FIRST ATTEMPT TO CHARACTERIZE AN ASPERMATOGENIC SUBSTANCE IN THE SEMINAL VESICLE FLUIDS OF BULLS*

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(Received 25th March 1971, accepted 30th July 1971)

Summary. The biosynthesis of the aspermatogenic substance in the seminal vesicle fluid of bulls is dependent on the androgen level. The aspermatogenic substance is not present in sexually immature bulls; its synthesis can be evoked, however, by means of testosterone propionate. Castration is followed by a loss of libido and a decline in the production of fluids from the accessory genital glands; injections of testosterone propionate restore libido, the secretion of fluids and their aspermatogenic effect.

The aspermatogenic substance is thermostable, it is not digested by a number of enzymes including proteolytic enzymes, but it must evidently be bound to a protein complex in order to induce aspermatogenesis.

Rabbits developed several antibodies against bull vesicular fluid treated with enzymes, as judged by double diffusion, immunoelectrophoresis and haemagglutination tests. The number and character of the precipitation and haemagglutination antibodies in immunized animals did not differ whether the testes were damaged or undamaged. The fluorescence technique did not detect any binding of antibodies either to epididymal spermatozoa or to histological sections of the testes of rabbits and bulls.

INTRODUCTION

The aspermatogenic effect of all bovine accessory sexual gland fluids, including testicular fluid and blood serum, was analysed in a previous paper (Matoušek, 1969). The fraction causing aspermatogenesis in the testes of heterologous species was detected in the seminal vesicle fluid. Injections of bovine seminal vesicle fluid produced a decrease in the index weight of the testes, a disturbance of spermatogenesis and, in more severe cases, a distortion and contraction of the seminiferous tubules.

Further study was directed to the characterization of the substance(s) responsible for the aspermatogenic effect. The effect of sexual maturation and androgenic hormones on the synthesis of the aspermatogenic substance in the seminal vesicles, and the sensitivity of the substance towards temperature

* This paper reports on part of a project within the International Biological Programme.

changes and towards enzymes were investigated, initial attempts being carried out at its isolation. The results obtained from these studies are presented in this paper.

MATERIAL AND METHODS

Collection of vesicular fluids and determination of the aspermatogenic effect

Bovine seminal vesicle fluid was collected at the slaughterhouse from healthy bulls of various ages (Matoušek, 1969) and from castrated bulls by means of an artificial vagina.

The determination of the aspermatogenic effect of a single intratesticular injection of the vesicular fluid of sexually immature, mature, castrated and testosterone-propionate-injected bulls, of vesicular fluid treated with enzymes and of the fluid fractions, was carried out by weight and histological analyses of testes from mice and guinea-pigs.

The index weight, i.e. testis weight in $g \times 1000/g$ body weight, was used to express testis weight. The degree of testicular damage was assessed on a scale from 0 (normal histological appearance) to 4 (disappearance of all cells of the spermatogenic layers and of the convoluted tubules) 35 days after intratesticular injections (Matoušek, 1969).

Each of the tested fluids or vesicular fluid fractions was injected into the testes of four mice and two guinea-pigs. The dose injected was 0.02 ml for mice and 0.2 ml for guinea-pigs. The fractions were administered by resuspending 40 mg freeze-dried and dialysed material in 1 ml 0.9% NaCl. Alcohol-ether fractions (precipitates and extracts) and enzymatically-split fractions of vesicular fluid were resuspended in 0.9% NaCl to give a 3 to 4% concentration of proteins. Protein concentration was determined by the biuret procedure.

Each enzyme-treated fluid was administered subcutaneously to two rabbits, at a dose of 1 ml per injection, the number of injections ranging from four to twenty-two at 2- to 11-week intervals. The antispermatogenic effect of the administered fluid was investigated by measuring the amount of the ejaculate and the number of spermatozoa per ejaculate. Ejaculates were obtained from rabbits by means of an artificial vagina. Some rabbits were hemicastrated at the time of spermatogenic depression and the testis was examined histologically.

Injections of testosterone propionate into bulls

The dependence of the biosynthesis of the aspermatogenic substance in the seminal vesicles on androgenic hormones was investigated in four bulls.

(1) Two bulls were treated at the age of 2 to 4 months with 50 mg testosterone propionate (Spofa, Prague), and at the age of 4 to 6 months with 100 mg per injection twice a week. They were killed at the age of 6 months. The developmental stage of the spermatogenic epithelium of the testes in these two bulls was determined histologically, and the antispermatogenic effect of their vesicular fluid was measured by a single intratesticular injection of this fluid into two guinea-pigs.

(2) Two bulls were castrated at the age of $3\frac{1}{2}$ years and their ejaculates were collected into an artificial vagina. When Bull 717 stopped ejaculating accessory

genital gland fluids, daily injections of 50 mg testosterone propionate were given. Injections were continued until the bull began ejaculating accessory genital gland fluids again (2 to 4 weeks). After repeated loss of fluids, the procedure was repeated. The fluids ejaculated into the artificial vagina were investigated for protein content and for aspermatogenic effect by means of intratesticular injections into mice and guinea-pigs.

Sensitivity of the aspermatogenic substance towards temperature change and enzymatic action

A mixture of vesicular fluids from twenty bulls was heated for 30 min in a water bath to 56, 65 or 75° C. It was also frozen eight times to -20° C and repeatedly thawed to room temperature.

The vesicular fluid was treated with papain (3.4.4.10), ficin (3.4.4.5), trypsin (3.4.4.4), chymotrypsin (3.4.4.5), lysozyme (3.2.1.17), pronase, α -amylase (3.2.1.1), β -amylase (3.2.1.2) and hyaluronidase (4.2.99.1) (Koch-Light Laboratories Ltd, England) according to the method of Amano & Behrman (1968). The concentration of the enzyme was 20 mg/ml phosphate buffer, pH 7.1. After enzymatic splitting (24 hr at 37° C), alcohol-ether was added to the mixture of vesicular fluid and enzyme and both precipitate and extract suspended in 0.9% NaCl were used for study.

Methods of fractionation of vesicular fluid

Alcohol-ether method. Vesicular fluid was dialysed against phosphate buffer, pH 7.2, and proteins were precipitated by a mixture of 96% ethyl alcohol and ether (3:1) at a ratio of 1:5. The filtered precipitate was cleared of the rest of alcohol-ether by air drying at laboratory temperature. Alcohol-ether was removed from the extract by submerging the sample into a water bath at 60 to 63° C. Precipitate and extract were resuspended in 0.9% NaCl to give a 3 to 4% concentration of proteins.

Ammonium sulphate method. Vesicular fluid was diluted with an equal volume of redistilled water and the pH was adjusted to 7.0 by means of 1 M-NaOH. Ammonium sulphate was added to a concentration of 1.5 M. After 24 hr, the mixture was centrifuged and the precipitate obtained was designated ASI_p; after resuspension in distilled water, it was dialysed for 48 hr against water and freeze dried. Ammonium sulphate was added to a concentration of 2 M to the supernatant and the resulting precipitate was designated ASII_p and the supernatant ASII_s. By the further addition of ammonium sulphate to a concentration of 2.5, 3 and 3.5 M to the corresponding supernatants II, III and IV, precipitates and supernatants were gained which were designated ASIII, IV and V, respectively.

Rivanol (6,9-diamino-2-ethoxyacridinelactate)-ammonium sulphate method. After diluting vesicular fluid with an equal volume of redistilled water, the pH was adjusted to 8.0 by means of 1 M-NaOH, and part of the proteins was precipitated from the solution by adding an equal volume of 1.68% rivanol. After centrifugation, the precipitate, designated RI_p, and the supernatant, RI_s, were obtained.

Precipitate RI was resuspended in 0.9% NaCl to the volume of the original

mixture, and rivanol was precipitated by adding crystalline NaCl. After centrifugation, a supernatant was obtained to which ammonium sulphate was added until it reached a concentration of 2.45 M in the solution. After 18 hr at 5° C, the supernatant was centrifuged and the precipitate, RIIp, and the supernatant, RIIs, were gained. Precipitate RII was dissolved in 0.9% NaCl, dialysed against water and freeze dried. Supernatant RII was also subjected to dialysis and freeze drying. The same procedure was applied to the supernatant RI which, after adjustment of the pH to 7.0 and the removal of rivanol, yielded precipitate RIIP and supernatant RIIs.

Chromatography on Sephadex G-100. Vesicular fluid was diluted 1:4 with ammonium carbonate (pH 7.85). Chromatographic separation was carried out on a column 6 × 91 cm, 0.1 M-ammonium carbonate being used as eluant. The flow rate amounted to 54 ml/hr and fractions were collected at 20-min intervals. After chromatographic separation, the fractions with separated proteins were dialysed for 48 hr against distilled water and freeze dried. The protein content was determined spectrophotometrically at 280 nm.

Electrophoretic analysis of fractions of vesicular fluid

Electrophoretic separation was carried out in starch gel in a glacial acetic acid + formic acid buffer (pH 1.7) according to the method of Aschaffenburg (1966). Urea (47 g/120 ml of buffer) was added to prevent formation of protein aggregates. Electrophoresis was carried out at 300 to 350 V and 42 to 47 mA for about 16 hr. The gels were cooled with running water.

Detection of antibodies

The production of antibodies in rabbits immunized subcutaneously was investigated by means of the haemagglutination test (Stavitský, 1954), the agar-gel double diffusion technique (1% agar-gel in borate buffer), microimmunoelectrophoresis (Scheidegger, 1955), and direct and indirect fluorescent methods (Batty & Walker, 1967).

The vesicular fluid used as antigen for the haemagglutination test was freed from haemolytic factor by means of absorption with bovine ampullary spermatozoa. Epididymal spermatozoa and ultrathin sections (4 µm) of testes from bulls and rabbits were used for fluorescent tests.

RESULTS

The effect of sexual maturation and androgen hormones on biosynthesis of the antispermato-genic substance

Vesicular fluid from sexually immature bulls (4 to 6 months old with testicular tissue differentiated maximally to the spermatocyte stage) did not evoke symptoms of aspermatogenesis when injected into the testes of four mice and eight guinea-pigs.

When spermatozoa appeared in the testes of maturing bulls, their vesicular fluid, which already contained the antispermato-genic substance, also caused the death of some of the injected animals. Out of seven guinea-pigs treated with a single intratesticular injection of 0.2 ml fluid, four died within 3 days. The

injected testes of the three remaining guinea-pigs 35 days after injection had an index weight of 0.62 ± 0.04 , and their seminiferous tubules contained only spermatogonia and sporadically occurring spermatocytes.

The aspermatogenic effect of vesicular fluid, however, is not dependent on the degree of spermatogenesis. The testes of two young bulls which were injected with testosterone propionate at 2 to 6 months only contained cells up to the primary spermatocyte stage at 6 months, but the vesicular fluid of both contained the aspermatogenic substance. The testes from four guinea-pigs injected with fluid from these young bulls had a significantly lower index weight (1.09 ± 0.09) and the degeneration of the testicular epithelium reached Grade 3 to 4.

In two bulls, castrated at the age of $3\frac{1}{2}$ years, the accessory genital gland fluids (obtained by ejaculate collection from an artificial vagina) contained the antispermatogenic substance almost until the ejaculatory capacity disappeared.

TEXT-FIG. 1. Ejaculate characteristics of the castrated Bull 715. Volume of ejaculated fluids (—), percentage of proteins in ejaculated fluids (-----), their aspermatogenic effect (A.E.) and the period of testosterone propionate application (T.P.).

After castration, one of these bulls (No. 715) ejaculated for a period of 16 weeks without any androgen treatment (Text-fig. 1). During this whole period, all ejaculated fluids except one possessed the aspermatogenic substance, even though the protein content was reduced from the original value of 8.45 g/100 ml biuret reactive material to values between 1 and 2 g/100 ml. Testosterone propionate injections considerably increased the protein content but not the amount of ejaculated fluids in this bull (Text-fig. 1). Bull No. 717 stopped ejaculating fluids into the artificial vagina by 3 weeks after castration (Text-fig. 2). Testosterone propionate injections regenerated the secretion of the accessory genital glands and also the antispermatogenic substance in these fluids. The suppression of accessory genital gland fluid production occurred three times within 7 months in Bull 717. Each time, the secretory capacity and the original protein content of the ejaculates were renewed by means of testosterone propionate and the antispermatogenic effect of the ejaculated fluids was proved (Text-fig. 2).

The effect of different temperatures and enzymes on the antispermatogenic substance

Temperatures of 56, 65 and 75° C did not alter the antispermatogenic effect of vesicular fluid. Injections of heated samples of the fluid into the testes of mice and guinea-pigs caused significant changes in the seminiferous tubules (Table 1). Fluid frozen and defrosted eight times still retained its antispermatogenic effect.

The substance was not affected by any of the enzymes which were used (Table 1). Alcohol-ether extracts of fluid treated with enzymes had a very weak antispermatogenic effect. Only the extract obtained by the action of lysozyme on the fluid was active (Table 1).

Some fractions of fluid were also tested for an antispermatogenic effect by subcutaneous injection into rabbits. The results confirmed those obtained by direct intratesticular injections into mice and guinea-pigs. All protein fractions obtained after enzymatic action with trypsin, chymotrypsin, papain, pronase and lysozyme and by precipitation with alcohol-ether dissolved in 0.9% NaCl

TEXT-FIG. 2. Ejaculate characteristics of the castrated Bull 717. Volume of ejaculated fluids (—), percentage of proteins in ejaculated fluids (-----), their aspermatogenic effect (A.E.) and the period of testosterone propionate application (T.P.).

caused loss of libido in both, or at least one, of the two injected rabbits. Text-figure 3 illustrates the results obtained after injecting rabbit No. 580 with an alcohol-ether precipitate from fluid subjected to the action of lysozyme. Histological examination of testes from rabbits hemicastrated during sexual rest always showed impairment of spermatogenesis. In some tubules, spermatogenic cells were present up to the secondary spermatocyte stage (Pl. 1, Fig. 1).

EXPLANATION OF PLATE 1

FIG. 1. Cross section of rabbit testis after subcutaneous injections with alcohol-ether precipitate of bull seminal vesicle fluids treated by lysozyme. Only spermatogonia are visible in the seminiferous tubules shown. $\times 220$.

FIG. 2. Electrophoretic separation of bull seminal vesicle fluids (SVFB) and their fractions on starch gel. (1) Mixture of SVFB, (2) alcohol-ether precipitate of SVFB treated by pronase, (3) Peak 2 after chromatographic fractionation of SVFB, (4) Peak 4 after chromatographic fractionation of SVFB, (5) alcohol-ether extract of SVFB treated by chymotrypsin, (6) supernatant V (ASVs) after ammonium sulphate precipitation of SVFB, (7) Peak 5 after chromatographic fractionation of SVFB (negative in AE).

PLATE 1

FIG. 1

FIG. 2

(Facing p. 184)

Fluid extracts treated with lysozyme, chymotrypsin and papain, also induced aspermatogenesis in rabbits, but it was necessary to double or treble the injection doses compared to those required with the corresponding precipitates: precipitates caused aspermatogenesis after four to ten injections; enzyme-treated extracts after ten to twenty-two injections.

TABLE 1

INFLUENCE OF TEMPERATURE AND ENZYMES ON THE ASPERMATOGENIC ACTIVITY OF THE SEMINAL VESICLE FLUID OF BULLS

Temperature and enzymatic treatment	Aspermatogenic activity			
	Index weight of testes		Degree of testis damage	
	Mice	Guinea-pigs	Mice	Guinea-pigs
Control (0.9% NaCl)	3.30	2.55	0	0
SVFB without heating	1.03*	0.63*	4	4
SVFB heated to 56° C	1.14*	0.71*	3 to 4	4
SVFB heated to 65° C	1.26*	0.35*	4	4
SVFB heated to 75° C	1.34*	1.05*	4	3 to 4
SVFB frozen and thawed eight times	1.27*	0.84*	3 to 4	4
SVFB* papain-precipitate	1.41*	0.68*	3 to 4	3 to 4
SVFB* papain-extract	2.39	1.89	0 to 1	0 to 1
SVFB* ficin-precipitate	1.68*	0.64*	3 to 4	4
SVFB* ficin-extract	2.53	2.08	1 to 2	0 to 2
SVFB* trypsin-precipitate	1.46*	0.97*	2 to 4	1 to 2
SVFB* trypsin-extract	3.09	2.16	0 to 1	0 to 1
SVFB* chymotrypsin-precipitate	1.45*	1.20	2 to 4	2 to 3
SVFB* chymotrypsin-extract	3.16	1.62	0	0 to 2
SVFB* pronase-precipitate	1.31*	0.61*	2 to 4	3 to 4
SVFB* pronase-extract	3.36	2.49	0	0
SVFB* lysozyme-precipitate	1.23*	0.57*	4	3 to 4
SVFB* lysozyme-extract	1.34*	0.72*	1 to 2	1 to 3
SVFB* α -amylase-precipitate	1.57*	0.54*	3 to 4	3 to 4
SVFB* α -amylase-extract	2.38	1.52	0 to 1	0 to 1
SVFB* β -amylase-precipitate	1.70*	0.90*	2 to 4	2 to 3
SVFB* β -amylase-extract	3.28	2.48	0	0
SVFB* hyaluronidase-precipitate	1.38*	1.12*	2 to 3	2 to 3
SVFB* hyaluronidase-extract	3.22	2.52	0	0

SVFB = bull seminal vesicle fluids.

* Statistically highly significant, $P < 0.01$.

The suppression of spermatogenesis was reversible. After 12 to 28 weeks, even in the case of a unilaterally castrated rabbit, sexual activity including the capacity to ejaculate spermatozoa was regained (Text-fig. 3).

Aspermatogenic effect of vesicular fluid after fractionation

It is evident from the results of fluid fractionation that the antispermatogenic substance is bound to a protein complex (Table 2). It does not pass through the dialysis membrane but forms a portion of the proteins obtained by precipitating fluid with alcohol-ether, 1.5 to 3 M-ammonium sulphate, and rivanol.

However, even after ammonium-sulphate-induced precipitation of the proteins causing aspermatogenesis, the supernatant still contains a protein with similar activity. In starch-gel electrophoresis, this fluid forms a similar pattern to that formed by the alcohol-ether precipitate of vesicular fluid treated with

TEXT-FIG. 3. Ejaculate characteristics of the Rabbit 580 injected with the alcohol-ether precipitate of bull seminal vesicle fluids treated by lysozyme. (Period of the subcutaneous injections: S.C.I.)

TABLE 2

ASPERMATOGENIC ACTIVITY OF SEMINAL VESICLE FLUID OF BULLS AFTER THEIR FRACTIONATION

Fractionation method	Designation of fraction	Aspermatogenic activity			
		Index weight of testes		Degree of testis damage	
		Mice	Guinea-pigs	Mice	Guinea-pigs
Control (0.9% NaCl)	Contr	3.30	2.55	0	0
SVFB dialysed in dist. H ₂ O	Dial	1.07*	0.40*	4	4
SVFB* alcohol-ether-precipitate	AEP	1.13*	0.68*	4	3 to 4
SVFB* alcohol-ether-supernatant	AEs	2.94	2.50	0 to 1	0
SVFB* (NH ₄) ₂ SO ₄ 1.5 M-precipitate	ASIp	1.09*	—	4	—
ASIs* (NH ₄) ₂ SO ₄ 2 M-precipitate	ASIIp	0.98*	0.58*	4	4
ASIIIs* (NH ₄) ₂ SO ₄ 2.5 M-precipitate	ASIIIp	1.08*	0.62*	4	3 to 4
ASIIIs* (NH ₄) ₂ SO ₄ 3 M-precipitate	ASIVp	1.58*	0.73*	2 to 4	2 to 4
ASIVs* (NH ₄) ₂ SO ₄ 3.5 M-precipitate	ASVp	2.82	—	0 to 2	—
ASIVs* (NH ₄) ₂ SO ₄ 3.5 M-supernatant	ASVs	1.56*	—	1 to 3	—
SVFB* rivanol-supernatant	RIIs	1.46*	1.62	2 to 3	1 to 2
RIp* (NH ₄) ₂ SO ₄ 2.45 M-precipitate	RIIp	1.23*	0.56*	4	4
RIp* (NH ₄) ₂ SO ₄ 2.45 M-supernatant	RIIs	1.55	2.14	0 to 3	0 to 1
RIIs* (NH ₄) ₂ SO ₄ 2 M-precipitate	RIIIP	1.47*	2.04	2 to 3	0 to 3
RIIs* (NH ₄) ₂ SO ₄ 2 M-supernatant	RIIIs	1.76	1.89	0 to 2	0 to 2
SVFB chromatography Peak 1	CH1	2.52	2.38	0 to 1	0
SVFB chromatography Peak 2	CH2	1.21*	1.18*	1 to 3	2 to 3
SVFB chromatography Peak 3	CH3	1.27*	1.53*	2 to 4	2
SVFB chromatography Peak 4	CH4	1.16*	1.04	3 to 4	1 to 2
SVFB chromatography Peak 5	CH5	2.96	1.93	0 to 1	0 to 1

SVFB = bull seminal vesicle fluids.

* Statistically highly significant, $P < 0.01$.

pronase. The protein group in question is mainly the one concentrating on the gel in one or two strong zones, designated A (Pl. 1, Fig. 2). Electrophoretic analysis of the other fractions showed that a significant antispermatogenic effect was present in those fractions (including alcohol-ether precipitates and extracts of fluid treated with enzymes) which had part of their proteins in location A.

After chromatographic separation of fluid on Sephadex G-100, the anti-spermatogenic substance was contained in three peaks out of five. These peaks (Text-fig. 4—CH₂, CH₃, and CH₄) display protein staining in zone A after starch-gel electrophoresis.

Antibodies after injections

One-ml doses of alcohol-ether extract of vesicular fluid injected twelve times into two rabbits induced antibodies reacting in two and three lines on double

TEXT-FIG. 4. Chromatography of the seminal vesicle fluids of bulls on Sephadex G-100.

diffusion plates, in two arcs after immunoelectrophoresis (α -globulin region) and in the haemagglutination test at a titre of 1:2 and 1:32, respectively. The same alcohol-ether extracts of fluid treated with proteolytic enzymes induced very weak antibodies: no lines on double diffusion plates or arcs after immunoelectrophoresis and the titres of haemagglutinins ranged from 0 to 1:4.

Alcohol-ether precipitates of vesicular fluid induced antibodies showing six to eight lines on double diffusion plates and six to nine arcs after immunoelectrophoresis (in albumin and globulin regions); haemagglutination was observed at a titre of 1:4 to 1:32, respectively. Alcohol-ether precipitates of fluid treated with proteolytic enzymes induced rabbit antibodies showing one to five lines on double diffusion plates and two to five arcs after immunoelectrophoresis (in the α - and β -globulin regions); titres of 1:4 to 1:16 were found in haemagglutination tests. The other enzymes, α - and β -amylases and hyaluronidase, did not change the production of antibodies in injected rabbits.

There were no differences in the number or character of the precipitation and haemagglutination antibodies between immunized animals with damaged testes and those with normal ones.

It was not possible, by means of the fluorescence technique, to detect any antibody from the sera of eight injected rabbits, which reacted with epididymal spermatozoa or to show any reaction in histological sections of the testes of

rabbits and bulls. The reactions were also negative with antisera obtained from animals with damaged testes. A positive reaction by means of fluorescence was obtained with antisera from rabbits immunized in the same way, using a mixture of fluids from bovine epididymides. The antibodies from these rabbits reacted with rabbit epididymal spermatozoa, the cells within the seminiferous tubules and even with the peripheral part of the tubular membrane.

DISCUSSION

One of the principal aims of the present work was to obtain information about the effect of androgenic hormones on the biosynthesis of the antispermatogenic substance. The absence of this substance from the vesicular fluid of sexually immature bulls, and its synthesis in prepuberal bulls treated with testosterone propionate, provide evidence for the dependence of the substance on androgenic hormones. Renewal of the secretion of accessory sex glands and of the synthesis of the substance in castrated bull No. 717 treated with testosterone propionate further confirms our hypothesis. The prolonged sexual activity and the biosynthesis of the antispermatogenic substance in castrated bull No. 715 in the absence of supplementary hormone is remarkable. It appears that in this bull the androgens secreted by the adrenal glands were sufficient.

The antispermatogenic substance in the seminal vesicle fluid of bulls is not only highly thermostable but also resistant towards the treatment with various enzymes. Fractionation of vesicular fluid suggested that the aspermatogenic effect is probably due to proteins. Nevertheless, none of the proteolytic enzymes used succeeded in splitting the substance. There can be two explanations for this phenomenon. Either the substance is not a protein, and the protein acts merely as carrier, or it is a protein that resists the enzymes used. The principal bearer of activity is evidently the protein region designated A, obtained after starch-gel electrophoresis of vesicular fluid and its fractions. Another chromatographic separation of fluid treated with pronase, not mentioned in this paper, showed that this protein region is not homogeneous but that it separates into at least two zones.

In an earlier paper (Matoušek, 1969), the aspermatogenic activity of vesicular fluid was considered from an immunological aspect and its cytotoxic aspect was mentioned only marginally. Our present results indicate the necessity of changing this order, and of adopting a more critical and circumspect approach towards the immunological version. Negative results on testes sections were obtained with fluorescence methods using sera of rabbits with induced aspermatogenesis. No difference in antibody response was observed in rabbits with damaged or undamaged testes after injections of the fractions of fluid. The sudden onset of degenerative changes in the testis after injection of the aspermatogenically-active fraction of the fluid does not suggest an immunological process. Degenerative changes could be observed in the seminiferous tubules by the 3rd day after intratesticular injection.

The possibility of a relationship between, or even the identity of, the aspermatogenic substance and the haemolytic factor which is also present in the seminal plasma of bulls (Millar, 1956) has been considered. On the basis of previous

work (Matoušek & Staněk, 1970; Č. Kysilka, personal communication), we can assume that the two factors are not identical.

There is evidently a relationship between the aspermatogenic and antifertilizing effect of vesicular fluid. The antifertilizing effect of this fluid was manifested by a decrease in the number of embryos in mice and a reduction of egg-laying in hens (Matoušek & Petrovská, 1969). Results obtained so far indicate that both of these phenomena are caused by one and the same substance. This problem will be dealt with in our next study. Other unsolved problems are the relationship of the antispermatogenic substance to the substance in seminal vesicle fluid that sensitizes spermatozoa to cold shock (Matoušek, 1968), and to the haemagglutinating factor in boar seminal plasma (Bournsnel & Coombs, 1966).

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