duces an acute systemic reaction in the rabbit which can be followed by means of the animal's temperature. Temperatures exceeding 104° are considered definitely pathognomonic. In animals which survive the disease it is usually difficult to precipitate it again, or as severely, by shock, compared to those which survive after infection through the leg route. On the other hand, the latter group do not usually develop the acute symptoms and high fever that characterize the former during the initial sickness.

These influences are reflected in the changes in the inhibitor concentration. Thus, in Fig. 3, the effect of the corneal infection can be seen to produce a consistent elevation which has a characteristic double peak during the period of acute illness. After recovery, sensitization and shock failed to cause important changes in either the infective state or the inhibitor level.

When the virus was injected into the leg, no large increases in the inhibitor concentration were found, Fig. 4, and the animals were not acutely ill. The sensitization was begun on the same day that the rabbits were infected in order to save time, as these experiments continue for months. From Figs. 1 and 3, it is apparent that sensitization *per se* has no appreciable influence on the inhibitor level. A prompt elevation of the inhibitor level followed shock in those animals in which precipitation of the disease occurred. Animals No. 1372, 1373 showed the most severe symptoms, No. 1366 little effect, and No. 1369 none. The changes in the inhibitor show a correlation with these symptomatic effects.

Summary. Hyaluronidase inhibitor in the blood serum of the rabbit was found to undergo no consistent change in concentration as the result of anaphylaxis induced by egg white. An elevation of the inhibitor level was observed during the acute phase of infection by *Herpes simplex* virus administered via the corneal route. Anaphylactic shock in rabbits which had recovered from the herpetic infection, administered via the leg route, resulted in elevated inhibitor values which were correlated with the severity of the disease thus precipitated from the latent state by the shock.

16814

Effects of Heterologous Seminal Plasma and Sperm Cells on Fertilizing Capacity of Rabbit Spermatozoa.

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In a previous experiment,[1] the beneficial effects of rabbit seminal plasma on the fertilizing capacity of rabbit spermatozoa was demonstrated. This paper reports the effects of human, bull, and rabbit seminal plasma, as well as of dead sperm of these species, on the fertilizing capacity of rabbit spermatozoa.

Methods: Human semen was collected into a sterile tube and stored at 2°C no more than 12 hours. Bull semen and vasectomized rabbit semen were obtained by means of an artificial vagina[2,3] about 1 to 2 hours before use. Human and bull samples were centrifuged at 2,000 R.P.M. for about 30 to 40 minutes and the supernatant fluid was used as seminal plasma.

The semen of a single rabbit, collected at

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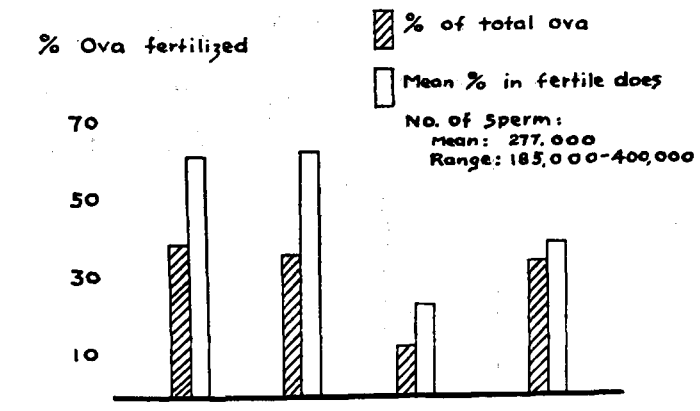


Treatment	Human Sem. plasma	Rabbit Sem. plasma	Bull Sem. plasma	Fructose Ringer Solution
No. of does	16	14	14	16
Sterile does	7	4	7	4
Total ova	498	412	517	507

FIG. 1.
Effects of heterologous seminal plasma on fertilizing capacity of rabbit spermatozoa (immediate insemination).

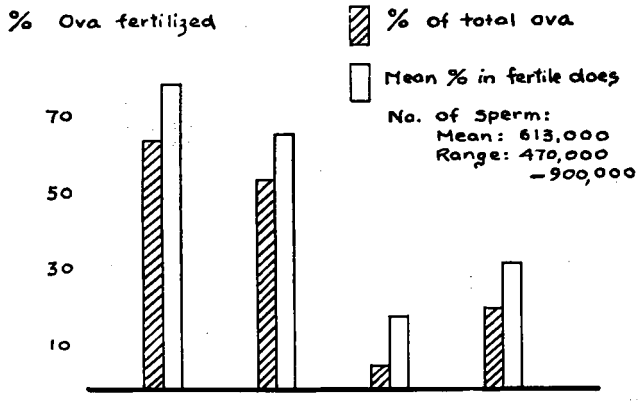
intervals of 7 days, was used throughout this investigation. About 0.01 to 0.03 ml of semen, depending on the concentration of sperm, was first suspended in 10 ml of Fructose Ringer solution (NaCl, 0.85 mg; KCl, 0.042 g; $CaCl_2$, 0.024 mg; $MgCl_2$, 0.02 g; $NaHCO_3$, 0.1 g; Fructose, 0.1 g; Double glass distilled water, 100 ml). The sperm concentration of this suspension was immediately and quickly examined by means of a haemocytometer technic in order to obtain a minimal effective number of sperm (Number of spermatozoa required to fertilize only a portion of the ova ovulated). Then 0.5 ml of this suspension was added to 0.5 ml of human, bull, rabbit seminal plasma, or to 0.5 ml of Fructose Ringer solution which was used as a control. The mixture (all 1 ml in volume) was inseminated, immediately in the first series of experiments, or after storage at room temperature for one hour in the second series of experiments. After insemination, the number of spermatozoa was counted again 4 to 6 times to obtain an accurate number of spermatozoa inseminated.

Six to 8 doe rabbits (superovulated according to Pincus[4]) were used at a time. Two does each were inseminated with the same sperm mixture. The ovulation injection of pituitary extract was given just after insemination. The animals were sacrificed about 25 hours later. The ova of each rabbit were flushed from fallopian tubes with undiluted rabbit serum and the fertilized and unfertilized ova were counted.

In a third series of experiments, sperm cells of human, bull or rabbit were obtained by centrifugation of semen samples and then resuspended in saline and centrifuged again. The washed sperm cells were deep frozen with a mixture containing solid carbon dioxide and acetone and then suspended in Fructose Ringer solution (about 800 millions of dead sperm per ml). A minimal effective number of rabbit spermatozoa was suspended in this fluid and stored at room temperature for one hour before insemination. In this series, one part of fresh egg yolk was suspended into one part of Fructose Ringer solution as a control.

Results: Fig. 1-3 illustrate the results. The fertilizing capacity of spermatozoa under different treatments was expressed in terms of percentage of ova fertilized. A few of the does (31%) had no ova fertilized, due to female infertility and/or to the small number

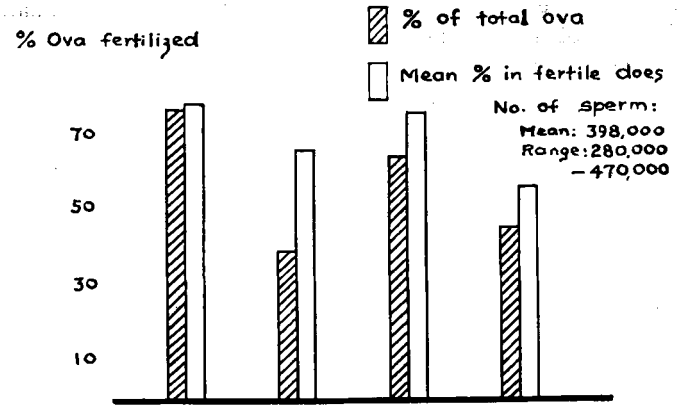
[4] Pincus, G., *Anat. Rec.*, 1940, 77, 1.



Treatment	Human Sem. plasma	Rabbit Sem. plasma	Bull Sem plasma	Fructose Ringer Solution
No. of does	8	8	8	8
Sterile does	1	1	3	5
Total ova	179	191	372	239

FIG. 2.

Effects of heterologous seminal plasma on fertilizing capacity of rabbit spermatozoa (stored one hour before insemination).



Treatment	Human Sperm	Rabbit Sperm	Bull Sperm	Egg yolk
No. of does	6	10	8	10
Sterile does	0	3	1	3
Total ova	145	273	208	303

FIG. 3.

Effects of dead sperm of different species on fertilizing capacity of rabbit spermatozoa (stored for one hour before insemination).

of sperm inseminated. Thus, the percentages of all ova, including the ova of sterile does, in each group and the mean percentage of ova fertilized in the fertile does were presented in figures. In the calculation of statistical significance between each group, the mean percentages of fertilized ova of all does, including sterile does, was taken.

Fig. 1 illustrates the results of 60 inseminations performed immediately after prepara-

tion of the mixture. The percentage of ova fertilized was low when rabbit sperm were suspended in bull seminal plasma or in Fructose Ringer solution. Statistical analysis of the data (by t test) however, shows that there is no significant difference between human and rabbit seminal plasma and Fructose Ringer solution, but there is a significant difference between bull seminal plasma and other sperm mixtures ($t = 2.3$, 2.78, or 2.4, $p < .05 > .01$). This clearly demonstrates that bull seminal plasma has an adverse effect on the fertilizing capacity of rabbit sperm.

Fig. 2 illustrates the results of another 32 inseminations performed when the sperm mixtures were allowed to stand for one hour before insemination. The beneficial effects of human or rabbit seminal plasma as compared with Fructose Ringer solution or bull seminal plasma is clearly shown. There is a very significant difference between human and bull seminal plasma ($t = 3.9, p < .01$), human and Ringer solution ($t = 3.55, p < .01$), rabbit and bull plasma ($t = 3.75, p < .01$) and rabbit and Ringer solution ($t = 3.12, p < .01$).

It is clear by comparison of Fig. 1 and 2, that human and rabbit seminal plasma have a beneficial effect, while bull seminal plasma has an immediate harmful effect, on the fertilizing capacity of rabbit spermatozoa. Fructose Ringer solution has no immediate ill effect, but the fertilizing capacity of rabbit spermatozoa decreases after one hour in Fructose Ringer solution.

Fig. 3 illustrates the result of another 34 inseminations when the minimal effective number of rabbit spermatozoa were suspended in Fructose Ringer solution containing dead human, bull and rabbit sperm cells or egg yolk. The percentage of ova fertilized was high when rabbit spermatozoa were mixed with human or bull sperm cells, but it was rather low when mixed with rabbit sperm cells or with egg yolk. There is a significant difference between human sperm cells and egg yolk ($t = 2.8, p < .02 > .01$); the difference between other treatments is not significant. Since egg yolk is considered the best medium for the preservation of fertilizing capacity of spermatozoa in the practice of artificial insemination,[5] it is of interest to note that sperm cells of human and bull are better than or equal to egg yolk.

The results presented in Fig. 2 and 3 are comparable because the sperm mixtures were stored for one hour before insemination. In this respect, human, rabbit, or bull sperm cells and human or rabbit seminal plasma are beneficial to the fertilizing capacity of rabbit spermatozoa (No statistical significant difference) while bull sperm cells are much better than bull seminal plasma (55% with significant difference). Fructose Ringer solution with egg yolk is definitely better for the preservation of fertilizing capacity than without egg yolk (28%, with significant difference). Human sperm cells are better than Fructose Ringer solution containing egg yolk (39%, with significant difference).

Discussion. In the previous experiment,[1] the beneficial effect of seminal plasma was demonstrated when 0.9% NaCl was used as control. It was thought that 0.9% of NaCl may have an adverse effect on spermatozoa. The present study once again demonstrated the same fact when a balanced salt solution with the metabolic substrate, fructose[6] was used.

In a study of the effects of high dilution on fertilizing capacity[7] Chang has postulated that there might be a beneficial substance in the seminal plasma or in the spermatozoa. The present study clearly verifies this assumption. Whether the beneficial effect of seminal plasma or egg yolk is to prevent the escape of essential substances in the spermatozoa as suggested by Emmens and Swyer[8] or to contribute an essential substance for the prolongation of fertilizing capacity is hard to say at present. If sperm cells, seminal plasma or egg yolk contribute this essential substance, the sources of this substance may be widely distributed in animal tissues. Consid-

[5] Anderson, J., The Semen of Animals and Its Use in Artificial Insemination. 1947: Edinburgh, Genetic Institute.

[6] Mann, T., *Nature*, 1946, **157**, 79.

[7] Chang, M. C., *Science*, 1946, **104**, 361.

[8] Emmens, C. W., and Swyer, G. I. M., *Nature*, 1947, **160**, 718.

ering the beneficial effect of bull sperm cells on rabbit spermatozoa and the harmful effect of bull seminal plasma, the presence of this essential substance in the spermatozoa of different species rather than in the seminal plasma is indicated.

It is of biological interest to note the compatibility of rabbit sperm and human seminal plasma and the incompatibility of rabbit sperm and bull seminal plasma.

A high dilution of sperm was employed in the present study in order to obtain a minimal effective number of spermatozoa. It was observed that the progressive movement of sperm lasted for 5 to 6 hours in seminal plasma (only 3 to 4 hours in bull seminal plasma) or in the solutions containing added dead sperm. But only sluggish movement was observed in Fructose Ringer solution from the beginning for 2 to 3 hours.

Summary. Superovulated does were inseminated with a minimal effective number of sperm suspended in Fructose Ringer, or seminal plasma of human, bull or rabbit; or in Fructose Ringer suspension of egg yolk, or deep frozen sperm. It was found that the seminal plasma of human or rabbit had a beneficial effect while that of bull had an ill effect on the fertilizing capacity of rabbit sperm as compared with Fructose Ringer. Fructose Ringer containing dead sperm of human, rabbit or bull was found as good or better media for the preservation of sperm as egg yolk; which indicates the presence of essential substances in the sperm cells for the maintenance of their fertilizing capacity.

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16815 P

Occurrence of Streptomycin Resistant Tubercle Bacilli in Mice Treated with Streptomycin.*

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Tubercle bacilli readily become resistant to streptomycin *in vitro*[1-3] and in patients being treated with streptomycin.[1,4-6] Feldman, Karlson and Hinshaw[7] reported the isolation of streptomycin resistant tubercle bacilli from 3 of 8 guinea pigs treated for a prolonged period with 6.0 mg streptomycin per day, after these had been infected with a streptomycin sensitive strain of *M. tuberculosis*.

Methods. One hundred mice were infected intravenously with 1.0 mg of a suspension of a culture of *M. tuberculosis* var. hominis (strain no. 24)[3] which we had previously

* This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

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3 Williston, E. H., and Youmans, G. P., *Am. Rev. Tuberc.*, 1947, **55**, 536.

4 Youmans, G. P., and Karlson, A. G., *Am. Rev. Tuberc.*, 1947, **55**, 529.

5 Streptomycin Committee, Veterans Administration, Report to the Council on Phar. and Chem., *J.A.M.A.*, 1947, **135**, 634.

6 Streptomycin Committee, Veterans Administration, Report to the Council on Phar. and Chem., *J.A.M.A.*, 1948, **138**, 584.

7 Feldman, W. H., Karlson, A. G., and Hinshaw, H. C., *Am. Rev. Tuberc.*, 1948, **57**, 162.