	10	—	1
10	111, 113, 114	R	M	3	GPVF	10	3	1

* Intraperitoneal injection, all others intradermal except for the animals of group 8 which received subcutaneous injections.

§ Fortified complete Freund's adjuvant used.

Appropriate controls were always included, using tanned but uncoated cells with immune serum and diluent plus coated cells.

Inhibition of Passive Hemagglutination.—Inhibition of tanned cell hemagglutination was performed at a constant antiserum dilution which corresponded to approximately 27 agglutinating units, using the same microtechnic described for passive hemagglutination. The various antigens being compared as inhibitors were serially diluted, starting at the same initial concentration of protein. The initial mixtures (antigen and antibody) were left for 30 min at room temperature, and then 0.025 ml of the tanned and coated red blood cells was added to each mixture. Readings of agglutination were made in the usual way.

Immunodiffusion.—Double diffusion gel precipitation was performed in disposable Petri dishes (Falcon Plastics, Culver City, Calif.) with tightly fitted covers. A 1% suspension of agarose (Mann Research Laboratories, Inc., New York) or agar in saline solution, with 0.1% sodium azide added as preservative, was used in these studies. 3 ml of this material was

poured into the bottom of each plate and allowed to harden. The desired pattern was placed as a sketch under the plate. Wells were cut with the use of a corkborer. The wells were usually 5 mm in diameter and the inside edges of the peripheral wells were 3 mm from the edge of the tenter well.

The wells were filled with the desired solutions and kept at room temperature. Plates were sketched and photographed for permanent record. Plates were sometimes washed in saline followed by distilled water. The agar was then dried and stained by the Amidoschwarz technic.

Skin Testing for Delayed Hypersensitivity.—These tests were made at 19 days after the initial injection. Into one site on the shaved back of the animal was injected 10 μg of test material, and into the other, 100 μg. All injections were contained in a 0.1 ml volume. Normal serum of the species tested was injected into one site in a 100 μg amount. The test sites were observed at 2, 24, and 48 hr. The size of any erythema and induration was measured in two diameters at right angles to each other.

The following gradation scale was used:

$<$3 mm, $\pm$; 3–5 mm, +; 5–10 mm, ++; 10–15 mm, +++; $>$ 15 mm, ++++

Histologic Sections.—Tissues were fixed in buffered formalin, and imbedded in paraffin wax after dehydration. Thin sections were cut with a microtome, placed on slides, and stained with hematoxylin and eosin.

Starch Block Electrophoresis.—A slurry of potato starch, in barbital buffer (pH 8.60, $\Gamma/2$ 0.05) was poured into a plastic frame. A trough was cut at an appropriate distance from the cathode end. This trough was filled with the desired protein solution, mixed in a slurry with a small amount of potato starch. A constant voltage of 250 v was utilized for a duration of 24 hr at 4°C. The current varied between 10 and 15 ma, depending on the dryness of the block. The block was sliced into 1 cm sections. Each segment was placed in a 15 ml capacity Büchner funnel with a coarse sintered glass disc. These were placed in 50 ml polyethylene centrifuge tubes, which were spun at 2000–3000 rpm. Protein concentration of the eluate was determined by the biuret procedure (12). A graph of optical density vs. segment number was prepared. Appropriate fractions were pooled and concentrated by pervaporation.

Cellulose Acetate Electrophoresis.—A Beckman Microzone apparatus was utilized for this procedure. The cellulose acetate membrane, after being soaked in barbital buffer (pH 8.60, $\Gamma/2$ 0.075), was placed in a cell filled with the same buffer. The sample in microliter amounts was applied to the membrane. The run was for 20 min at a constant 250 v at room temperature. The current varied from 3 to 6 ma. The membrane was stained with Ponceau S for protein, photographed, and finally cured for storage as a permanent record or for densitometer scanning.

Velocity Ultracentrifugation.—Experiments were carried out in the Spinco Model E analytical ultracentrifuge, using a single-sector cell with a 12 mm light path, at a nominal speed of 60,000 rpm. Sedimentation coefficients (s) were calculated in the usual way and corrected to standard conditions by the usual procedure. Ultracentrifugal experiments were also used as an aid in determining the homogeneity of purified extracts.

Diffusion Coefficient Determination.—The analytical ultracentrifuge, equipped with a synthetic boundary cell, was used at 8000 rpm. Diffusion coefficients (D) were calculated by plotting $(A/H)^2$ vs. time of the schlieren peak, where A is the area of the peak and H is its height at a given time. The slope (m) of the line obtained from the data was applied to the following equation: $D = m/4\pi$). This diffusion coefficient was then corrected to standard conditions by the usual procedure.

Molecular Weight Determination.—Molecular weights (MW) were calculated by use of the equation: $MW = RTs/D(1 - \bar{v}\rho)$, where R is the universal gas constant, T is absolute temperature, $\bar{v}$ is the partial specific volume of the protein solution, and ρ is the density of water at 20°C.

RESULTS

Biochemical Analyses

Electrophoretic Analysis.—A pool of the GPVF was subjected to cellulose acetate electrophoresis at pH 8.6. When 0.25 μl of a 3% solution was examined, three distinct components were revealed. To make this analysis more sensitive, a larger amount of protein was used in subsequent tests. The application of 1.0

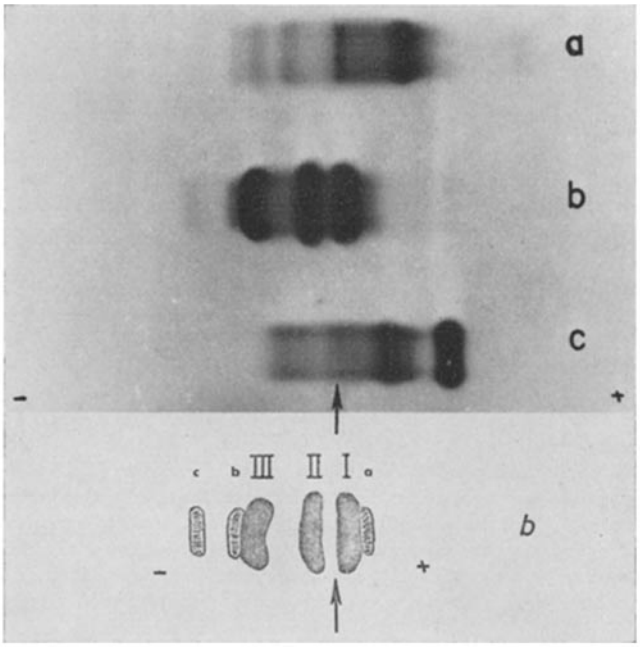

FIG. 1. Electrophoretic patterns on a cellulose acetate membrane. The applied samples were: (*a*) guinea pig seminal plasma (pooled), (*b*) guinea pig vesicular fluid (pooled), (*c*) normal guinea pig serum (pooled). The arrow indicates the point of application of the protein solutions. The sketch shows the essential features of the pattern for vesicular fluid.

μl of this material to the membrane resulted in the detection of three additional components. The three major components were numbered from anode to cathode: *I*, *II*, and *III*. The minor components, being designated in a similar sequence, were called *a*, *b*, and *c*. This is shown in Fig. 1 along with a sketch and, for comparison, the patterns of serum and seminal plasma. None of the major or minor components of VF was seen in normal guinea pig serum.

Densitometer tracings of the major components of VF as separated on the cellulose acetate membrane have revealed that these three componens are present in approximately equal proportions in whole vesicular fluid. Furthermore, these components make up approximately 99% of the total soluble protein as

seen in electrophoresis; a typical tracing is shown in Fig. 2. Only the major components have been studied in any further detail.

The electrophoresis has also been carried out in buffers at higher and lower pH values than 8.6. This included a 0.1 M sodium phosphate buffer, pH 7.5, and a 0.2 M sodium phosphate buffer, adjusted to pH 9.7 with NaOH. In addition, the amount of protein applied to the membrane was varied at these different pH values. At the more acidic pH value, poor separation of the major components was revealed. Likewise at the more basic pH value, poor separation and distorted patterns were seen. No new components were ever resolved in any of these experiments.

Ultracentrifugal Analysis of Whole GPVF.—Analysis in the analytical ultra-

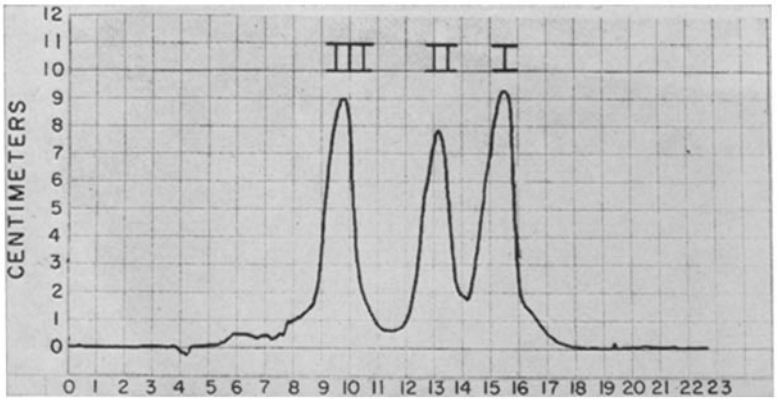

FIG. 2. A densitometer tracing of an electrophoresis pattern (cellulose acetate membrane) of pooled guinea pig vesicular fluid.

centrifuge suggested that this fluid is homogeneous, as determined by this technic. Fig. 3 illustrates one of the runs made on such a preparation. A number of runs were made using pooled or individual preparations of GPVF. In each experiment the fluid looked homogeneous and revealed a sedimentation coefficient of approximately 1.5 S. Changes in the concentration of the material used had no effect upon the schlieren pattern seen. Even the use of a 2% solution of GPVF did not reveal the presence of a second component.

Electrophoretic Fractionation.—The major components of GPVF were then isolated by means of starch block electrophoresis. Reproducible results were obtained in 11 different runs. The elution curve from this type of electrophoresis showed three peaks, as shown in Fig. 4. Appropriate tubes in each peak were pooled, and these pools were then analyzed by cellulose acetate electrophoresis. The purity of each of the three components, obtained by starch block electro-

phoresis, is revealed in the cellulose acetate membrane pictured in Fig. 5. No mixtures of the major components were revealed, even when as much as 4.0 μl of a 0.5% solution of a preparation of each component was applied to the membrane.

Ultracentrifugal Analysis of Fractionated GPVF.—Each purified component of VF was again analyzed in the analytical ultracentrifuge. There was no difference in the shape of the schlieren patterns seen, or in the sedimentation coefficients calculated, as compared with those obtained with whole GPVF. Variations in concentration also had no effect on the sedimentation coefficient of each of the purified components.

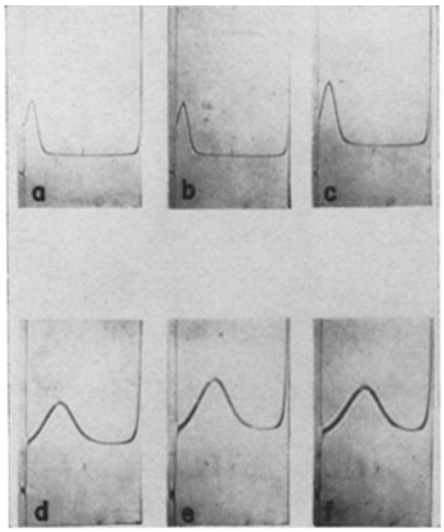

FIG. 3. The schlieren peaks seen during velocity ultracentrifugation of pooled guinea pig vesicular fluid (total protein, 1.0%). Sedimentation was to the right. Successive pictures were at the indicated minutes and bar angles: (*a*) 28′, 70°, (*b*) 30′, 70°, (*c*) 46′, 60°, (*d*) 151′, 50°, (*e*) 191′, 40°, (*f*) 202′, 40°.

Technic for Determination of Diffusion Coefficient and Molecular Weight.—The method for determining the diffusion coefficient was first tested on human serum albumin (HSA). A 1.0% solution of HSA was used, and measurements and calculations were made for a series of pictures. The points fell on a straight line, and from the slope of this line was calculated the value of D. The diffusion coefficient of HSA was thus found to be 7.1×10^{-7} cm²/sec (at 1.0% concentration), as determined by this approximate method. This is reasonably close to the published value of 6.1×10^{-7} cm²/sec, representing the results at infinite dilution, as determined by more precise methods (14). The molecular weight of HSA was then also calculated. The values used for $\bar{v}$ and ρ were 0.75 cm³/g and 0.998 g/cm³, respectively. The molecular weight of HSA was found in this way

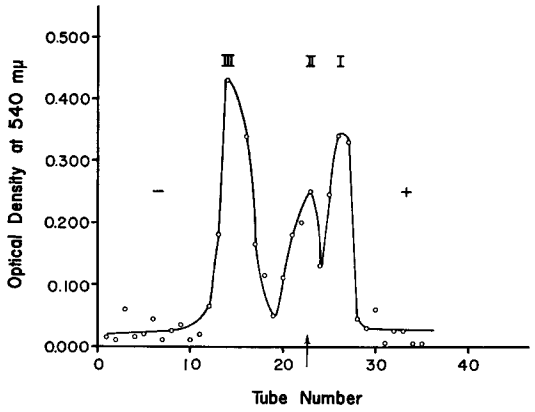

FIG. 4. Graph of the optical density of starch block electrophoresis eluates of guinea pig vesicular fluid, plotted against tube number. Arrow indicates the point of origin.

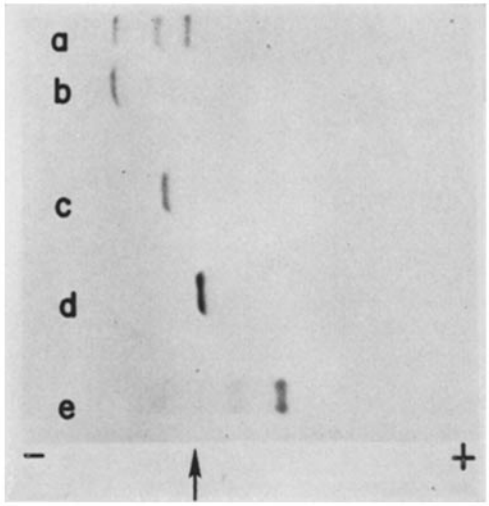

FIG. 5. Electrophoretic patterns on cellulose acetate membrane. The applied samples were: (*a*) guinea pig vesicular fluid, (*b*) component III of GPVF, (*c*) component II of GPVF, (*d*) component I of GPVF, (*e*) normal guinea pig serum. The arrow indicates the point of application of the protein solutions.

to be 58,000 and thus within reasonable agreement with the published value of 68,000 (14).

Diffusion Coefficient and Molecular Weight of Component II.—This method was then used to determine the diffusion coefficient of component II, at a protein concentration of 0.5%. The plot of $(A/H)^2$ vs. time gave a straight line, and D for component II was found to be 11.4×10^{-7} cm²/sec. From this value, the

value of s at 0.5%, namely, 1.5 S, and a value for $\bar{v}$ of 0.75 cm^3/g, an approximate molecular weight of 13,000 was calculated for this purified antigen.

Immunological Analyses

Heteroimmunization.—Whether one (Group 9) or several (Group 10) injections were given, antibody formation was induced only by component II. This was shown by the technics of gel diffusion and tanned cell hemagglutination.

Fig. 6 shows the tanned cell hemagglutination titers obtained in rabbits 5 (Group 9) and 113 (Group 10), respectively. In both cases, the indicator cells were coated with a pool of GPVF. When purified components I, II, or III (at ap-

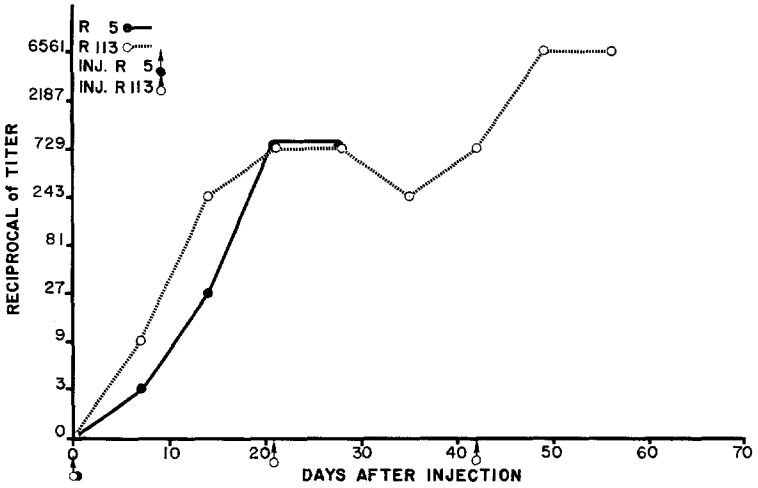

FIG. 6. Graph of the reciprocal of the hemagglutination titer plotted against time of bleeding for rabbits 5 and 113.

propriate concentration) was coated on indicator cells, a positive reaction was obtained only with the cells coated with component II (Table II). The gel diffusion plate pictured in Fig. 7 shows that these distinctions are also true for gel diffusion. Only component II and whole GPVF are antigenic, and each gives a line of precipitation with the antiserum, although sometimes this line appears to be split. These lines, as seen for the different preparations, merge in an identity reaction.

Tissue- and species-specificity studies were performed on the final bleeding of rabbit 113. Extracts of guinea pig kidney, liver, spleen, lung, heart, testis, and epididymis, as well as guinea pig seminal plasma, normal guinea pig serum, the guinea pig accessory glands of reproduction, rabbit, rat, and mouse male accessory gland extracts, human seminal plasma and dog prostatic fluid, were tested against the final bleeding of rabbit 113 by means of gel diffusion. Ex-

tensive dilutions of each fluid were made and tested. In no case did a line of precipitation appear for any of the extracts used, except those of the guinea pig accessory glands of reproduction. These extracts could be diluted to a reasonable degree and still produce a line of precipitation. This is shown in Fig. 8

TABLE II

Tanned Cell Hemagglutination of the Final Bleedings of Rabbits 5 and 113. Indicator Cells Were Coated as Shown

Antiserum dilution	Cells coated with component I		Cells coated with component II		Cells coated with component III	
	R5,d*	R113,h	R5,d	R113,h	R5,d	R113,h
1:3	±	+	+++	+++	−	±
1:9	−	−	++	++	−	±
1:27	−	−	+	++	−	−
1:81	−	−	+	+	−	−
1:243	−	−	+	+	−	−
1:729	−	−	−	+	−	−
1:2187	−	−	−	−	−	−
1:6561	−	−	−	−	−	−

* Trial bleedings are designated by consecutive letters of the alphabet.

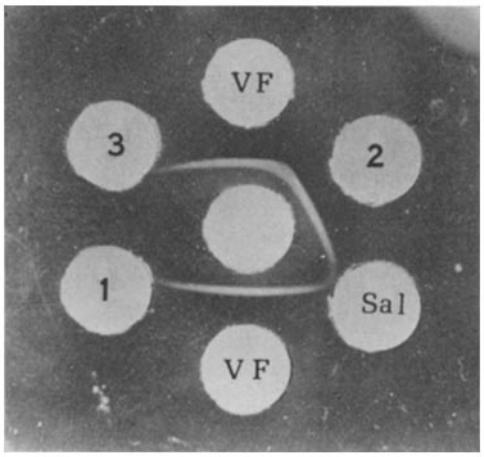

FIG. 7. Gel diffusion precipitation pattern. Central well: rabbit anti-guinea pig vesicular fluid (R113, h). Peripheral wells: VF, vesicular fluid; Sal, saline; 1, 2, 3, components I, II, III, respectively.

where the lines produced by component II, whole VF, whole SP (seminal plasma), prostate-coagulating gland extract, and seminal vesicle extract all merge in a reaction of identity.

Tissue- and species-specificity studies were also done by the more sensitive technic of inhibition of tanned cell hemagglutination. Only the extracts which

gave precipitation lines in gel diffusion, as just mentioned, gave significant inhibition titers in this test. This is shown in Table III. The antigenic component(s) of GPVF are, therefore, highly tissue and species specific. These tests also rule out the possibility that component II is a type of Forssman antigen, because guinea pig kidney, which contains a high concentration of the Forssman antigen, gave negative results when reacted with rabbit anti-GPVF by the various immunological tests described.

Isoimmunization: Antibody Response.—Eight groups of guinea pigs were immunized with material from the guinea pig seminal vesicle. Groups 1–7 were

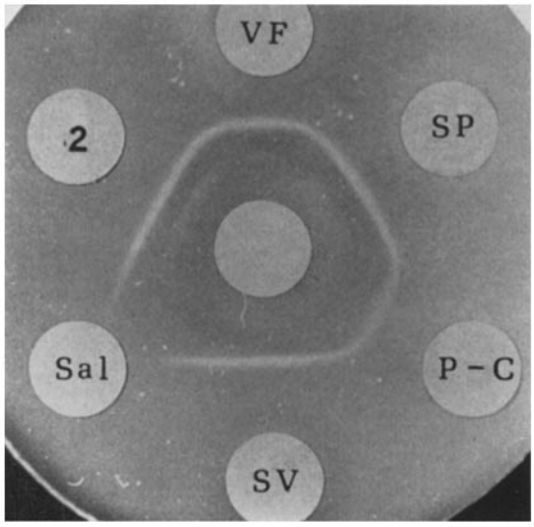

FIG. 8. Gel diffusion precipitation pattern. Central well: rabbit anti-guinea pig vesicular fluid (R113, h). Peripheral wells: VF, vesicular fluid; SP, seminal plasma; P-C, prostate-coagulating gland extract; SV, seminal vesicle extract; SAl, saline; 2, component II.

injected with soluble GPVF only; group 8 was injected with soluble and insoluble antigens of GPVF. Group 1–4 were male animals injected by different routes and with different amounts of protein, to compare the effects of these variables.

The animals of groups 1 and 2 were injected intraperitoneally with 10 mg and 1 mg of VF protein, respectively, per injection. Complete Freund's adjuvant was used in both of these experimental situations. When tested by tanned cell hemagglutination, with VF used as the coating antigen, animals in both groups gave low (1:81) but significant titers, produced after three or four injections. All of the animals in group 1 responded to the injections, and three of the four animals of group 2 reacted.

The animals of groups 3 and 4 were injected with the same material as the

animals of groups 1 and 2, respectively, but intradermally. Both groups were tested as before and gave quicker responses (after only one injection) than in groups 1 and 2. The animals of group 3 (injected with 10 mg of protein per injection) responded with higher titers (1:243) than the animals of group 4 (1:81). Three of the four animals in group 3 responded to the injections, and all of the rabbits in group 4 reacted by forming humoral antibodies. The amount of protein injected did not make a significant difference, 10 mg being only slightly better, but the route of injection made quite a difference, that of the intradermal method being far superior.

TABLE III

Inhibition of the Reaction between Rabbit Anti-Guinea Pig Vesicular Fluid (R113, h–1:243 Dilution) and Human Tanned Red Blood Cells Coated with Guinea Pig Vesicular Fluid

Antigen dilution	Prostate-coagulating gland or fluid	Seminal vesicle	Vesicular fluid	Seminal plasma	Kidney	Liver	Spleen	Heart	Lung	Testis	Epididymis	Rabbit male accessory gland	Rat male accessory gland	Mouse male accessory gland	Human seminal plasma	Dog prostatic fluid	Normal guinea pig serum
1:2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
1:4	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
1:8	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
1:16	+	±	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
1:32	+	+	−	±	+	+	+	+	+	+	+	+	+	+	+	+	+
1:64	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:128	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:256	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:512	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:1024	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1:4096	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The undiluted protein concentration of each inhibitor was adjusted to 1.5%. Dilutions of inhibiting specimens are listed below. All are guinea pig extracts unless otherwise mentioned.

The animals of group 5 were male guinea pigs injected intradermally with 10 mg of GPVF protein per injection, using fortified complete Freund's adjuvant. All of these animals responded with significant titers, as tested in tanned cell hemagglutination, after the first injection. Two of the animals produced the highest titers seen in this system, 1:729.

The animals of group 6 were female guinea pigs injected intradermally with 10 mg of GPVF protein per injection, using complete Freund's adjuvant. 7 of the 10 animals of this group responded with significant titers, as tested in tanned cell hemagglutination, after the first injection.

The animals of group 7 were female guinea pigs injected in the same way as the animals of group 6, except that fortified complete Freund's adjuvant was

used. All of the animals of this group responded after the first injection, as tested by tanned cell hemagglutination, and with significantly high titers, 1:729.

There were 10 male guinea pigs in group 8. These were injected with soluble and insoluble antigens of the seminal vesicle incorporated into complete Freund's adjuvant. 7 of these animals were positive for humoral antibody, as tested by tanned cell hemagglutination.

The male and female animals in the foregoing groups reacted equally well to the series of injections given them. The best route of injection was consistently the intradermal one, using fortified complete Freund's adjuvant. None of the animals in these groups formed detectable precipitating antibodies, as shown by extensive gel diffusion tests which were always negative.

TABLE IV

Tanned Cell Hemagglutination for Guinea Pigs 22 and 37. Indicator Cells Were Coated as Shown

Antiserum dilution	Cells coated with component I		Cells coated with component II		Cells coated with component III		Cells coated with GPVF from GP22
	GP22,a*	GP37,d	GP22,a	GP37,d	GP22,a	GP37,d	GP22.a
1:3	–	–	++	+++	–	–	+++
1:9	–	–	++	++	–	–	++
1:27	–	–	+	+	–	–	+
1:81	–	–	+	+	–	–	+
1:243	–	–	–	–	–	–	–
1:729	–	–	–	–	–	–	–
1:2187	–	–	–	–	–	–	–

* Trial bleedings are designated by consecutive letters of the alphabet.

The effective antigen was again shown by tanned cell hemagglutination to be component II, and the antibodies produced in the male guinea pigs have been shown to be autoantibodies. These findings can be seen in the data of Table IV.

Skin Testing.—The animals of groups 1–7 (injected with soluble antigen) were skin tested with GPVF for delayed hypersensitivity. Positive tests were obtained after the second or third injection, but never after only one injection. Two of the three animals of group 1 gave a positive test. Three of the four animals tested in group 2 were negative. One animal in group 3 was tested and was found to be positive. Two animals in group 4 were tested and one was positive. All of the animals of group 5 were positive. Six animals in group 6 were tested, and all were positive. Three animals in group 7 were tested, and all were positive. Only the animals injected intraperitoneally, with only 1 mg of vesicular fluid protein per injection, were negative upon skin testing. When the guinea pigs of groups 1 and 2 which were injected intraperitoneally were omitted, and

only groups 3–7 (injected intradermally) were considered, 15 animals were found with positive skin tests of the 16 tested.

The animals of group 5 were also skin tested for delayed hypersensitivity with 10 and 100 μg of each of the purified major components of GPVF. Components I and III did not induce any positive skin reactions. Only component II gave positive skin tests, which were well correlated with those obtained with whole GPVF.

The animals of group 8 (injected with soluble and insoluble antigen) were skin tested with GPVF for delayed hypersensitivity. Positive results were obtained, after only one injection, in the same seven animals which were positive for humoral antibody.

Guinea pigs injected with rabbit vesicular fluid and fortified CFA were never induced to form antibodies which reacted with GPVF, or other guinea pig components.

Histologic Results.—The accessory glands and many other tissues from the animals of groups 1–5 and 8 were examined for histologic abnormalities. Two types of abnormalities were noted, and these seemed to be specific for the seminal vesicle. The first of these abnormalities was a white cell infiltration (lymphocytes, plasma cells, etc.). This infiltration was confined to the epithelial lining and lamina propria of the seminal vesicles. Although not of an overwhelming nature, this infiltration was definite, and one to three cells per high power field could be observed. The second kind of abnormality was an atrophy of the tubules of the seminal vesicle. This was seen only in the guinea pigs of group 8. Cellular infiltration was also seen to some extent in the prostate and coagulating glands, but seemed limited to the area immediately surrounding the seminal vesicle. Fig. 9 illustrates the types of seminal vesicle abnormalities that have been observed.

It is important to note that if the guinea pigs that were injected with soluble antigen showed any seminal vesicle abnormalities, these abnormal appearances were only a matter of cellular infiltration. In contrast, many of the guinea pigs injected with soluble and insoluble antigen showed seminal vesicle tubular atrophy, as well as cellular infiltration. There seemed to be a good correlation of the immune response in general with tissue destruction.

Control guinea pigs (those injected with vesicular fluid) showed no abnormalities of their seminal vesicles or other tissues.

DISCUSSION

The antigens and autoantigens of the accessory (or adnexal) glands of reproduction have been much studied in recent years by our group (3, 4, 8, 9, 15, 16) and by others (17–20). Although it had been quite clearly shown that autoantibody formation could be induced in the rabbit (8, 9), there did not seem to

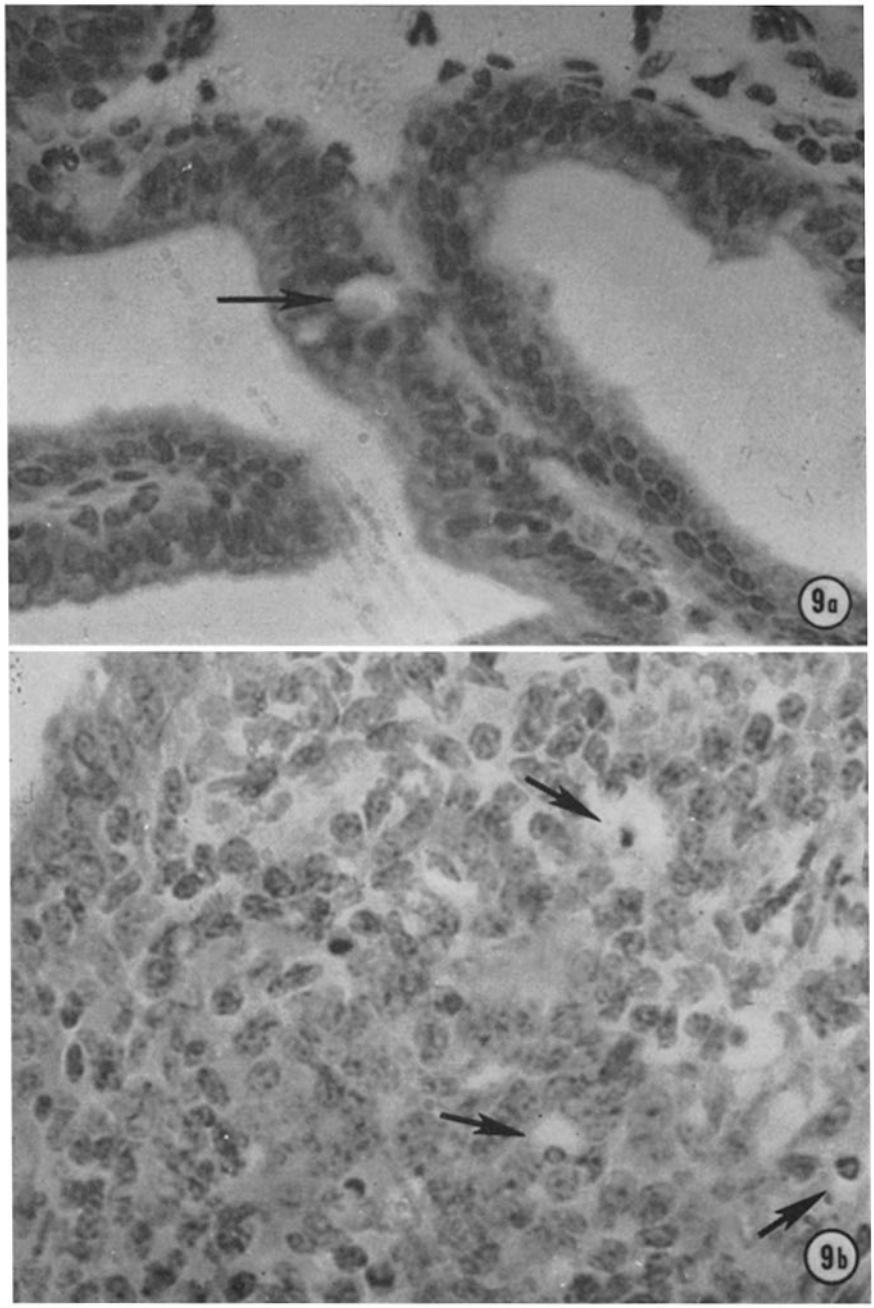

FIG. 9. Histological sections. (*a*) Seminal vesicle of guinea pig 3677, showing lymphocytic infiltrate, arrow. × 400. With the exception of this infiltrate, the appearance of this section was normal. (*b*) Seminal vesicle of guinea pig 79, showing destruction of normal seminal vesicle tubule histology, together with white cell infiltration, arrows. × 400.

be any definite appearance of tissue lesions in the target organ accompanying the immune response. This negative finding was of some concern since one might well ask whether the prostate diseases in man might include an autoimmune factor. It has in fact been claimed in one report (21) that some humans exhibited autoimmunity to prostate, but this claim has apparently not yet been confirmed. It was felt that the lack of tissue damage in the experimental animal might have involved factors of species responsiveness, since the rabbit is notably poor in the production of experimental aspermatogenesis (10), compared with other species.

Because of these earlier observations, the guinea pig was chosen for study, with the hope of producing an experimental model for autoimmune disease of the accessory glands of reproduction. A number of individual glands, as well as pooled tissues, were studied both biochemically and immunologically to gain some insight into such an experimental model.

Biochemical Analyses.—Vesicular fluid was found to have some unusual properties. Electrophoresis revealed six components, three major and three minor. All of these components, at least the major ones, had to have the same sedimentation rate because this fluid was homogeneous upon analytical ultracentrifugation. It is likely that the minor components were not present in high enough concentration for resolution in the analytical ultracentrifuge, and so their sedimentation rates might well have been different from that of the major components. Other studies of biochemical interest on these proteins have been carried out by Mányai and his colleagues (22–24) and by Williams-Ashman and his colleagues (25–27). In particular, guinea pig seminal vesicle secretion was fractionated by use of Sephadex CM-50 to give six components (26). A basic protein was then isolated in a homogeneous form (27), and was shown to have a molecular weight of about 17,900. It was proposed that this protein was the major substrate for the enzyme action leading to seminal clot formation. The purification procedure of Ballard et al. (26) was attempted by us, but it was abandoned because of difficulties, usually deriving from clot formation and blockage at the top of the column. We therefore found the method of starch block electrophoresis to be preferable in our hands. It seems likely that the "basic protein" is essentially the same as our "component III."

The diffusion coefficient of our isolated component II was found to be 11.4×10^{-7} cm^2/sec, after analysis of the values obtained in the analytical ultracentrifuge. From this value and a sedimentation coefficient of 1.5 S, an approximate molecular weight of 13,000 was calculated. With the above values being known, the shape of the molecules of component II would be estimated, using the following equation (28):

$$f/f_0 = 1.00 \times 10^{-8} \left(\frac{1 - \bar{v}\rho}{D^2 s \bar{v}} \right)^{1/3}.$$

This will give us the frictional ratio (f/f_0) of the molecule, where f is the fric-

tional coefficient of the particle, and f_0 is the frictional coefficient of a sphere having a volume equal to that of the particle.

If we assume that the molecules of component II are globular proteins, and that they assume one of three possible shapes, spherical, prolate ellipsoid, or oblate ellipsoid, then we can calculate their axial ratios from tabulated calculations (28) using the values of f/f_0 . The frictional coefficient of the molecules of component II was found to be 1.20. The axial ratios of these molecules were then calculated to be 4, if they were prolate ellipsoids, or 5, if they were oblate ellipsoids. These molecules could not be spherical at any rate, since their axial ratio was not unity. Further details on the molecular parameters of this substance await additional study.

Immunological Analyses: Heteroimmunization.—The rabbits immunized with GPVF are included in groups 9 and 10. Whether one (Group 9) or several (Group 10) injections were given, antibody formation was induced only by component II of the VF. This was shown by the technics of gel diffusion and of tanned cell hemagglutination.

Components I and III are biochemically similar in many ways to component II. In spite of this, components I and III were apparently nonantigenic. It is possible that certain amino acids, which are needed to render a molecule antigenic, are absent from components I and III. An amino acid analysis of the basic component of vesicular fluid has shown that this component, which is probably the same as our component III, is devoid of tyrosine, proline, and cysteine (27). A number of investigators have shown, in work with synthetic polypeptides, that certain amino acids, notably tyrosine, are needed for antigenicity (29, 30). Therefore, the absence of tyrosine from component III may contribute to the fact that this molecule is nonantigenic. Even less information is available, so far, concerning component I.

By the use of rabbit anti-guinea pig vesicular fluid, component II has been shown to be present antigenically in only the accessory glands and the seminal plasma of the guinea pig. We conclude that component II is highly tissue and species specific.

Isoimmunization.—Male and female guinea pigs were isoimmunized with this VF. Some male animals were also immunized with insoluble material, derived from a homogenate of the total seminal vesicle. The great majority of these animals formed antibodies to the antigen(s) of vesicular fluid. The effective antigen was shown by tanned cell hemagglutination to be component II, and the antibodies produced in male animals have been shown to be autoantibodies. Both male and female guinea pigs reacted equally well to the immunizations to which they were submitted. Autoantibody responses of a similar kind were also found by Vulchanov (31).

When the foregoing animals were skin tested for delayed hypersensitivity to vesicular fluids, many of the animals showed positive reactions. It is of interest

to note that in this kind of immune response, also, positive tests were induced by component II only, but not by components I or III.

The guinea pigs immunized with soluble material were examined for the presence of histologic abnormalities within their accessory glands. Many of these animals showed white cell infiltration, but no other abnormalities. These instances of cellular infiltration consisted generally of lymphocytes and plasma cells, but further detailed study is needed with larger numbers of animals. The effects were never extensive, and only several invading cells could be seen per high power field. This infiltration was confined to the epithelial cells and the lamina propria of this gland.

Because it was thought possible that insoluble antigens would be better able to induce lesions than would soluble antigens, a group of guinea pigs (Group 8) was injected with both the soluble and the insoluble antigens of the guinea pig seminal vesicle. It was thought that even more significant immunological results and/or histologic abnormalities might be seen with this type of isoimmunization. These animals were skin tested with soluble antigens, and the sera tested in vitro with only soluble antigens. These animals did exhibit a humoral antibody response that was not very different from that obtained in the earlier animals. The new findings here were a quicker sensitization to delayed hypersensitivity (after only one immunizing injection) and a rather pronounced atrophy of the seminal vesicle tubules, along with white cell infiltration. The cellular infiltration was essentially the same as those induced with only soluble antigen. Other organs in these and other animals, besides the accessory gland, were unaffected. A number of control guinea pigs were injected with rabbit vesicular fluid and fortified complete Freund's adjuvant. None of these animals showed any histologic abnormalities in the accessory glands of reproduction.

The more intensive tissue lesions that resulted from injection of total homogenate, rather than fluid alone, may be due to a distinctive insoluble antigen. Alternatively, in the more complex mixture, component II itself may have been modified to form a much more active antigen. In any event, some new lines of exploration could now follow in the immunology of reproduction (32, 33). This is especially true, since it is known that sperm cells interact with certain seminal vesicle antigens.

This study has strongly suggested that an autoimmune disease of the seminal vesicle has been discovered. This experimental model could possibly be of some interest in the etiology of some human urogenital diseases. Although human seminal vesiculitis is not nearly so common nor disturbing as the prostate disease, it does occur as a clinical and histological entity, as shown, for example, in a recent report (34). Furthermore, there may be possibilities that the vesiculitis model may yet shed some light on mechanisms in some forms of human prostate disease.

SUMMARY

Guinea pig vesicular fluid was characterized both biochemically and immunologically. Biochemical analyses showed this fluid to be homogeneous by ultracentrifugal analyses, revealing a single boundary with a sedimentation coefficient of 1.5 S. In contrast, electrophoretic separation methods revealed six components, of which three were major components, of approximately equal proportions. They were termed I, II, and III. One of these components (II) was shown to be strongly antigenic in heteroimmunization, whereas components I and III failed to show any antigenicity, even after diverse attempts. This antigen (component II) was found to be highly tissue specific and species specific.

Through procedures of isoimmunization, component II was also found to be immunogenic, giving rise (in male animals) to autoantibodies. A high proportion of injected guinea pigs showed positive skin tests and many revealed tissue lesions when the seminal vesicles were examined histologically. It is therefore concluded that experimental autoimmune disease of the seminal vesicle has been induced.

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