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

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TABLE I.
Occurrence of Streptomycin-Resistant Tubercle Bacilli in Mice Treated with Streptomycin.

| No. of mice | Amt streptomycin sulphate in mg per day | % mortality | Avg survival time in days* | No. of mice which developed streptomycin resistant strains | | | Range of sensitivity to streptomycin sulphate† (in mg per ml of medium) |
|---|---|---|---|---|---|---|---|
| | | | | Total % | Sacrificed % | Died % | |
| 19 | 1500 | 26.3 | 131.6 | 17 (89.4) | 14 (100) | 3 (60) | 12.5 to >1000 |
| 20 | 750 | 85 | 73.5 | 13 (65.0) | 3 (100) | 10 (58.8) | 12.5 to >1000 |
| 18 | 375 | 100 | 40.1 | 4 (22.2) | 0 | 4 (22.2) | 12.5 to 500 |
| 18 | 187.5 | 100 | 19.7 | 1 ( 5.5) | 0 | 1 ( 5.5) | 12.5 |
| 20 | 0 | 100 | 14.6 | 0 | 0 | 0 | 0.19 to 1.56 |

* Includes mice sacrificed at 155 days.

† Indicates difference in sensitivity to streptomycin of cultures from individual mice. No significant difference in range of sensitivity was noted between mice sacrificed and those that died.

shown readily became resistant *in vitro*.[3] This culture, however, was sensitive *in vitro* to between 0.19 and 1.56 μg per ml of streptomycin sulphate.[3] The mice were divided into groups and treated by subcutaneous injection twice daily with one-half the amounts of streptomycin shown in Table I. Experimental attrition reduced the number of mice on which complete data were obtained to 95. Treatment was continued for a period of 155 days, at the end of which time, surviving mice were sacrificed. At the time each mouse died, or was sacrificed, it was autopsied, and one lung fixed in 3.7% formaldehyde, and gross and microscopic examination made as previously described.[8-11] Isolation of tubercle bacilli was accomplished by grinding the other lung in a sterile mortar, and treating the residue with oxalic acid, as described by Corper and Uyei,[12] and planting on Herrold's medium.[13] When sufficient growth was obtained, these cultures were tested in serum synthetic liquid media for their sensitivity to streptomycin.

*Results.* Table I shows the results obtained. It is readily apparent that a large proportion of the mice developed streptomycin resistant tubercle bacilli. The incidence of the occurence of streptomycin resistant strains of tubercle bacilli in the different groups of mice was directly related to the amount of streptomycin administered. A similar relationship existed between the development of resistance and the average survival time. The two smaller doses of streptomycin apparently were not sufficiently large to suppress the infection for a period which would permit the multiplication of the resistant organisms to a detectable number.

Results essentially similar to these have also been obtained with H37Rv strain of *M. tuberculosis*.

In many of the mice, the pathological findings confirmed the bacteriological evidence of the presence of streptomycin resistant tubercle bacilli. Evidence of active necrotic disease superimposed upon healed or healing areas was common in many of the animals from which streptomycin resistant tubercle bacilli were isolated.

Since we have found that streptomycin resistant tubercle bacilli appear in a large proportion of mice treated with adequate amounts of streptomycin, an experimental method is now available for the determination of the effect of various factors, including combined therapy, on the development of resistance to streptomycin *in vivo*.

*Summary.* Streptomycin resistant tubercle bacilli were obtained from the majority of intravenously tubercularized mice treated with amounts of streptomycin which permitted a relatively long survival time.

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