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PROTEINS IN THE SEMINAL PLASMA AND THE ACCESSORY SEXUAL GLAND FLUIDS OF THE BOAR

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Summary. The protein spectrum of boar seminal plasma is formed mainly by the seminal vesicle fluid proteins, and to a lesser extent by the proteins in the fluids of the cauda epididymidis and prostate.

The proteins of the seminal plasma, the fluids of the cauda epididymidis, prostate, seminal vesicles and the blood serum were divided into five peaks by column chromatography on Sephadex G-100. The proteins of Peak I in boar seminal plasma (mol. wt at least 150,000) were mostly derived from the seminal vesicle fluid and to a small extent from the fluids of the cauda epididymidis and prostate. The proteins of Peak II (mol. wt 120,000) were found in the blood serum only, and those of Peak III (mol. wt 69,000) in the seminal vesicle fluid, the fluid of the cauda epididymidis and the prostate. Peak IV (mol. wt 34,000) was formed by proteins originating mainly from the seminal vesicles and partly from the prostate. The proteins of Peak V (mol. wt 12,000 to 14,000) were derived from the fluids of the cauda epididymidis and the prostate.

These conclusions were confirmed by comparing the activity of acid and alkaline phosphatase and esterase by means of disc electrophoresis on polyacrylamide gel at pH 9·4 and 4·3 and by immunoelectrophoresis of all the fluids and fractions obtained by column chromatography of proteins on Sephadex G-100.

INTRODUCTION

On the grounds of an electrophoretic comparison of the protein mobility in the seminal plasma and the seminal vesicle fluid, Boursnell, Johnson & Zamora (1962) concluded that the principal protein components of boar seminal plasma originate in the seminal vesicle fluid. By the use of gel filtration, Nelson & Boursnell (1966) divided the proteins of boar seminal plasma into two groups. Boursnell & Briggs (1969) determined the isoelectric point of Group A proteins at pH 8·8 and the sedimentation coefficient (S_{20w}) at 2·35. The isoelectric point of proteins from Group B was determined at pH 4·6 and their sedimentation coefficient at 6·6S. Protein H with haemagglutination activity was isolated by Boursnell & Coombs (1966) and Boursnell (1967) and its isoelectric point was determined by Boursnell & Briggs (1969) at pH 9·4.

Boursnell, Briggs & Cole (1968) have reported that completely fresh vesicular secretion kept at 37° C, and studied at this temperature within 30 min of slaughter, presents an identical gel filtration pattern.

While investigating the protein polymorphism of the accessory sex gland fluids of boars, Dostál (1968) found a striking similarity between the electrophoretic patterns of seminal plasma proteins and those of the seminal vesicles.

Schellpfeffer & Hunter (1970) analysed the protein composition of boar seminal plasma before and after removal of the accessory sex glands. Gel filtration on Sephadex G-200 separated three groups of proteins in boar seminal plasma. Peak A (mol. wt approximately 55,000) was primarily of seminal vesicular origin. Most of the proteins in Peak B (155,000) were of epididymal or testicular origin. Peak C (34,000) was found in approximately equal amounts in all segments of the boar reproductive tract.

The aim of this work was to determine the contribution of the cauda epididymidis, prostate and seminal vesicles in the formation of the protein spectrum of boar seminal plasma. The blood serum was also investigated in order to obtain a more detailed picture of the protein component character.

The similarity of antigenic reaction in the protein components of individual peaks after chromatography on Sephadex G-100 was also investigated with the aim of determining the share of the individual reproductive tract fluids in the formation of the antigenic spectrum of boar seminal plasma.

MATERIAL AND METHODS

Collection of fluids

Boar semen was collected by means of an artificial vagina and the secretion of the bulbo-urethral (Cowper's) glands was removed by filtration through gauze. The spermatozoa were separated from the seminal plasma by centrifugation at 5600 ***g*** for 15 min.

The fluid from that portion of cauda epididymidis defined as Segment F by Crabo (1965) and prostatic and seminal vesicle fluids were obtained from the slaughterhouse according to the techniques described by Sedláková, Dostál & Matoušek (1968).

Column chromatography

Chromatographic separation of proteins was carried out on Sephadex G-100 (Pharmacia, Uppsala, Sweden) on a column of 2·2 × 72 cm, using an automatic column chromatography apparatus. A solution of 0·1 M-NaCl was used as eluant and the flow rate was fixed at 3·7 ml/15 min. The separation of proteins was evaluated spectrophotometrically at 280 nm.

Protein samples were applied after dissolving 50 mg of previously dialysed and freeze-dried material in 2 ml of a 5% solution of saccharose in 0·1 M-NaCl. The column was calibrated by means of Blue Dextran (Pharmacia, Uppsala, Sweden), human serum albumin (Léčiva, Praha; mol. wt 69,000), ovalbumin (Koch-Light; mol. wt 45,000) and cytochrome C (Koch-Light; mol. wt 13,000).

Disc electrophoresis

Disc electrophoresis (Ornstein, 1964) was used as a control technique for the

column separation procedure and for investigation of the character of the proteins. It was carried out on an apparatus devised by Davis (1964). Standard conditions, i.e. 7·5% gel for electrophoresis at pH 9·4 and 15% gel at pH 4·3, were chosen according to instructions provided by the firm Canalco. After dialysis, 0·3 mg dry protein was applied to 0·5 × 8 cm acrylamide gels.

Immunoelectrophoresis and preparation of antisera

The immunoelectrophoretic method described by Scheidegger (1955) was used with a tris-EDTA buffer, pH 8·9 in a 1% solution of Bacto agar Difco.

Antisera were obtained by the immunization of rabbits by intracutaneous injections of seminal plasma, blood serum and individual accessory gland fluids of boars without adjuvant at doses of 0·5 ml/rabbit, applied four times at 7-day intervals. Each rabbit received two more immunization doses, again administered at 7-day intervals 3 months after ending the first course of injections. On the 10th day after the last injection, blood was collected from the ear vein.

Staining procedures

Staining for proteins was performed in the usual manner with Amido Black 10B.

Staining for esterase activity was performed by the technique of Veselský & Kúbek (1970) in 0·15 M-phosphate buffer at pH 7·2 with Diazo Blue B salt and alpha-naphthyl acetate. Samples were incubated in the medium for 30 to 60 min at laboratory temperature.

The detection of acid phosphatase activity was carried out by means of a modified procedure of Lawrence, Melnick & Weiner (1960) with acetate buffer, pH 5·0, in which the Fast Blue RR salt had been dissolved. Incubation in that medium lasted 6 to 12 hr at a temperature of 37° C.

Alkaline phosphatase activity in 0·2 M-tris-HCl buffer, pH 9·3, was detected by the modified method of Gomori (1948). Fast Blue RR salt and alpha-naphthyl sodium phosphate were dissolved in this buffer. The samples were incubated for 2 to 6 hr at laboratory temperature.

RESULTS

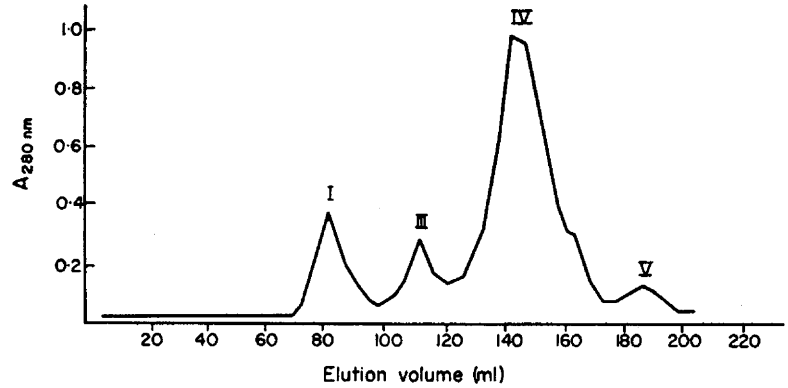
Chromatography on Sephadex G-100

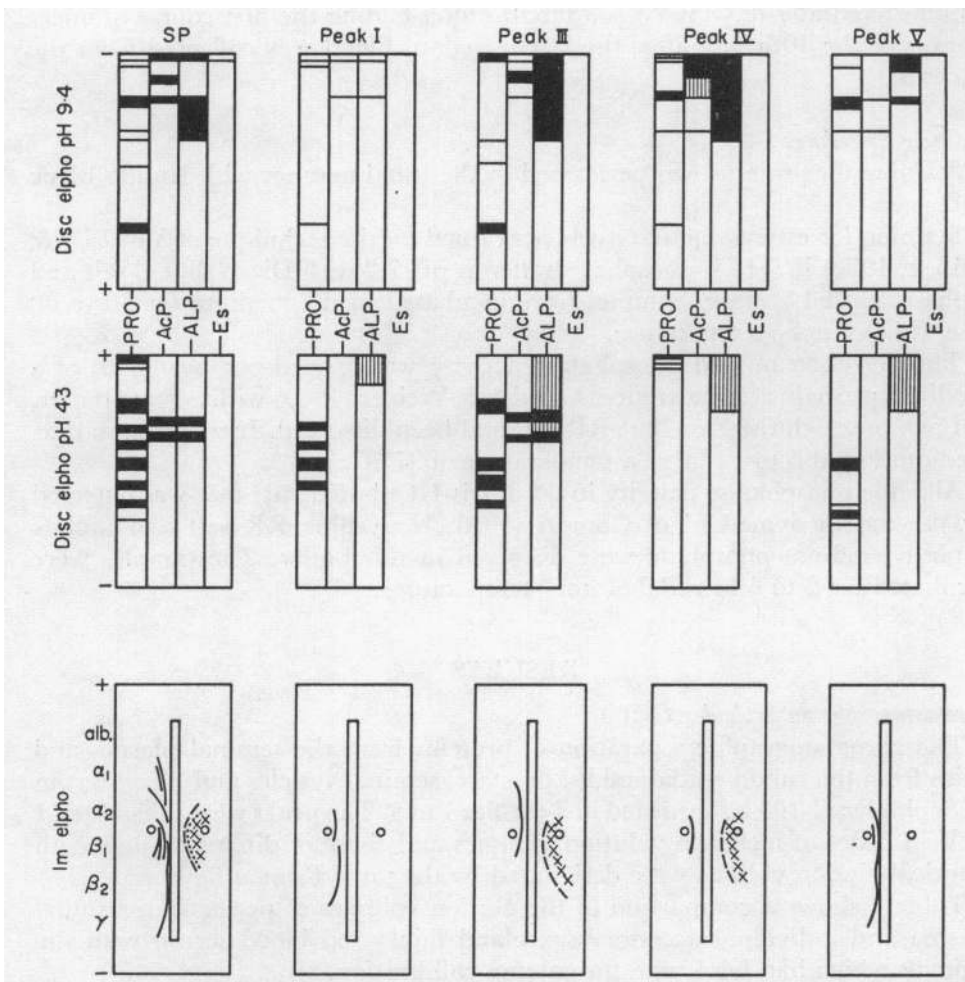
The chromatographic separation of proteins from the seminal plasma and fluids from the cauda epididymidis, prostate, seminal vesicles and blood serum on Sephadex G-100 is illustrated in Text-figs 1 to 5. The peaks were designated I to V in order of increasing elution volumes and those of different fluids with identical elution volume were designated by the same Roman figure.

Table 1 shows a comparison of the elution volumes of peaks from seminal plasma and individual accessory sex gland fluids and blood serum with the proteins which had been used for column callibration.

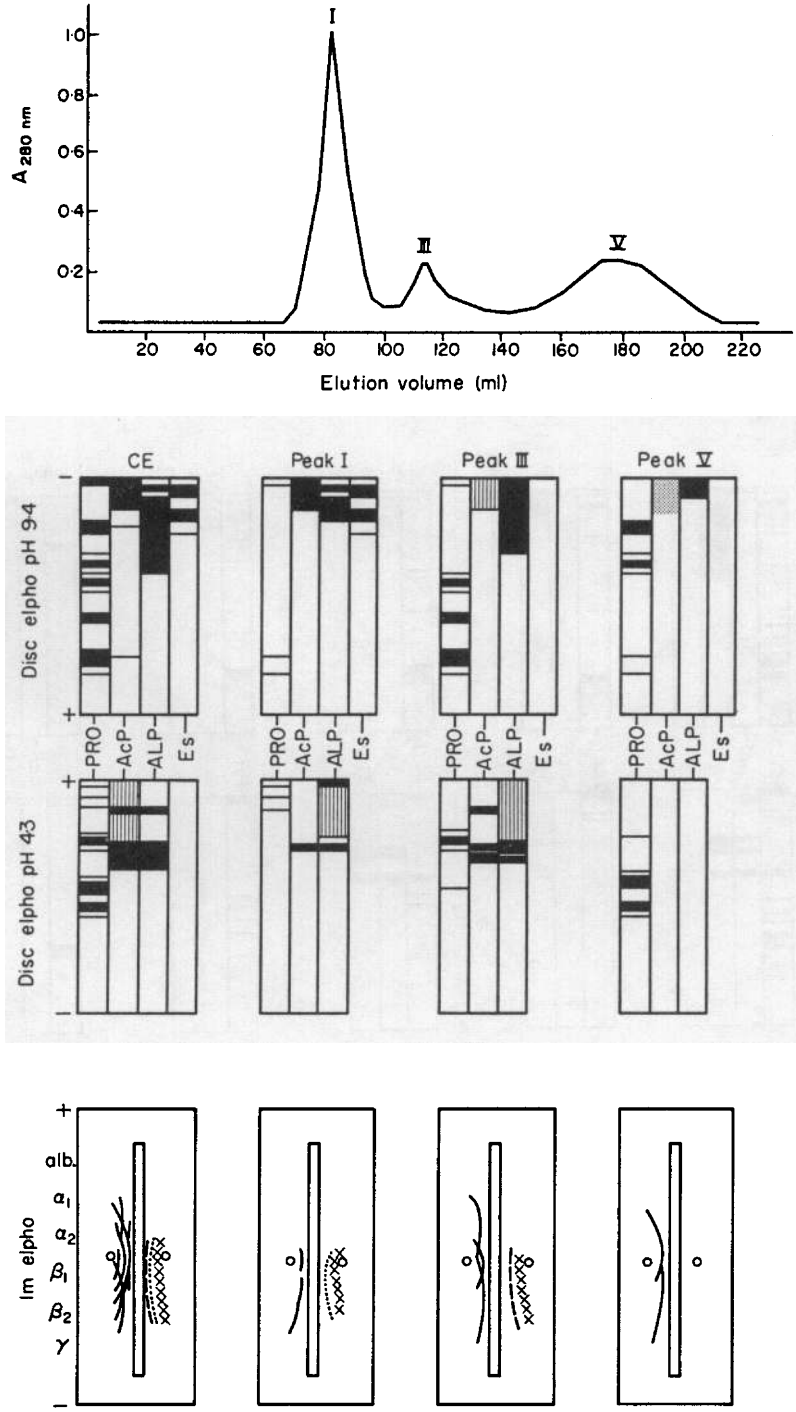
Disc electrophoresis

The total number of protein fractions detectable by disc electrophoresis at

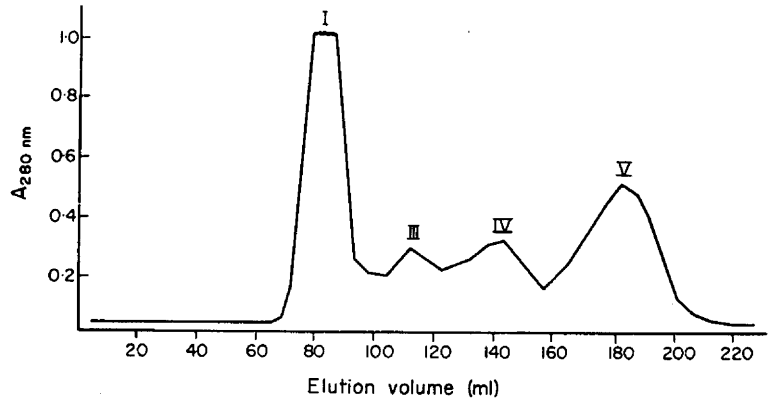


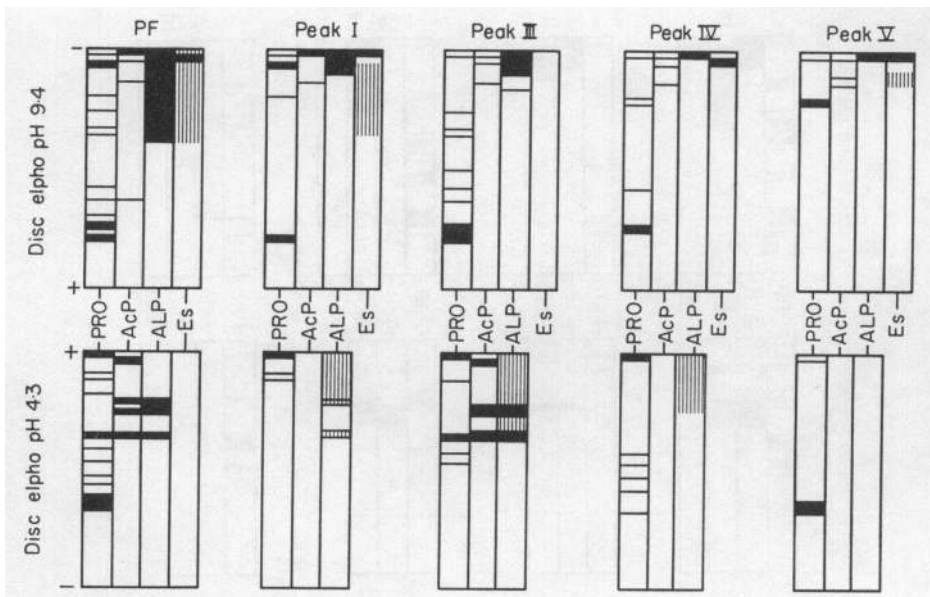


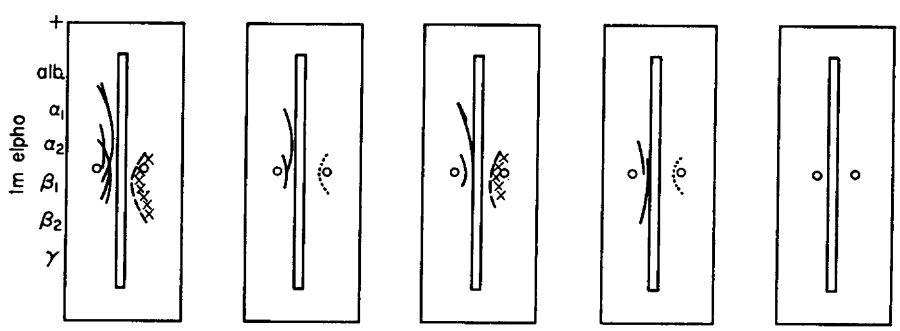
TEXT-FIG. 1. Sephadex G-100 column chromatography of proteins from boar seminal plasma (SP). Protein (PRO), ——; acid phosphatase activity (AcP), – – – –; alkaline phosphatase activity (AlP), × × ×; esterase activity (Es), · · · ·. The uninterrupted (——) interrupted (– – –), crossed (× × ×) and dotted (· · · ·) lines refer to the immunoelectrophoretic observations only.



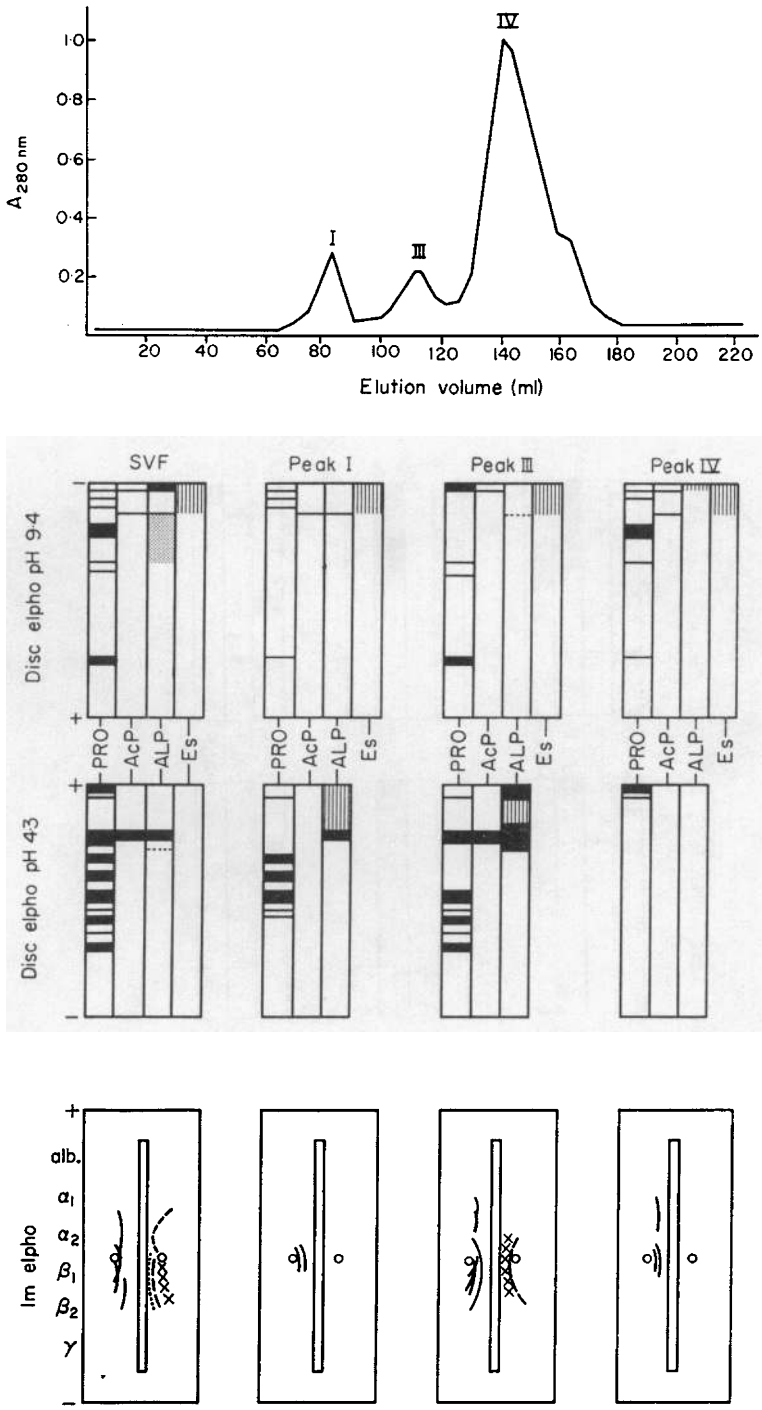
TEXT-FIG. 2. Sephadex G-100 column chromatography of proteins from the fluid of cauda epididymidis in boars (CE). Abbreviations as for Text-fig. 1.



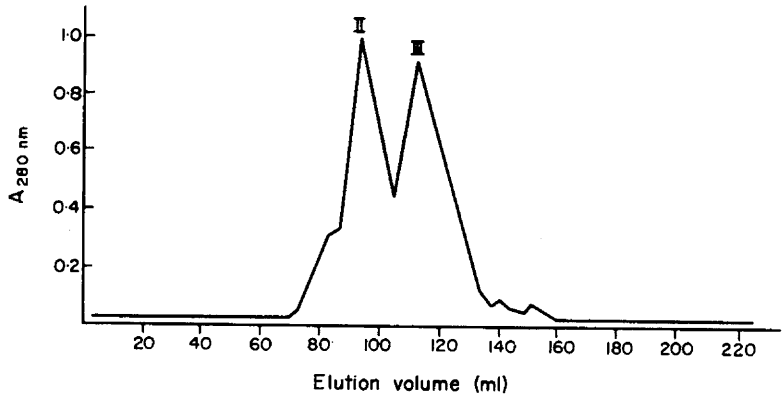


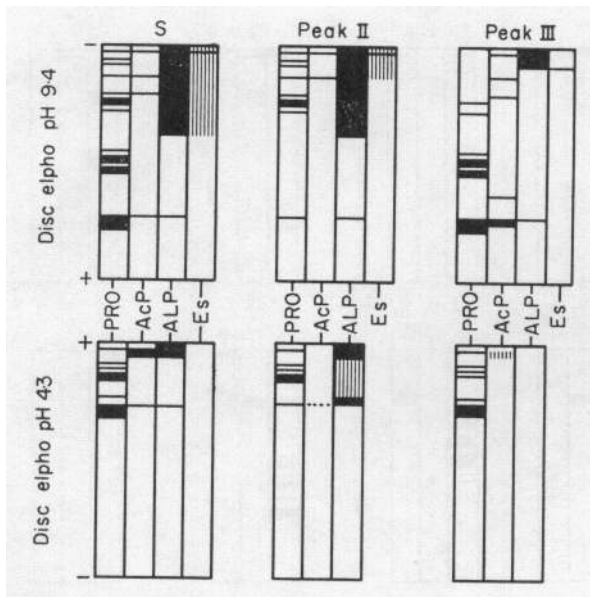


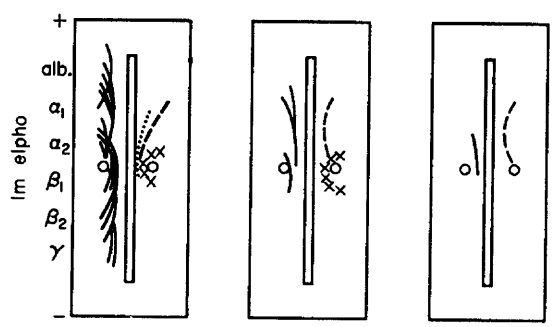
TEXT-FIG. 3. Sephadex G-100 column chromatography of proteins from the prostatic fluid of boars (PF). Abbreviations as for Text-fig. 1.



TEXT-FIG. 4. Sephadex G-100 column chromatography of proteins from boar seminal vesicle fluid (SVF). Abbreviations as for Text-fig. 1.







TEXT-FIG. 5. Sephadex G-100 column chromatography of proteins from boar blood serum (S). Abbreviations as for Text-fig. 1.

pH 9·4 and 4·3 in the seminal plasma and fluids from the cauda epididymidis, prostate, seminal vesicles and blood serum is given in Table 2.

In the seminal plasma and in the other fluids, the activity of acid and alkaline phosphatase and esterase were detected. Esterase activity was not found in any fluid on acrylamide gel during disc electrophoresis at pH 4·3 (Text-figs 1 to 5).

TABLE 1

ELUTION VOLUMES OF PEAKS FROM BOAR SEMINAL PLASMA AND INDIVIDUAL ACCESSORY SEX GLAND FLUIDS AND THE CORRESPONDING MOLECULAR WEIGHTS OF PROTEINS

	Elution volume (ml)	*Mol. wt*
Blue Dextran 2000	83	2,000,000
Human serum albumin	113	69,000
Ovalbumin	130	45,000
Cytochrome C	184	13,000
Peak I	83	150,000
Peak II	94	120,000
Peak III	113	69,000
Peak IV	142	34,000
Peak V	184	13,000 to 14,000

TABLE 2

THE NUMBER OF PROTEIN FRACTIONS FOUND BY MEANS OF DISC ELECTROPHORESIS ON ACRYLAMIDE GEL AT pH 9·4 AND 4·3 IN BOAR REPRODUCTIVE TRACT FLUIDS

Origin of reproductive tract fluid	*Disc electrophoresis*	
	pH 9·4	*pH* 4·3
Seminal plasma	7	9
Cauda epididymidis	10	10
Prostate	11	10
Seminal vesicles	7	10
Blood serum	11	6

Immunoelectrophoresis

The antigenic reactions of the seminal plasma, the fluids from the cauda epididymidis, prostate and seminal vesicles and the blood serum of boars, as well as the reaction of acid and alkaline phosphatase with rabbit antisera against the individual fluids are illustrated schematically in Text-figs 1 to 5.

Cross-reactions of protein components in the genital tract fluids and the blood serum of boars with antisera against the individual fluids are surveyed in Table 3. The proteins of the blood serum dissolved only partly after chromatography on Sephadex G-100 in the buffer used for immunoelectrophoresis so that there were fewer precipitation arcs in these samples.

The proteins of boar seminal plasma were separated by column chromatography on Sephadex G-100 into four peaks (I, III, IV and V). Peak II was found only in the blood serum, indicating either that these proteins were not present in reproductive tract fluids or that they were not present in levels which could be detected.

The proteins of Peak I of boar seminal plasma were present in high concentration in the fluids of the cauda epididymidis and prostate and could also be

TABLE 3

A SURVEY OF IMMUNOELECTROPHORETIC REACTIONS OF PROTEINS IN THE SEMINAL PLASMA, ACCESSORY SEX GLAND FLUIDS AND BLOOD SERUM OF BOARS

Origin of reproductive tract fluid	*Rabbit anti-boar serum against:*				
	Seminal plasma	*Fluid from cauda epididymidis*	*Prostatic fluid*	*Seminal vesicle fluid*	*Blood serum*
Seminal plasma	7	$1\beta_1$ 2γ	$1\beta_1$	$2\beta_1$ $1\alpha_2$-β_1	$1\alpha_2$
Peak I	2	0	$1\alpha_2$-β_1	$1\alpha_2$-β_1	$1\alpha_1$
Peak III	2	0	0	$1\beta_1$	0
Peak IV	1	$1\beta_2$	$1\beta_1$	$1\beta_1$	$1\beta_1$
Peak V	3	$1\beta_2$	0	0	0
Fluid from cauda epididymidis	$3\beta_1$	6	$1\beta_1$-β_2	$1\alpha_2$-β_1	$1\alpha_2$
Peak I	$1\beta_1$	2	0	0	0
Peak III	0	3	0	0	0
Peak V	0	2	0	0	0
Fluid of prostate	$1\alpha_2$	$1\beta_1$-β_2	6	$1\alpha_2$	$1\alpha_1$-β_1
Peak I	1γ	0	2	0	0
Peak III	1γ	0	2	0	0
Peak IV	0	0	2	0	0
Peak V	0	$1\beta_1$-β_2	0	0	0
Seminal vesicle fluid	$2\beta_1$ 1γ	$1\alpha_2$	$1\beta_1$	4	$1\beta_1$
Peak I	$2\beta_1$	0	$1\beta_1$	2	0
Peak III	$1\alpha_2$ $1\beta_1$ 1γ	0	$1\beta_1$	4	0
Peak IV	$1\alpha_2$ $1\beta_1$ 1γ	0	$1\alpha_2$-β_1	3	0
Blood serum	0	0	$1\alpha_2$-β_1	0	13
Peak II	0	0	0	0	3
Peak III	0	0	0	0	1

The figures in front of the fraction symbols indicate the number of arcs found in the area mentioned.

detected in the fluid from the seminal vesicles (Text-figs 2 and 3). It cannot be said, however, that the bulk of the proteins in Peak I originated in the epididymis or the prostate, because both fluids form only a small part of the seminal plasma and the total protein concentration in these fluids was low (1 to 2%).

The main protein in Peak I of the seminal plasma was derived from the seminal vesicle fluid which contains about 10% total proteins and forms about a quarter of the whole volume of boar seminal plasma.

These conclusions were confirmed by the results of investigations on alkaline phosphatase and esterase activity. Alkaline phosphatase was detectable by means of immunoelectrophoresis and disc electrophoresis in Peak I only in the fluid of the cauda epididymidis (see Text-fig. 2). Esterase activity was found in Peak I of the cauda epididymidis and prostate (Text-figs 2 and 3). In Peak I of

the seminal plasma and seminal vesicle fluid, none of these activities was determined (Text-figs 1 and 4).

Cross-reactions proved that the antigens in Peak I of the seminal plasma were formed primarily by the proteins of the seminal vesicle fluid, and to a lesser extent by those of the cauda epididymidis and prostatic fluids. The number of cross-reacting antigens of the individual fluids is given in Table 3.

Peak III of the boar seminal plasma represented proteins from the fluids of the cauda epididymidis, prostate and seminal vesicles. The presence of acid and alkaline phosphatases in this peak was also proved by disc electrophoresis and immunoelectrophoresis in all the boar reproductive tract fluids examined. Cross-reactions showed that the highest number of antigenic components were also derived from the seminal vesicles (Table 3).

A further group of proteins in Peak IV of the seminal plasma originated primarily from the seminal vesicle fluid. The fluid of the cauda epididymidis either did not contain these proteins, or only contained them in undetectable amounts. Peak IV represented the finding in the prostatic fluid. The relative amount of these proteins, however, was low and since the share of the prostate in the formation of boar seminal plasma volume is small, the share of these proteins in the formation of Peak IV of the seminal plasma must also be small. The results obtained by comparing esterase activity by disc electrophoresis and immunoelectrophoresis in Peak IV of the prostate and Peak IV of the seminal plasma supported these conclusions. Both acid and alkaline phosphatase activities, however, have been detected in Peak IV of the seminal plasma.

The characteristics of Peak IV of the seminal plasma were almost identical to those of Peak IV of the seminal vesicle fluid. That the seminal vesicle fluid proteins formed a substantial part of the proteins in Peak IV of the seminal plasma was confirmed by the cross-reactions of antigenic proteins in Peak IV of the seminal plasma, the prostatic and seminal vesicle fluids.

The group of proteins forming Peak V of the seminal plasma must originate from the fluids of the cauda epididymidis and prostate since these proteins were not present in the seminal vesicles. No acid or alkaline phosphatase or esterase activities were demonstrated immunoelectrophoretically in Peak V of the seminal plasma or the fluids from the cauda epididymidis and prostate.

The chromatographic records given in Text-figs 1 and 4 and the cross-reactions of the seminal plasma and the seminal vesicle fluid with rabbit antisera shown in Table 3 confirmed that the protein spectrum of boar seminal plasma is formed primarily by proteins originating from the seminal vesicles.

DISCUSSION

No precipitation reactions were observed in any of the reproductive tract fluids tested by immunoelectrophoresis with rabbit serum before the rabbits were immunized. It was therefore assumed that no natural antibodies or precipitins were present in rabbit blood before immunization.

No changes in the protein composition of the boar reproductive tract secretions were observed after freeze-drying. It is probable that there is no precipitation of 7S protein during freeze-drying as seems to occur on cooling samples of

seminal plasma from some boars (Boursnell, Nelson & Cole, 1966), but the occurrence of physico-chemical changes in the proteins themselves remains a possibility.

The finding of acid and alkaline phosphatase activities in Peak IV of the seminal plasma appears remarkable when no such activity could be proved in Peak IV of the other accessory sex gland fluids. Veselský (1972) reported a high activity of acid and alkaline phosphatase in the prostatic fluid of 23% of boars though in 77% no activity could be electrophoretically demonstrated. It is possible that phosphatase activation takes place only during certain physiological processes, one of which may be ejaculation. This would explain the presence of phosphatase in Peak IV of the seminal plasma. Another possible source of phosphatase activity, the urethral gland fluid, cannot be excluded, however, since it was not investigated in the present experiments. Schellpfeffer & Hunter (1970) found that the proteins of Peak C of the boar seminal plasma, of mol. wt 34,000, determined on Sephadex G-200, derived their origin from all parts of the boar reproductive tract. In our case, Peak IV of the seminal plasma corresponded in elution volume to a mol. wt of 34,000. Hence, it is possible that the activity of acid and alkaline phosphatases comes from the urethral glands. The elucidation of this problem, however, will require more experimental evidence.

It is difficult to compare our results with those of Schellpfeffer & Hunter (1970), because they used boar seminal plasma before and after removal of some accessory sex glands. This differs substantially in its protein composition from the accessory gland fluids obtained from slaughtered animals.

The finding that the proteins of the boar seminal plasma are primarily derived from the seminal vesicles is in full agreement with the findings of Boursnell *et al.* (1962) and our previously published results (Dostál, 1968). The presence of proteins originating from the fluids of the cauda epididymidis and prostate in Peak V of boar seminal plasma confirms the general conclusions of Subina (1964), who reported that the protein composition of boar seminal plasma reflected that of the seminal vesicle and prostate gland fluids. It also confirms the general conclusions of Schellpfeffer & Hunter (1970).

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