at 500 R.P.M. for 10 minutes. The serum was removed with Pasteur bulb pipettes and 50 cc. of sterile glycerol infusion broth were added to each clot. The centrifuge tubes containing the clot and medium were then placed in the incubator. The tubes were removed and the medium examined daily for evidence of growth. At weekly intervals, for 6 weeks, a loopful of the broth was streaked over the surface of NNN medium and subsequently incubated. No growth was obtained.

The negative results obtained as a result of this rather large series of experiments are rather hard to interpret in the light of many recent studies[2] on the isolation of organisms from the blood stream in health and disease. It is possible that the therapeutic measures instituted against the Hansen infection, namely, the weekly injection of 4-5 cc. of the iodized ethyl esters of *Hydnocarpus wightiana* oil, may have had a direct influence on the incidence of blood stream invasion. It is also true that these studies were made during the rainy season so that the possibility of air borne contamination was at a minimum.

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Effect of Testicle Extract on Primary Tuberculous Infection and Reinfection in Guinea Pigs.

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1. *Effect on Primary Infection.* Testicle extract greatly increased the extent of the local lesions resulting from the intradermal or subcutaneous injection of virulent human tubercle bacilli into guinea pigs. When compared with lesions resulting from the injection of an equal number of tubercle bacilli suspended in salt solution, the increase was estimated at from 10 to 15 fold. The testicle extract was prepared by mincing bull testicle in 2 volumes of physiological salt solution, straining the material through cloth and finally filtering through a Berkefeld V candle. Kept at ice-box temperature, the extract maintains its activity for a long period of time. Two groups of 6 guinea pigs were used in the first experiment, in which the injection was made intradermally. One group

[2] Callow, Bessie R., *J. Inf. Dis.*, 1933, **52**, 779.

was injected with a mixture of 1.0 cc. of testicle extract and 1.0 cc. of a suspension of H-37 strain of human tubercle bacilli in salt solution, containing 0.1 mg. of organisms, moist weight. The other group of 6 guinea pigs was injected with 2.0 cc. of a suspension of tubercle bacilli in salt solution, containing 0.1 mg. of organisms. The lesions resulting from these intradermal infections in the case of the testicle extract group were very large, spreading over the entire flank, from 5 to 6 cm. in width and from 7 to 8 cm. in length. The controls showed small nodules which ulcerated and drained, at the most 1.5 to 2.0 cm. in diameter.

A second experiment was performed using 2 groups of 10 guinea pigs, in which the injections were made subcutaneously in the groin. The dosage and quantities of testicle extract were the same as in the first experiment above.

Both experiments were terminated from 60 to 70 days after infection. The animals that had been infected with the addition of testicle extract showed, besides a more extensive primary infection, a much greater amount of generalized tuberculosis. The increase in the extent of visceral lesions was apparently due to the greater extent of the primary lesion, resulting from the spreading of the injected organisms, rather than to a systemic effect of the testicle extract, since in experiments which are still being carried on, there is evidence that the parenteral injection of testicle extract after infection has been established does not cause any appreciable increase in the amount of tuberculosis which develops.

2. *Effect on Tuberculin Reaction.* Testicle extract was added to solutions of tuberculo-protein MA-100 (Mulford) in the proportion of 0.7 cc. of testicle extract to 0.3 cc. of a solution containing from 0.25 to 3.0 mg. of tuberculo-protein. Injected intradermally into tuberculous guinea pigs, the skin reactions were spread over a large area, 4.5 cm. in diameter, in comparison with the smaller but more intense reactions resulting from the injection of a like amount of tuberculo-protein without the testicle extract. The reactions, while increased in extent, due to the spreading factor in testicle extract, were less intense and of much shorter duration than in the controls.

3. *Effect on Reinfection.* Guinea pigs that had been infected subcutaneously with 0.01 mg. of H-37 tubercle bacilli from 4 to 6 weeks previously, were reinfected with 0.1 mg. of H-37 bacilli, suspended in 1.0 cc. of salt solution and 1.0 cc. of testicle extract. Another group of guinea pigs that had been infected at the same

time were reinfected with 0.1 mg. of H-37 bacilli suspended in 2.0 cc. of salt solution. A total of 36 animals were reinfected, 18 with the addition of testicle extract and 18 without. The lesions in the animals reinfected with testicle extract were 4 or 5 times larger than those of the controls and were characterized by a progressive induration which, after 2 to 3 weeks, resulted in the formation of a hard, dry, necrotic area which healed very slowly from the edges. Sloughing of the surface of the necrotic mass occurred in some instances, leaving a shallow indolent ulcer. At no time did abscess formation occur, nor did the lesions discharge any material.

An interesting and striking result of such reinfection was that the progress of the disease in the group with testicle extract was greatly retarded in comparison with the controls, and with the lesions in untreated controls of primary infections. The animals were sacrificed from 4 to 6 weeks after reinfection, and in 3 separate experiments the same condition and results prevailed.

The animals reinfected with testicle extract showed from 40 to 60% less tuberculosis than did the controls or untreated controls of primary infection.

In each of the 3 experiments, also, the ulcers of primary infection showed healing more frequently in the animals reinfected with testicle extract. Further experiments on the nature of this effect are in progress.*

No benefit was found to derive from the reinfection of tuberculous guinea pigs with BCG, with or without testicle extract, nor from the repeated injection of heat-killed human tubercle bacilli, with or without testicle extract.

* In an additional experiment recently completed, 5 guinea pigs with far advanced tuberculosis were reinfected by intracutaneous injections of living H37 bacilli with testicle extract. For some reason probably connected with the far advanced state of the disease the resultant skin lesions were practically of the same size and appearance as those which occurred in four animals reinfected at the same time with a saline suspension of bacilli. Also there was no appreciable difference in the extent of the general lesions in the two groups, when sacrificed 34 days later.