

2.5 cc. Tyrode solution and 0.5 cc. fresh guinea pig serum and placed at 37°C. After 8 days a heavily infected endothelial cell was found in one of the flasks. The culture failed, however, to infect a guinea pig.

*Experiment 2.* Cultures were set up as above on 16/6 and 19/6 respectively and incubated at 30°C. After 8 and 9 days, respectively, fairly large numbers of rickettsia were found within cells as well as free near ruptured endothelial cells. After 13 days two of the positive cultures were mixed, the tissue ground, mixed with fresh minced tunica from a normal guinea pig and allowed to stand 30 minutes. The subcultures were set up as above and incubated at 30°C. Fourteen days later rickettsia were found in endothelial cells in the subculture. A guinea pig was infected with material from the positive cultures and after an incubation period of 12 days it developed a typical infection. A passage was made to another guinea pig which came down after 8 days and lice infected with the brain emulsion of this animal showed after 7 days numerous rickettsia in the epithelial cells of the intestine.

A successful transplant was, therefore, made from the first culture and the subculture was viable and infective 27 days after the initial culture was made. It should be noted that a suspension of rickettsia—infected louse intestines in serum tyrode solution without guinea pig tunica loses its infectivity for guinea pigs in 2 days at 30°C. Moreover, since the endothelial cells of the tunica in the cultures were found heavily packed with rickettsia it is evident that growth must have taken place. It seems reasonably certain that the European type of Typhus rickettsia can be grown in flask cultures containing guinea pig tunica and incubated at 30°C.

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### Spectral Absorption of Purified Tuberculin.

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The characteristic spectral properties of normal proteins and their derivatives stimulate similar studies with material showing special

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biological activity. Previous investigations along a similar line were hampered by the use of impure material.<sup>1</sup> Therefore, for the studies here presented several preparations of tuberculin, purified according to the methods of Seibert and Munday,<sup>2</sup> were used.

Preparations made from human, bovine and avian tubercle bacilli were studied. In the case of the human tubercle bacillus there were 5 different fractions: (1) 3 purified unheated tuberculins, (2) a purified Old Tuberculin, and (3) a purified Old Tuberculin made from cultures in a synthetic medium. Products made similarly from the non-pathogenic acid-fast bacilli, timothy, butyricum and smegma, and purified protein, proteoses, and a cyclic amino acid, served as controls. The initial concentration of all of these slightly alkalinized solutions was one per cent. In these studies a small Quartz Hilger spectrograph was used. Carbons containing iron provided the spectrum.

In all preparations made from the 3 strains of tubercle bacilli, the spectrograms showed a specific absorption band with a maximum at 265  $m\mu$ . The concentration at which this band appeared varied in the different preparations and seemed to correspond closely to their respective tuberculin potencies. The more potent preparations showed the special absorption band at a higher dilution than the less active samples. This was observed not only in the case of the 3 different tubercle bacillus strains but also in different fractions from the same strain (human). The preparations made from the 3 non-pathogenic strains of bacilli failed to show this special absorption band.

Irradiation with ultra-violet light, known to destroy the potency of tuberculin, seems to increase the total absorption and at the same time destroys the specific absorption band.

The detailed data and spectrograms with a discussion concerning their significance and suggestions for further practical use will be presented elsewhere.

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<sup>1</sup> Fischer, A., and Hausmann, W., *Z. Tuberk.*, 1930, **58**, 328.

<sup>2</sup> Seibert, F. B., and Munday, B., *Am. Rev. Tub.*, 1932, **25**, 724.