Inhibition by Seminal Plasma of Acrosomal Enzymes in Intact Sperm (34648)

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The acrosomes of rabbit spermatozoa contain various enzymes including a trypsin-like enzyme (TLE) (1, 2), a corona penetrating enzyme (CPE) (3) and a new type of neuraminidase (4). CPE and TLE may be involved in the penetration of spermatozoa through the corona radiata and the zona pellucida (1–3). Seminal plasma contains a trypsin inhibitor (2, 5) and decapacitation factor (DF), a substance which functionally reverses the capacitation process (6). DF (7) inhibits CPE (3, 8) and sperm neuraminidase (9). The trypsin inhibitor inhibits TLE using benzoyl arginyl ethyl ester (BAEE) as substrate (2).

Acrosomal extracts of epididymal sperm prepared by treatment with Hyamine (3, 10) have a high trypsin activity, whereas no trypsin activity can be found in similarly treated ejaculated sperm (2). Present experiments indicate that TLE, CPE, and sperm neuraminidase activities are high in epididymal sperm extracts and are decreased by contact with seminal plasma.

Materials and Methods. Seminal plasma was obtained by centrifugation of rabbit semen at 900*g* for 15 min. Rabbit epididymal spermatozoa were divided into portions of 4×10^9 sperm and incubated for 2 hr at 37° with 3 ml of either Krebs–Ringer phosphate (KRP) buffer, untreated seminal plasma, and seminal plasma boiled for 15 min and centrifuged to remove the insolubles. Each sample was washed by centrifugation at 900*g* for 15 min and the supernatant solution was removed. The sperm were resuspended in KRP buffer and recentrifuged. This was repeated three times. The washed sperm samples were suspended in KRP buffer, the acrosomes detached with detergents according to the method of Hartree and Srivastava (10) and the particulate matter removed by centrifugation. The resulting enzyme solution was treated with alcohol, the precipitate dialyzed and finally lyophilized. The lyophilized material was reconstituted in KRP and assayed for TLE activity on BAEE by the method of Schwert and Takenake (11) for CPE activity by incubation with ova at 37° for 24 hr (3) and for neuraminidase activity by the Warren method (12). The ova were obtained by injection of 75 units of human chorionic gonadotropin (HCG) in the ear vein of New Zealand white rabbits. The rabbits were sacrificed 13 hr later and the oviducts were flushed with KRP. The ova were incubated with hyaluronidase at 37° to remove the cumulus cells and washed with KRP by repeated transfer. Pancreatic trypsin inhibitor (200 μg) was added to each assay tube to prevent possible corona cell removal by TLE. A numerical scoring system was used for the degree of denudation of the ova: 4, if the corona was completely dispersed; 3, if most of the corona was removed; 2, if only some corona cells were absent; and 1, if no corona cells were removed. The grades for the ova were added and divided by the total number of ova assayed. This figure is entered under "degree of denudation" (Table II). A number of 3 or more indicates an active CPE preparation. Protein determinations were performed by the modified Lowry method (13).

Results. The TLE activity of untreated epididymal sperm was high whereas the epididymal sperm treated with seminal plasma possessed a very low TLE activity. The boiled and centrifuged seminal plasma did not significantly alter the TLE activity of the epididymal sperm extracts. Sperm neuraminidase activity was inhibited by both prepara-

TABLE I. Trypsin and Neuraminidase Activity of Epididymal Sperm Treated with Various Seminal Plasma Solutions.

Epididymal sperm incubated with the following before preparation of acrosomal extracts	TLE[a] (units/mg of acrosomal protein)	Neuraminidase (μmoles of sialic-acid/mg of acrosomal protein/min)
Krebs–Ringer phosphate buffer	151	0.25
Untreated seminal plasma	8	0.16
Boiled, centrifuged seminal plasma	122	0.17

[a] Average amount from 8 experiments.

TABLE II. Corona Penetrating Enzyme Activity of Epididymal Sperm Treated with Various Seminal Plasma Solutions.[a]

Epididymal sperm incubated with the following before preparation of acrosomal extracts	Degree of denudation	No. of ova recovered
Krebs–Ringer phosphate buffer	3.9	11
Untreated seminal plasma	2.0	12
Boiled, centrifuged seminal plasma	2.0	11

[a] Each assay system employed 4 ova. These were incubated with the various solutions (ranging in protein content from 7.6 to 42 μg—constant for each assay) for 24 hr at 37°.

tions although the untreated seminal plasma has the greatest inhibitory effect (Table I). CPE activity of the epididymal sperm was similarly inhibited by seminal plasm and boiled, centrifuged seminal plasma (Table II).

Discussion. The experiments reported here provide the first demonstration of a direct effect of seminal plasma inhibitors on enzymes located in the sperm acrosome. Since the acrosome is essentially a modified lysosome (14), it is reasonable to assume that penetration of the outer acrosomal membrane by these inhibitors occurs. It is interesting to note that although epididymal plasma contains both the TLE inhibitor and DF in lower amounts than in seminal plasma (15) the enzyme-inhibitor complexes do not seem to form until the epididymal sperm come in contact with the seminal plasma. It is likely that during capacitation these inhibitors are removed from their respective enzymes so that penetration of ova can occur.

Boiled seminal plasma lost its ability to inhibit sperm TLE although the trypsin inhibitor is heat stable (2). It is possible that another factor in the seminal plasma is necessary for the transfer of the trypsin inhibitor across the acrosomal membrane. The presence of such a heat-labile factor may also explain why acrosomal enzymes are not inhibited completely until the sperm come in contact with seminal plasma. Apparently this factor is absent in epididymal fluid.

Summary. Epididymal sperm have high trypsin-like enzyme (TLE) activity, CPE activity and neuraminidase activity whereas these enzyme activities are much lower in ejaculated sperm. Incubation of epididymal sperm with seminal plasma caused a decrease in each of these enzyme activities, indicating that seminal plasma inhibitors may transfer to the acrosomal enzymes during ejaculation. Boiled and centrifuged seminal plasma did not inhibit the TLE activity of epididymal sperm but did decrease the CPE and neuraminidase activity.

1. Stambaugh, R., and Buckley, J., Science **161**, 585 (1968).

2. Zaneveld, L. J. D., Srivastava, P. N., and Williams, W. L., J. Reprod. Fert., in preess.

3. Zaneveld, L. J. D., McRorie, R. A., and Williams, W. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 567 (1968).

4. Srivastava, P. N., Zaneveld, L. J. D., Gould, K., and Williams, W. L., Abstr. Soc. Study of Reprod. Meeting, Sept. 8, 1969, Davis, Cal., Biol. Reprod., in press.

5. Fritz, H., Trautschold, I., Haendle, H., and Werle, E., N. Y. Acad. Sci. **146**, 400 (1968).

6. Chang, M. C., Nature (London) **179**, 258 (1957).

7. Robertson, R. T., Pinsker, M. C., Caster, W. O., and Williams, W. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 705 (1969).

8. Zaneveld, L. J. D., McRorie, R. A., and Williams, W. L., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **28**, 705 (1969).

9. Srivastava, P. N., Zaneveld, L. J. D., and Williams, W. L., Biochem. Biophys. Res. Commun., in press.

10. Hartree, E. F., and Srivastava, P. N., J. Reprod. Fert. **9**, 47 (1965).

11. Schwert, G. W., and Takenake, Y., Biochim. Biophys. Acta **16**, 570 (1955).

12. Warren, L., J. Biol. Chem. **234**, 1971 (1959).

13. Lowry, O. H., Rosebrough, W. J., Farr, A. L., and Randall, R. J., J. Biol. Chem. **193**, 265 (1951).

14. Austin, C. R., "Fertilization," Prentice Hall, Englewood Cliffs, New Jersey (1965).

15. Robertson, R. T., master's thesis, Univ. of Georgia, 1969.

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