

red cell count above and below normal are still evident 3 months after cessation of the stimulus. They become less and less marked, however, until shortly after the fourth or fifth month, the normal count or a figure near this, is maintained.

The results of this study shed further light on the delicate nature of what Krumbhaar⁴ has called the hemolyto-poietic equilibrium. It demonstrates that both the blood-forming and blood-destroying sides of the system possess, when stimulated excessively, a momentum which carries them beyond normal limits, this momentum becoming less and less marked, in pendulum-like fashion, as the stimulus (in this case, either more or fewer cells than normal) becomes less intense.

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Sensitization and Antibody Formation after Injection of Tubercle Bacilli and Paraffin Oil.*

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The synergistic effect of various substances upon immunization was noted a long time ago; the literature has been admirably reviewed by Swift and Schultz.¹ The experiments of Dochez² have direct bearing on our problem. He succeeded in promoting immunization by the incorporation of streptococci into agar and production of persistent foci. Coulaud³ sensitized 2 rabbits by the injection of melted paraffin containing killed tubercle bacilli. Ramon and others⁴ and Eisler and Gottdenker⁵ found that adding paraffin oil to diphtheric toxin accelerates antitoxin-production. Saenz⁶ discovered that one cc. of paraffin oil containing killed and dried tubercle bacilli injected into the subcutaneous tissue of guinea pigs produces a very

⁴ Krumbhaar, E. B., *Cowdry's Special Cytology*, 2nd edition, 1932, Vol. 2, Ch. XV.

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¹ Swift, F. H., and Schultz, M. P., *J. Exp. Med.*, 1936, **63**, 703.

² Dochez, A. R., *Harvey Lectures*, 1924-25, 131.

³ Coulaud, E., *Rev. de la Tuberculose*, 1934, **2**, 850.

⁴ Ramon, G., Lemetayer, E., and Richou, R., *Rev. d'Immun.*, 1937, **3**, 205.

⁵ Eisler, M., and Gottdenker, F., *Z. Immunitätsforsch.*, 1937, **90**, 427; **91**, 49.

⁶ Saenz, A., *Compt. rend. Soc. Biol.*, 1935, **120**, 870, 1050.

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intense and lasting sensitiveness to tuberculin. He⁷ found that in animals so treated the dissemination of living tubercle bacilli from the subcutaneous tissue through the draining lymph nodes is slower than in guinea pigs immunized with a saline suspension of killed tubercle bacilli. Guinea pigs immunized with paraffin-oil suspensions, however, were no more resistant to infection with tubercle bacilli than untreated controls.⁸ Hensel⁹ reported that when 5 mg. of killed tubercle bacilli incorporated in one cc. of lanolin or vaseline are injected into guinea pigs, very intense sensitization develops and persists for at least one year. Guinea pigs treated with saline suspension become sensitive also, but their sensitiveness is slight and transient.

We injected 8 guinea pigs subcutaneously with 2 cc. of paraffin oil containing 10 mg. of tubercle bacilli (dry weight), in doses undivided or divided into from 4 to 10 simultaneous injections, and tested them for hypersensitiveness and antibodies. One-tenth cc. of 10% tuberculin was used. The tuberculin-reactions were graded according to the largest diameter over the areas of edema: from 0 to 4 mm. = 0; from 5 to 9 mm. = 1 plus; from 10 to 19 mm. = 2 plus; from 20 to 29 mm. = 3 plus; from 30 to 39 mm. = 4 plus; from 40 to 49 mm. = 5 plus. When the area of swelling was ill-defined, one-half was deducted from the grade. In the titration of complement-fixing antibodies, heat-killed tubercle bacilli were used as antigen; the technic was described previously.¹⁰ All animals developed intense and persistent sensitiveness. Necrotic tuberculin-reactions occurred as shown in Table I.

TABLE I.

After the injection of tubercle bacilli Months	No. of guinea pigs	
	With necrosis	With no necrosis
3	1	7
6	4	3
11	5	3
16	1	1
17	0	2
18	1	1

Of 3 animals immunized with killed tubercle bacilli suspended in saline solution instead of oil, one reacted with necrosis once; the other 2 pigs reacted slightly, if at all, in tests performed at intervals

⁷ Saenz, A., *Ibid.*, 1937, **124**, 1161.

⁸ Saenz, A., *Ibid.*, 1937, **125**, 495.

⁹ Hensel, G., *Beitr. z. Klin. Tuberk.*, 1937, **90**, 387.

¹⁰ Freund, J., Laidlaw, E. H., and Mansfield, J. S., *J. Exp. Med.*, 1936, **64**, 573.

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of 2 weeks during 2 months. In these 3 animals serum-tests were not made.

In guinea pigs injected with killed tubercle bacilli suspended in paraffin oil, the first antibody-titrations were carried out 11½ months after immunization. The high titers found at that time did not decrease during the following 8 months (see composite graph, Fig. 1).

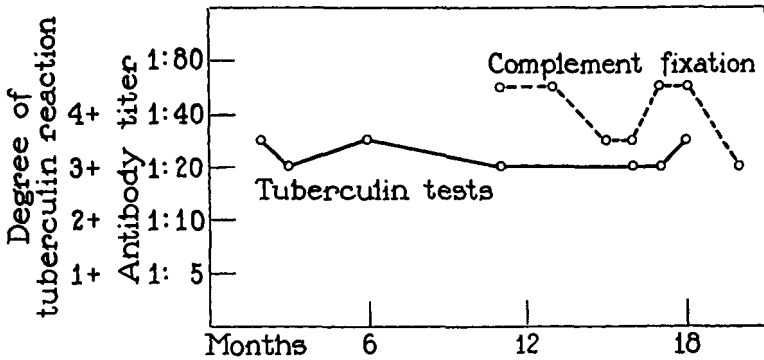


FIG. 1.

Tuberculin reactions and antibody titers in guinea pigs injected with heat-killed tubercle bacilli and paraffin oil.

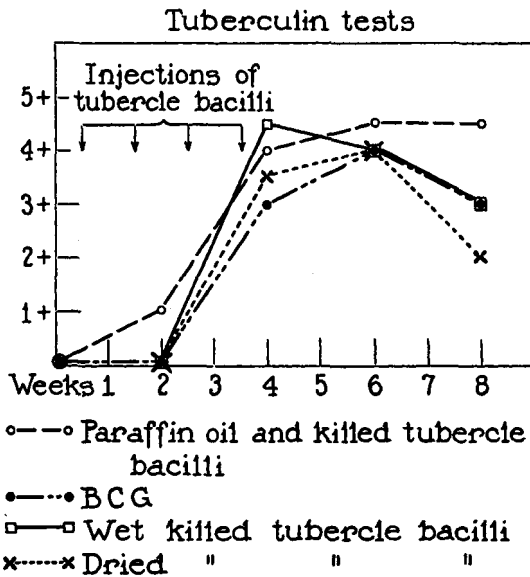


FIG. 2.

Tuberculin reactions in groups of rabbits injected with killed bacilli suspended in paraffin oil, or salt solution, and in rabbits injected with BCG.

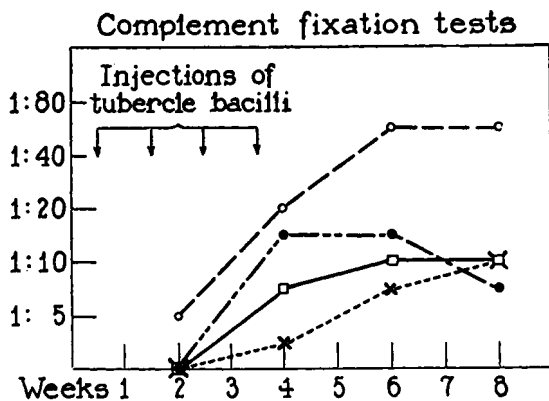


FIG. 3.
Antibody titers in rabbits of Fig. 2.

Guinea pigs treated with oil alone or not treated at all did not react to tuberculin; nor did their sera fix complement.

Tuberculin-sensitiveness and antibody-formation were studied in 30 rabbits immunized with killed and dried tubercle bacilli suspended in paraffin oil, in 9 immunized with killed and dried bacilli suspended in saline, in 9 immunized with killed and undried bacilli suspended in saline, and in 18 immunized with living BCG also suspended in saline. The animals received 4 simultaneous injections of 0.025 mg. of dried tubercle bacilli (equivalent to 0.1 mg. of wet bacilli) in 0.1 cc. of oil or salt solution, or 0.1 mg. of BCG in salt solution, into the subcutaneous tissue at weekly intervals for 4 weeks. Tests for hypersensitiveness and antibody were made 2 weeks apart. (Unlike guinea pigs, rabbits were injected with 0.2 cc. of tuberculin diluted one in 5.)

In the composite graphs the first 3 tests do not reveal appreciable differences in the intensity of reactions to tuberculin. It should be noted, however, that necrosis was frequent in the oil-group and exceptional in the others. The last tests showed that 4 weeks after the last injection of tubercle bacilli, sensitiveness was fully maintained in the oil-group and diminished in all other groups.

The composite graphs of antibody-formation show that paraffin strikingly promotes this function. Rabbits treated with a saline suspension of BCG seem to produce antibodies somewhat more readily than those treated with killed tubercle bacilli suspended in saline. The graphs also suggest that drying of killed tubercle bacilli is harmful to their capacity to produce sensitization and formation of antibody. Comparison of results with BCG and killed tubercle bacilli is uncertain because results with BCG vary greatly in different experiments.

Our results show that the addition of paraffin oil to killed tubercle bacilli augments sensitization and antibody-formation in both guinea pigs and rabbits. In guinea pigs, intense sensitization and antibody formation are astonishingly persistent after the injection of paraffin oil containing killed tubercle bacilli.

The synergistic effect of paraffin oil is probably connected with the nature of the cellular reaction produced by tubercle bacilli coated with an inert oil. (As to the surface-properties of tubercle bacilli in relation to oil and water, see Mudd and Mudd, Mudd and Nugent.¹¹) Paraffin oil alone causes only a transient cellular response. The addition of a small amount of paraffin oil (0.02 cc.) intensifies the reaction to killed tubercle bacilli very conspicuously and makes it very persistent. (In a node removed from a guinea pig one year after the injection, large numbers of acid-fast bacilli were found.) Indeed, the local lesions are so extensive that it seems very desirable to modify the preparation of the suspension so that the excess of the amount of oil in relation to the bacteria be reduced. Approximately 20 mg. of killed tubercle bacilli were triturated in an agate mortar alone, later with one drop of oil, and then after addition of saline. The suspension that was formed contained oil droplets up to a few microns in diameter; these are loaded with tubercle bacilli. Immunization employing such a suspension is in progress.

Conclusions. 1. The addition of paraffin oil to killed tubercle bacilli promotes sensitization and antibody-formation in both guinea pigs and rabbits. 2. In rabbits, killed tubercle bacilli incorporated in paraffin oil produce sensitization and antibody-formation with greater intensity than BCG. 3. Twenty months after the injection of killed tubercle bacilli in paraffin oil into guinea pigs, evidence of sensitization still persists and complement-fixing antibody is present in the serum.

¹¹ Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, **50**, 647; 1926, **53**, 127; Mudd, S., Nugent, R. L., and Bullock, L. T., *Colloid Symposium Monograph*, 1931, **9**, 229.