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Agar-Slant Tissue Cultures of Spotted Fever Rickettsiae.*

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During June of 1938 our attention was called by the Massachusetts Department of Public Health to a patient on Cape Cod, a girl of 8 years, who was suffering from a disease clinically resembling spotted fever. The patient came from a region infested with ticks, and not far from East Brewster, where 2 cases of spotted fever were reported during 1937.[1] Blood was taken for us on the 8th day of illness by Dr. Rowley of the Hyannis Hospital, and 2 guinea pigs were inoculated intraperitoneally. One of these animals ran a fever, beginning the 5th day and continuing for 6 days, the temperature reaching 105.6°. Blood and spleen from the other guinea pig, taken on the 7th day (temperature 105.5°), were passed on to normal guinea pigs, the strain being maintained for 4 generations. A total of 13 animals was inoculated, all of them reacting with temperatures and 4 of them with a noticeable scrotal swelling, but without necrosis. Though there was no mortality, the animals were found to be immune when reinoculated with a virulent strain of

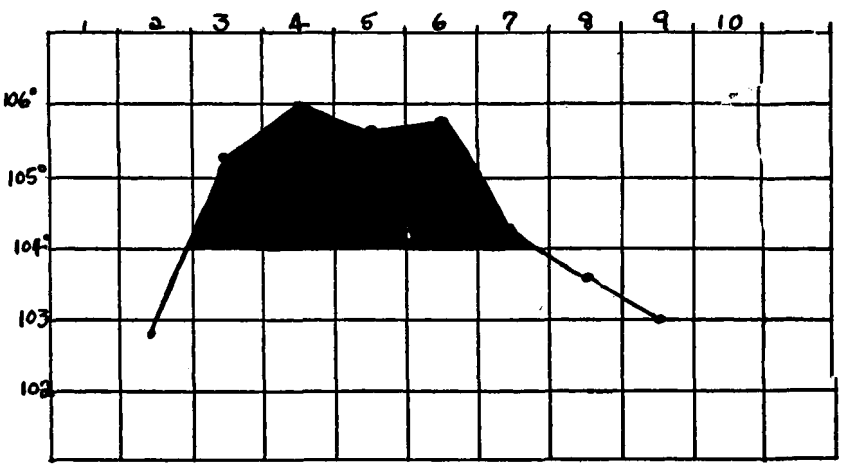

CHART 1.
Temperature curve of guinea pig inoculated with ½ a slant, 6th generation culture.

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1 *Rhode Island Med. J.*, 1937, **20**, 139.

spotted fever obtained through the courtesy of Dr. Dyer of the National Institute of Health.

Cultures were initiated with spleen and tunica by the agar slant method[2, 3] from one of the original guinea pigs. Almost all tubes showed good growth with some intranuclear Rickettsiae by the 10th to 14th day, at which time transfers were made to new slants, with mouse embryo as the fresh tissue. Growth has continued very rich through 10 generations, and transplants may now be made as early as the 6th day. Minced chick embryo may also be used with equally good results.

Guinea pigs inoculated with ½ a slant of the 6th generation mouse culture reacted as shown in Chart 1. Sections of tissues from these animals, examined for us by Dr. Henry Pinkerton, were characteristic of spotted fever. The cultures are still being maintained in series.

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Ascorbic Acid Content of Milk of Various Species as Influenced by Ascorbic Acid Injection and Diet.

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This paper reports studies of the ascorbic acid content of the milk of the ewe, cow, mare, and guinea pig. The determinations were made by the titration procedure, using 2,6-dichlorophenolindophenol. The samples were so handled in drawing them from the animal and thereafter as to exclude the light factor which Kon and Watson[1] found responsible for low values. A special microtitration method was devised for studying the guinea pig milk.

A ewe fed a ration of alfalfa hay and grain was milked twice daily for 9 weeks beginning the 12th day after lambing. The yields were recorded and samples were periodically taken for titration. During the last part of the experimental period the influence of ascorbic acid injection was studied. The results are presented in Fig. 1. The data reveal that during the period prior to ascorbic

[2] Zinsser, H., Wei, H., and FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

[3] Zinsser, H., Wei, H., and FitzPatrick, F., *J. Exp. Med.*, in press.

[1] Kon, S. K., and Watson, M. B., *Biochem. J.*, 1936, **30**, 2273.