tion in the thyroxine content of the 2 preparations would be almost 175%. The possibility of adulteration must also be considered. Addition of a cheap, water-insoluble, relatively stable substance such as iodized casein would be very difficult to detect by chemical means if determination of organic iodine or of the acid-insoluble iodine fraction is used for assay.

Standardization of thyroid by direct determination of the thyroxine content after the method of Leland and Foster appears to be the most satisfactory solution of the problem. The determinations can easily be made in cooperating commercial laboratories, an important consideration for any practical method. By maintaining the thyroxine content of thyroid tablets within specified limits, one of the most important of the variables encountered in thyroid medication may be eliminated.

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Retardation of Tuberculous Infection in Guinea Pigs Vaccinated with Killed Tubercle Bacilli as Shown by Cultural Method.

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The effect of vaccination with killed tubercle bacilli upon the resistance of animals to tuberculous infection has been studied extensively. It has been repeatedly shown that guinea pigs can be sensitzed by this means to the intracutaneous injection of old tuberculin (Römer,[1] Bessau,[2] Zinsser, Ward and Jennings,[3] Petroff[4]). Its influence upon the course of subsequent infection, however, has been studied with less conclusive results under widely varying experimental conditions. Römer,[1] Zinsser, Ward and Jennings,[3] Petroff,[4] and L. Lange[5] conclude that such treatment gives some protection; Dold,[6] and Seligmann and von Gutfeld,[7] that it gives none. B. Lange and his associates[8] believe that inoculation with heat-

[1] Römer, P. H., *Beitr. klin. Tub.*, 1909, **12**, 185.

[2] Bessau, G., *Berl. klin. Woch.*, 1916, 801.

[3] Zinsser, H., Ward, H., and Jennings, F. B., *J. Immun.*, 1925, **10**, 719.

[4] Petroff, S. A., and Stewart, *J. Immun.*, 1926, **12**, 97. Petroff, S. A., Branch, A., and Jennings, F. B., *Ibid.*, 1929, **16**, 233.

[5] Lange, L., *Zentralbl. Bakt.*, 1921, **85**, 26.

[6] Dold, H., *Klin. Woch.*, 1925, 1763.

[7] Seligman, E., and von Gutfeld, F., *Deitsche med. Woch.*, 1925, 1064.

[8] Lange, B., and Freund, R., *Zeitsch. Hyg.*, 1926, **105**, 571. Lange, B., and Jochimsen, E., *Ibid.*, 1927, **107**, 426. Lange, B., Jochimsen, E., and Magat, J., *Ibid.*, 1927, **107**, 645.

killed tubercle bacilli affords only a very slight protection, which can be as readily obtained with killed *B. coli* or a lipoid derived from animal tissue ("Helpin"). In the work thus far reported, the effect of immunization has been estimated by comparing in vaccinated and unvaccinated animals the duration of disease and the pathological changes observed at autopsy.

Tubercle bacilli injected into the tissues of guinea pigs penetrate the lymph ducts and cause formation of tubercles in the nearest lymph nodes, and the rate of spread of the bacteria in the regional lymphatic system is an important factor in the progress of the disease. Opie[9] has shown that soluble antigens such as crystalline egg albumin and horse serum, spread rapidly from the site of injection and enter the blood stream in normal animals, but that when rabbits have been immunized by repeated injections these antigens are fixed at the site of the acute local inflammation (Arthus phenomenon). Similarly Willis[10] has shown a delay in the dissemination of tubercle bacilli along the lymphatics draining the site of injection in guinea pigs immunized with *living avirulent* tubercle bacilli. They demonstrated the presence and estimated the quantity of bacilli in the organs by guinea-pig inoculation. The present study is an attempt to determine by the cultural method, whether inoculation with *heat-killed* tubercle bacilli retards the spread of living tubercle bacilli in the regional lymphatics.

Experimental. The vaccine was prepared from a very virulent strain of tubercle bacillus of human type (489 A). Three-weeks-old cultures removed from glycerin agar were dried over sulphuric acid *in vacuo*. One cc. of the suspension contained 2 mg. of tubercle bacilli (dry weight). The suspension was sealed in ampules and immersed in boiling water for 20 minutes. Six guinea pigs injected with 5 cc. of the vaccine were found free of tuberculosis when killed 6 months later. No growth was obtained on Dorset's medium.

Male guinea pigs weighing from 350 to 420 gm. were used. One cc. of vaccine was injected close under the skin about 1 cm. below the right clavicle three times 5 days apart. One month later, 0.5 cc. vaccine was similarly injected. One or two abscesses were found at the site of injection in all vaccinated animals. Five days after the last vaccination 6 of the vaccinated guinea pigs were injected with 0.1 cc. of a 1-in-10 dilution of old tuberculin. All of them showed tuberculin reactions with necrosis. This skin reaction differed in one respect from those seen in tuberculous guinea pigs: there was

[9] Opie, E. L., *J. Exp. Med.*, 1924, **39**, 659.

[10] Willis, H. S., *Am. Rev. Tub.*, 1925, **11**, 427.

relatively more necrosis and less edema. Five days later the vaccinated and control (not vaccinated) animals were infected with tubercle bacilli. The strain used was of human type (489 A); 0.00001 mg. killed guinea pigs within 6 months when injected subcutaneously. Two-tenths cc., containing 0.001 mg. of tubercle bacilli, was injected into the skin of the left groin of 3 vaccinated and 3 control guinea pigs, alternately. At short intervals guinea pigs of both groups were killed, the organs removed and cultured, and the macroscopic pathological changes noted. From some of them (Table I) one-half of the spleen was cultured. The following procedure was employed in culturing.

The guinea pigs were bled to death and the organs were removed aseptically, cut into small pieces and ground with sea sand alone and with 1.2 cc. of phosphate solution. Of the resulting suspensions, some prepared from the spleen were treated with Petroff's sodium hydroxide method to kill microorganisms other than tubercle bacilli. The suspensions were placed in test tubes containing glass beads and shaken, and 0.1 cc. was spread on each of 3 or 4 tubes of Dorset's medium, and on 3 or 4 tubes of Petroff's medium. To prevent evaporation paraffin was poured on the cotton plugs from a coffee pot. The colonies were counted at various intervals, and the final reading was made during the fourth month of incubation.

The following tables show in what numbers and at what intervals after infection, colonies of tubercle bacilli were obtained from the lymph nodes and spleen of vaccinated and control animals.

Tables I and II show 2 distinct differences in the vaccinated and unvaccinated animals. (1) Positive cultures were obtained from the lymph nodes of the unvaccinated animals after a shorter period of infection. Unexpectedly, this order seemed to be reversed in the spleen, but this result was probably only apparent, and due to the fact that the colonies were obtained from the spleen of one vaccinated animal by the direct method, from the others, by the sodium hydroxide method, which yields fewer colonies. (2) A larger number of colonies were obtained from the control group.

These observations show that immunization of guinea pigs with heat-killed tubercle bacilli, having produced hypersensitiveness to old tuberculin, retards dissemination of living tubercle bacilli through the regional lymphatics. Evidence as to whether the retardation is due to a mechanical fixation, or to destruction (killing) of the injected tubercle bacilli, or to both, could probably be obtained by culturing the tissue injected with tubercle bacilli.

TABLE I.

Number of Colonies Obtained from Vaccinated and Unvaccinated Guinea Pigs after Intracutaneous Injection with 0.001 mg. Tubercle Bacilli.

Interval after infection	Abdominal-inguinal lymph node†		Inguinal lymph node		Iliac lymph node		Spleen	
	v.	c.	v.	c.	v.	c.	v.	c.
days								
1	0	12	—	—	—	—	0*	0*
8	0	60	—	—	—	—	60	0*
10	0	0	—	—	—	—	0	0*
13	0	2,000	—	0	0	0	0	0*
16	—	2,160	—	1,320	—	—	—	438
21	80	—	0	—	0	600	0	0
23	—	—	0	0	0	60	—	60
29	—	—	—	4,800	—	500	—	—
33	—	—	—	0	—	0	—	0
35	4,800	—	0	—	0	—	0	—
36	—	9,600	—	—	—	8,400	—	—
37	—	—	0	—	0	—	0	—
42	2,200	—	—	20	—	0	0	9,600
43	1,200	—	—	—	780	—	180	—
44	—	0	—	4,800	—	720	—	1,020
48	2,400	—	1,440	12,000	24,000	—	0	24,000
50	35,000	—	1,200	12,000	—	—	0	—
55	6,720	18,000	31,000	24,000	4,200	12,000	600	2,400
64	1,800	—	22,800	—	36,000	12,000	54,000	48,000
67	24,000	—	16,800	—	100,000	—	—	—

v. vaccinated with heat-killed tubercle bacilli.
c. control.
0 no colonies found.
— not done or lost on account of contamination.
* treatment with sodium hydroxide.
† Also called superficial inguinal lymph node.

TABLE II.

Immunization with Heat-killed Tubercle Bacilli.

Number of Days Elapsed Between Infection and Positive Cultures.

Organ	Vaccinated	Unvaccinated
Abdominal-inguinal lymph node	21	1
Inguinal lymph node	48	16
Iliac lymph node	43	21
Spleen	8	16