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ANTIGENICITY OF BULL SEMINAL RIBONUCLEASE AND THE EFFECT OF ANTIBODIES ON ITS ACTIVITY

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Summary. The antigenicity of bull seminal ribonuclease (AS RNase) appears to depend on the species of the recipient.

Partly purified AS RNase formed haemagglutination and precipitation antibodies in rabbits against the original antigen and also against pancreatic RNase A. Absorption of these antisera by pancreatic RNase A demonstrated that the antisera to the seminal RNase were still present in the sera. Rabbit antibodies to bovine pancreatic RNase A reacted with its homologous antigen and AS RNase.

Antibodies did not prevent the action of AS RNase *in vivo*. Antigen–antibody complexes injected (i) into the testes of adult mice caused degeneration, (ii) into pregnant mice caused embryonic mortality, and (iii) into 6-day-old mice resulted in death.

Production of antibodies to AS RNase in mice also caused testicular damage, embryonic death and degeneration of Crocker tumour cells. Egg production in hens was reduced by similar treatment.

INTRODUCTION

The protein substance in bull seminal vesicle fluid (Matoušek, Staněk & Veselský, 1972; Dostál & Matoušek, 1972, 1973) responsible for the aspermatogenic activity (Matoušek, 1966, 1969) also exerts embryotoxic effects (Matoušek & Petrovská, 1969; Matoušek, 1973a; Matoušek, Fulka & Pavlok, 1973) and ribonuclease activity (AS RNase) (Matoušek, Pavlok, Dostál & Grozdanovič, 1973). In addition to the effects on testicular and embryonic tissue, it was shown (Matoušek, 1973b) to cause regression of Crocker tumour cells in mice.

The effects outlined above were employed in a study of the effect of antibodies on AS RNase activity and the seminal ribonuclease antigenicity in various animal species.

MATERIALS AND METHODS

Source of seminal ribonuclease

Pooled bull seminal vesicle fluid, partly purified AS RNase (Dostál & Matou-

šek, 1973) and pure AS RNase (Dostál & Matoušek, 1973) were used as antigenic material.

Injection procedures

Two bulls, eight rams and three boars were injected subcutaneously in the neck region with pooled bull seminal vesicle fluid once weekly for 4 weeks. Each injection comprised 10 ml (bulls) or 5 ml (rams and boars). Two other bulls (P_1 and P_2) each received 10-ml doses at intervals during 4-month and 8-month periods respectively. The total volumes injected were 250 and 490 ml respectively.

The rams received two further similar courses of injection at 3 and 5 months.

Semen was collected before and during the injection courses and sperm concentrations and percentage motilities were determined. The size of the testes was measured by a flexible ruler before and after the injection courses.

A total of sixty-six guinea-pigs received injections (0·2 ml) into the neck region of solutions in 0·1 M-trisodium citrate of either bull seminal vesicle fluid or 1% (w/v) of the partly purified AS RNase substance (BS AS RNase) (Dostál & Matoušek, 1973) once weekly for 4 weeks. A total of thirty-three rabbits were similarly treated, receiving 0·5 ml/dose.

In addition, one other rabbit received four 0·5-ml injections of a solution in 0·1 M-trisodium citrate of 0·5% (w/v) pure RNase. A second rabbit was similarly injected with 1% (w/v) bovine pancreatic RNase A (Koch-Light, England) and a third rabbit received two such injections separated by an 8-month interval.

In a further group of sixteen rabbits, nine received injections of pooled bull ampullary fluids and four of these were reinjected after 3 to 6 months. Three rabbits were injected with bull serum and four with fluid from bull cauda epididymidis (Matoušek, 1969).

Ten mice of C57BL and six of CBA strains were injected subcutaneously with BS AS RNase in five daily doses each of 0·1 ml 1% (w/v) protein. Similar courses of injections followed at intervals of not less than 60 days.

Determination of antibodies

These were carried out by the haemagglutinin titration tests described by Stavitský (1954) and by the microimmunoelectrophoresis method of Scheidegger (1955), using the fully purified AS RNase as the test antigen in both cases.

Binding of AS RNase antibodies to antigen in vitro

Rabbit antisera exhibiting the highest titre and immunoelectrophoretic response were pooled and 0·5-ml aliquots were mixed with 5 mg AS RNase. After 1 hr at 37°C, the antibody remaining was determined. Mouse antisera were similarly mixed in a 1 : 1 ratio with 1% BS AS RNase solution.

Effect of antibody on AS RNase activity

Samples (0·04 ml) of the antigen–antibody complexes (rabbits or mice), prepared as described, were injected into the left testis of mature mice. After

10 days, the testes were excised, weighed and compared in terms of mg testis/g body weight ('weight index'). Control tests involved injection of AS RNase plus normal rabbit serum and rabbit antisera.

Similarly, four subcutaneous injections of antigen–antibody complex (0·2 ml/dose) were given to female mice on Days 5 to 9 of pregnancy. At 13 to 17 days of pregnancy, the mice were killed and the number of live embryos determined. Control injections of 0·9% (w/v) NaCl were also given.

Following the method of Matoušek & Grozdanovič (1973), 0·2-ml quantities of the pooled rabbit antisera and AS RNase were subcutaneously injected into 6-day-old mice and the ratio of live/dead animals was determined after 48 hr. Control mice received either AS RNase solution or rabbit antisera, both diluted 1 : 1 with 0·1 M-trisodium citrate.

Effect of antibodies on RNase activity in mice and chickens

Sexually mature male mice were injected with several courses of BS AS RNase and the presence of antibodies against AS RNase was determined. The antitesticular activity of AS RNase in the presence of antibodies was also ascertained by the weight index of the testes. The experimental groups of animals were compared with the control groups of mice in which the last course of BS AS RNase injections was omitted and replaced by the 0·9% NaCl injections.

The embryotoxic effect of AS RNase and the effect of antibodies on the enzymatic activity *in vivo* was studied in mice injected once, twice or three times with AS RNase courses at different stages of pregnancy. The females were killed 4 to 7 days after the last AS RNase injection, serum was collected and the number of living embryos was determined. The embryotoxic effect in the experimental groups was compared with the control group of mice injected with 0·9% NaCl.

Injections (0·1 ml) of 1% BS AS RNase solution were started 7 to 14 days after the transplantation of Crocker tumour cells (Matoušek, 1973b). Before transplantation, mice received injections of RNase in one to three courses. Animals receiving transplants were killed 10 to 17 days after the first injection. Serum was collected and the gross appearance and histology of the tumours were examined. The extent of the cell degeneration in both cases was assessed on the following arbitrary grading: 0 (healthy tumour), 1, 2, 3, or 4 (total disintegration of tumour). Control groups received 0·9% (w/v) NaCl instead of BS AS RNase in the last course of injections.

Two hens, *Gallus domesticus* (LB × Cornish crosses), were examined daily for 22 months for egg production after five courses of injections, each course consisting of four injections of 1 ml of 1% BS AS RNase over a period of 2 weeks. Two similar control hens received corresponding injections of 0·9% NaCl.

RESULTS

Antibodies against RNase produced by bull seminal vesicle fluid antigen

Only one ram of the eight injected produced antibodies, as shown by the haemagglutinin titre (256) but not by immunoelectrophoresis. Six of the rams,

however, showed a reduction in the size of the testes after the first injection courses.

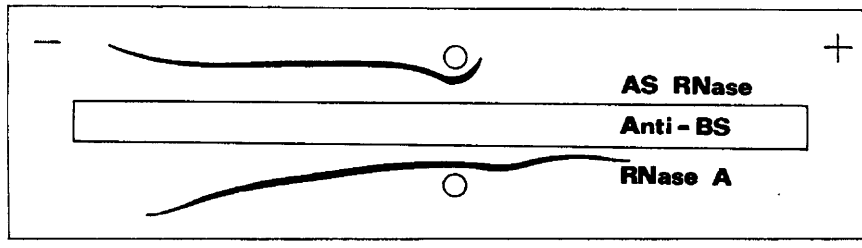
Neither bulls nor boars similarly treated gave haemagglutinin, immunoelectrophoretic or testicular size responses.

Only two of fifty-eight guinea-pigs showed precipitation antibodies and none showed a haemagglutinin reaction to only one course of immunization.

Of twenty-six rabbits injected, fifteen responded by giving haemagglutinin titres (4 to 128); immunoelectrophoretic precipitation lines were given by the same fifteen animals and by two others. Three rabbits given a second course responded positively in both tests. The testicular weights of four out of seven rabbits tested after a first injection course decreased by 48(±11)%.

Antibodies against RNase produced by partly purified (BS) and pure AS RNase

Seven rabbits were injected with BS AS RNase. Of these, three formed haemagglutination, and four precipitation antibodies. After reinjection, all formed antibodies (Text-fig. 1) with haemagglutination titres of 32 to 1024.



TEXT-FIG. 1. Precipitation line of AS RNase and pancreatic RNase A (RNase A) with rabbit antiserum against BS AS RNase (Anti-BS).

Rabbit No. 67, which was given two injection courses of pure AS RNase, formed antibodies as shown by immunoelectrophoresis and also by a haemagglutination titre of 128; after the third course, the titre rose to 2048.

This rabbit also gave both antibody reactions against pancreatic RNase A (titres of 128 and 512 according to the number of previous courses). One other rabbit (No. 40) reacted similarly after two courses of BS AS RNase (haemagglutinin titre, 256).

Absorption of the three antisera mentioned above with pancreatic RNase A (10 mg/ml serum) eliminated the reaction against the pancreatic RNase A but left the AS RNase reaction intact. By contrast, absorption with AS RNase removed AS and pancreatic RNase activities.

Of six rabbits receiving BS AS RNase injections, five reacted only weakly (titres of 6 to 64) and none gave immunoelectrophoretic reactions with pancreatic RNase A.

No immune reactions were found when pancreatic RNase A was used as the test antigen towards sera from bull, ram, boar, guinea-pig, mouse or chicken immunized with AS RNase.

All sixteen mice given four courses of BS AS RNase gave precipitin lines

and haemagglutinin titres from 8 to 256. Tests with various numbers of courses showed that four courses were necessary for a full antigenic response.

Antibodies against AS RNase after injections of further genital fluids of bulls, bull serum and bovine pancreatic RNase A

Nine rabbits received four 1-ml injections of bull ampullary fluid but only some of the animals developed antibodies (haemagglutination, three rabbits; precipitation, four rabbits).

All four rabbits receiving further courses of injection developed antibodies which were detected by both tests.

No antibodies against AS RNase were detected in rabbits injected with either bull blood serum or fluid from bull cauda epididymidis.

Two rabbits received one injection course and one rabbit received two injection courses of bovine pancreatic RNase A. The haemagglutinin titres against pancreatic RNase A test antigen were 32, 256 and 1024 respectively. As the test antigen, AS RNase gave similar titres of 16, 256 and 1024. In all three cases, the antibodies were fully absorbed both by pancreatic RNase A and by AS RNase (10 mg/ml serum).

The binding of antibodies on AS RNase antigen and the enzymatic efficiency of antigen–antibody complex

The AS RNase antibodies in the antisera of six rabbits were completely absorbed by the corresponding antigen as tested by haemagglutination and precipitation reactions.

Pooled rabbit antisera mixed with AS RNase solution were injected into the left testes of six mice. A statistically significant decrease in the weight index occurred ($1{\cdot}95 \pm 0{\cdot}63$) compared with the uninjected testes ($3{\cdot}82 \pm 0{\cdot}41$). Six control mice injected with AS RNase mixed with normal rabbit serum showed a similar decrease ($1{\cdot}88 \pm 0{\cdot}22$ compared with $3{\cdot}78 \pm 0{\cdot}31$). Six control mice injected with pooled rabbit antisera alone showed no decrease ($4{\cdot}25 \pm 0{\cdot}41$ compared with $4{\cdot}26 \pm 0{\cdot}47$).

The AS RNase mixture pooled with rabbit antisera was administered to twenty-seven pregnant mice on the 5th to 9th day of pregnancy and was found to be enzymatically effective. At autopsy, only one mouse was found to have two living embryos. The control group of twelve mice injected with 0·9% NaCl at the same period of pregnancy had $5{\cdot}91 \pm 0{\cdot}18$ embryos per mouse.

The same antigen/antiserum mixture was injected into seven 6-day-old mice and all died within 48 hr. Five mice of similar age injected with the 1% AS RNase solution alone also died, but a control group of five mice injected with the pooled rabbit antisera continued to develop normally.

Antisera to AS RNase raised in mice did not block the antitesticular effect of the enzyme in these animals. The left testes of eight mice, injected with a mixture of pooled mouse antisera and 1% AS RNase solution, showed a weight index of $1{\cdot}65 \pm 0{\cdot}66$, whereas the uninjected testes had a weight index of $3{\cdot}58 \pm 0{\cdot}66$. Three control mice in which the left testes were injected with pooled mouse antisera alone had weight indices of $4{\cdot}01 \pm 0{\cdot}56$ (left), and $3{\cdot}90 \pm 0{\cdot}56$ (right).

Table 1. Weight of testes in mice after repeated courses of AS RNase injections

Control groups			*Experimental groups*					
						Antibodies		
						Haemagglutinating		*Precipitating*
Treatment (No. of AS RNase and 0·9% NaCl courses)	*No. of males injected*	*Wt of both testes (mg/g body wt) (Mean ± S.E.)* †	*Treatment (No. of AS RNase courses)*	*No. of males injected*	*Wt of both testes (mg/g body wt) (Mean ± S.E.)* ‡	*No. of males with antibodies*	*Titre of antibodies*	*No. of males with antibodies*
One 0·9% NaCl	15	7·45 ± 0·91	One	10	3·40 ± 0·46**	0	0	0
One AS RNase and one 0·9% NaCl	8	4·38 ± 0·32	Two	8	3·42 ± 0·47**	3	4 to 16	3
Two AS RNase and one 0·9% NaCl	5	4·98 ± 0·34	Three	5	2·93 ± 0·92**	5	4 to 64	5
Three AS RNase and one 0·9% NaCl	4	4·67 ± 0·42	Four	3	2·40 ± 0·43**	3	16 to 128	3

** Statistically significant $P < 0·01$.
† Testes weighed 81 to 181 days after the last injection of AS RNase and 21 days after the injection of 0·9% NaCl.
‡ Testes weighed 21 days after the last injection of AS RNase.

Table 2. Embryonic mortality of mice after repeated AS RNase injection series

Groups	*No. of AS RNase injection courses*	*No. of days from mating to the first AS RNase injection*	*No. of females injected*	*No. of embryos/ injected female (Mean ± S.E.)*	*Antibodies*			
					No. of mice tested	*Haemagglutinating*		*Precipitating*
						No. of females with antibodies	*Titre of antibodies*	*No. of females with antibodies*
Control	0·9% NaCl	1 to 13	44	5·38 ± 3·45	7	0	0	0
	One	2 to 10 days before mating	29	2·65 ± 2·17	—	—	—	—
	One	1 to 13	94	0·73 ± 1·19	10	0	0	0
Experimental	Two	2 to 10 days before mating	25	4·00 ± 2·20*	—	—	—	—
	Two	1 to 5	30	2·36 ± 2·64**	8	4	4 to 32	3
	Three	1 to 5	8	1·50 ± 2·25**	4	3	8 to 128	3
	Four	1 to 5	4	1·00 ± 1·50**	—	—	—	—
	Two	6 to 8	30	3·10 ± 2·63**	7	1	8	1
	Three	6 to 8	17	0·12 ± 0·20**	6	5	4 to 128	5
	Four	6 to 8	10	0·00**	3	3	8 to 256	3
	Two	9 to 13	32	0·38 ± 0·60**	—	—	—	—
	Three	9 to 13	18	0·00**	6	4	8 to 64	4
	Four	9 to 13	16	0·00**	—	—	—	—

* Statistically significant $0{\cdot}05 > P > 0{\cdot}01$.
** Statistically significant $P < 0{\cdot}01$.

Table 3. Degeneration of Crocker tumour cells in mice after repeated AS RNase injection courses

No. of injection courses	*Control groups*			*Experimental groups*					
							Antibodies		
							Haemagglutinating		*Precipitating*
	No. of mice with tumour injected in the last courses with 0·9% NaCl	*Tumour wt (g) (Mean ± S.E.)*	*Degree of degeneration*	*No. of mice with tumour injected with AS RNase*	*Tumour wt (g) (Mean ± S.E.)*	*Degree of degeneration*	*No. of animals with antibodies*	*Titre of antibodies*	*No. of animals with antibodies*
Two	6	2·10 ± 0·58	0 to 1	8	0·92 ± 0·96*	1 to 4	3	4 to 8	3
Three	5	2·39 ± 0·54	0 to 1	9	0·80 ± 0·76*	1 to 4	8	4 to 128	9
Four	5	2·12 ± 0·64	0 to 1	10	0·53 ± 0·21**	2 to 4	10	8 to 256	8

* Statistically significant $0·05 > P > 0·01$.
** Statistically highly significant $P < 0·01$.

Courses of subcutaneous injections of AS RNase administered to groups of male mice confirmed the above results. In mice which were reinjected with AS RNase antigen and with proved anti-AS RNase antibodies, the weight index decreased while no such decrease occurred in control mice in which the last AS RNase course was omitted (Table 1). In control groups, no antibodies could be demonstrated, even with three courses of AS RNase injections. In these groups, there was a long delay (81 to 191 days) in the collection of blood after the last course.

The study of the effect of antibodies on antiembryonic activity of AS RNase (Table 2) gave a similar picture. There was a highly significant decrease in the number of living embryos, even in groups of mice in which antibodies were shown to be present.

Antibodies against AS RNase did not block the activity of this enzyme and were unable to prevent the degeneration of the Crocker tumour cells in mice (Table 3).

Table 4. Egg-laying of two hens after repeated AS RNase injection courses

No. of injection courses of 0·9% NaCl (C) or AS RNase (Exp)		*No. of eggs laid during 28 days before injections*	*No. of eggs laid during 28 days after the last injection*	*No. of eggs laid during 29 to 56 days after the last injection*	*Antibodies*		
					Haemagglutinating		*Precipitating*
					No. of hens with antibodies	*Titre of antibodies*	*No. of hens with antibodies*
One	(C)	34	26	23	—	—	—
	(Exp)	36	7	15	—	—	—
Two	(C)	26	21	21	0	0	0
	(Exp)	28	0*	22	2	32 to 64	0
Three	(C)	26	37	15	0	0	0
	(Exp)	30	27	16	2	16 to 32	0
Four	(C)	28	32	27	0	0	0
	(Exp)	26	5*	18	2	16 to 32	0
Five	(C)	24	25	37	0	0	0
	(Exp)	26	11	13	2	16 to 128	2

* Statistically significant $0{\cdot}05 > P > 0{\cdot}01$.

Although the investigation of the effect of the antigen–antibody complex on the egg-laying capacity in hens was limited to two experimental and two control animals, the results showed statistically significant decreases in egg production after the second and fourth course of AS RNase injections. Overall, the total egg-laying capacity of the treated birds showed a statistically significant fall (Table 4). The mean number of eggs ($\pm$S.E.) laid during 28 days after the second to fifth course of injections by the treated birds ($5{\cdot}4 \pm 4{\cdot}1$) was significantly different ($0{\cdot}05 > P > 0{\cdot}01$) from that of the control birds ($14{\cdot}3 \pm 2{\cdot}8$).

DISCUSSION

The production of antibodies against AS RNase differs from one animal species

to another. Of the species tested, rabbits, hens and mice (in that order) most easily formed antibodies. Immunological sensitivity and possibly the phylogenetic distance from the source of the seminal ribonuclease apparently play an important rôle. In rams, even though they were injected three times, the antigenicity of the bull enzyme was very small, and in bulls, despite long-term injection of seminal vesicle fluid, no success could be reported in the formation of antibodies against AS RNase.

Although the AS RNase molecule differs considerably in molecular weight from bovine pancreatic RNase A (22,000 and 13,600 respectively), a certain identity of some determinant groups is evident from the results. Antibodies obtained by injections of pancreatic RNase A into rabbits reacted in haemagglutination and immunoelectrophoresis tests both with its own antigen and with the corresponding AS RNase antigen. Antibodies against both ribonucleases were also found in the sera of several rabbits injected with AS RNase alone. It is evident, however, that antibodies in these sera against bovine pancreatic RNase A were considerably weaker than against the homologous antigen. It seems, therefore, that the corresponding common or cross-reacting determinant groups are antigenically more efficient in pancreatic RNase A than in AS RNase. When pancreatic RNase A was added to potent rabbit AS RNase antisera (which reacted against both AS RNase and pancreatic RNase A), the antigen absorbed pancreatic RNase antibodies but left intact AS RNase antibodies. By contrast, AS RNase antigen always absorbed all the antibodies present.

It would appear therefore that all the determinant groups of pancreatic RNase A are present in the AS RNase in addition to specific groups for AS RNase itself. It can be postulated that the AS RNase is formed from pancreatic RNase A plus a further polypeptide chain responsible for the extra AS RNase properties; this corresponds with the observations on the reactions *in vitro* of AS RNase by Holý & Grozdanovič (1972).

The main object of this paper was to study how AS RNase antibodies affect the efficiency of the enzyme in the living animal. Although it was shown that antigen–antibody binding occurred *in vitro*, apparently no similar effect occurs *in vivo*. This could be due to the fact that under conditions *in vivo* the active centre of the enzyme is unaffected by antibody, or the antigen–antibody complex is not stable.

D'Alessio & Floridi (1967) also isolated bull seminal ribonuclease and named it RNase BS_1 (D'Alessio, Floridi, de Prisco, Pignero & Leone, 1972). This enzyme resembles AS RNase in showing immunologically similar behaviour to bovine pancreatic RNase A (Floridi & Fini, 1972), but differs in that Floridi & Fini did not succeed in preparing antisera with antibodies against pancreatic RNase A by immunizing the rabbits with RNase BS_1.

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REFERENCES

D'Alessio, G. & Floridi, A. (1967) Compartamento cromatografico della ribonucleasi seminale. *Boll. Soc. ital. Biol. sper.* **43,** 32.

D'Alessio, G., Floridi, A., de Prisco, R., Pignero, A. & Leone, E. (1972) Bull semen ribonucleases. I. Purification and physico-chemical properties of the major component. *Eur. J. Biochem.* **26,** 153.

Dostál, J. & Matoušek, J. (1972) Purification of aspermatogenic substance in bull seminal vesicle fluid. *J. Reprod. Fert.* **31,** 273.

Dostál, J. & Matoušek, J. (1973) Isolation and some chemical properties of aspermatogenic substance from the bull seminal vesicle fluid. *J. Reprod. Fert.* **33,** 263.

Floridi, A. & Fini, C. (1972) Bull semen ribonuclease: immunological correlations with pancreatic ribonuclease. *Ital. J. Biochem.* **21,** 71.

Holý, A. & Grozdanovič, J. (1972) The substrate specificity of bovine seminal vesicle fluid ribonuclease (ribonuclease AS). *Biochim. biophys. Acta,* **227,** 556.

Matoušek, J. (1966) Antigenicity of the seminal vesicle fluid in bulls. *Proc. 10th Int. Congr. Anim. Blood Grps, Paris,* p. 513. Institut National de la Recherche Agronomique, Paris.

Matoušek, J. (1969) Effects on spermatogenesis in guinea-pigs, rabbits and sheep after their immunization with sexual organ fluids of bulls. *J. Reprod. Fert.* **19,** 63.

Matoušek, J. (1973a) Antiembryonic effect of bull seminal vesicle fluid in mice. *J. Reprod. Fert.* **32,** 175.

Matoušek, J. (1973b) The effect of bovine seminal ribonuclease (AS RNase) on cells of Crocker tumor in mice. *Experientia,* **29,** 858.

Matoušek, J., Fulka, J. & Pavlok, A. (1973) Effect of ribonuclease fractions isolated from bull seminal vesicle fluid on embryonic mortality in guinea-pigs, rabbits and pigs. *Int. J. Fert.* **18,** 13.

Matoušek, J. & Grozdanovič, J. (1973) Specific effect of bull seminal ribonuclease (AS RNase) on cell systems in mice. *Comp. Biochem. Physiol.* **46A,** 241.

Matoušek, J., Pavlok, A., Dostál, J. & Grozdanovič, J. (1973) Some biological properties of the bull seminal vesicle aspermatogenic substance in mice. *J. Reprod. Fert.* **24,** 9.

Matoušek, J. & Petrovská, E. (1969) Antifertilizing effect of the seminal vesicle fluid of bulls on females. *J. Reprod. Fert.* **20,** 189.

Matoušek, J., Staněk, R. & Veselský, L. (1972) First attempt to characterize an aspermatogenic substance in the seminal vesicle fluids of bulls. *J. Reprod. Fert.* **29,** 179.

Scheidegger, J. (1955) Une micro-méthode de l'immuno-électrophorèse. *Int. Archs Allergy appl. Immun.* **7,** 103.

Stavitský, A. B. (1954) Micromethods for the study of proteins and antibodies. I. Procedure and general applications of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. *J. Immun.* **72,** 360.