HYPERSENSITIVENESS AND ANTIBODY FORMATION IN TUBERCULOUS RABBITS*

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The purpose of these experiments was to study the relationship of tuberculin skin sensitiveness[1] to antibody formation and their relationship to the tuberculous infection. The plan of the experiments was to infect rabbits with bovine tubercle bacilli, test their sensitiveness to intracutaneous injections of tuberculin, and determine the antibody contents of their blood at weekly intervals throughout the course of the disease.

Material and Methods

Male rabbits weighing from 1800 to 2500 gm. were used. They were either Chinchillas or of the hare brown variety.

The strain of tubercle bacillus used was of bovine type, Ravenel, isolated more than 30 years ago. It is of high virulence; 0.00001 mg. injected into an ear vein kills rabbits with extensive lesions in the lungs and kidneys in about 3 to 6 months. The rabbits were infected by the intravenous or intratracheal route with from 0.000001 to 0.001 mg. of tubercle bacilli.

The tuberculin employed was a commercial preparation of high potency. It is about one and a half times as potent in skin tests as the international standard tuberculin. 0.2 cc. of a one in five dilution, *i.e.* 40 mg., was injected into the skin. The skin reactions were observed 48 hours after the injection of tuberculin. The areas of redness and edema were measured and the elevation of the skin was estimated. The 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;

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[1] There is no adequate knowledge of tuberculin sensitivity in the rabbit. Most workers doubt the occurrence of such sensitivity while others believe that its appearance is irregular.

from 40 to 49 mm. = 5 plus. When the area of swelling was ill defined, one-half was deducted from the grade. Hemorrhages or necrosis did not occur in the skin reactions.

The complement fixation tests were carried out in the following way. The rabbit sera were heated for 30 minutes at 55°C. As antigen a suspension of the Ravenel strain was used. It was prepared by grinding a culture of Ravenel strain and suspending it in normal saline so that 1 cc. of the suspension contained 10 mg. of tubercle bacilli. The suspension was heated for 30 minutes at 60°C. and 0.35 per cent tricresol was added as a preservative. About one-fourth of the self-inhibition dose was used for the complement fixation. The complement was either fresh or dried guinea pig serum. It was always freshly titrated and two and a half units were used. The concentration of sheep cells suspension was 5 per cent (in relation to whole blood). Two units of hemolysin were employed. 0.25 cc. of each ingredient was incubated in a water bath at 37°C. for 1 hour. To check the accuracy of the complement fixation tests, a standard serum was always included, which was obtained from a rabbit that was immunized by a series of injections of tubercle bacilli. 0.5 cc. portions of the serum were dried *in vacuo* in the frozen state by the method of Elser, Thomas, and Steffen (1), and a sample of dried serum (recovered by adding 0.5 cc. of distilled water to the dry material) was run in the complement fixation test. That the method of drying yielded uniform samples was shown by testing three samples of dried serum simultaneously. The variation in the complement fixation test from week to week was very slight, if any, since the highest dilution of the standard serum that fixed complement was always the same.

EXPERIMENTAL

In group 1 (Charts 1 and 2) rabbits were injected with 0.000001, with 0.0000025, and with 0.00001 mg. tubercle bacilli respectively, some intravenously and some intratracheally. The rabbits died with extensive tuberculosis from 102 to 159 days after the infection. The lungs weighed from 52 to 125 gm., most of them weighing from 70 to 80 gm. (the weights of normal lungs vary from about 10 to 12 gm.) and about nine-tenths of their cut surface was involved in tuberculosis. There were many tubercles in the kidneys and in some animals the spleen, liver, and cecum contained tubercles. Two to four rabbits in each group were tested weekly with tuberculin. The first tests were made 1 week before, and second tests 1 week after the inoculation with living tubercle bacilli. It is noteworthy that the duration of life and the character of lesions were about the same in rabbits subjected to weekly tuberculin tests and in those not tested.

The progress of sensitization as indicated by the results of tuberculin tests performed weekly followed a fairly uniform course. Sensitization was evident from 2 to 4 weeks after infection. In one instance the reaction was 4½ plus, at the end of 2 weeks, but in the remaining instances from 3 plus to 4 plus at the end of 3 or 4 weeks was usual. Subsequently for a period of about 10 weeks

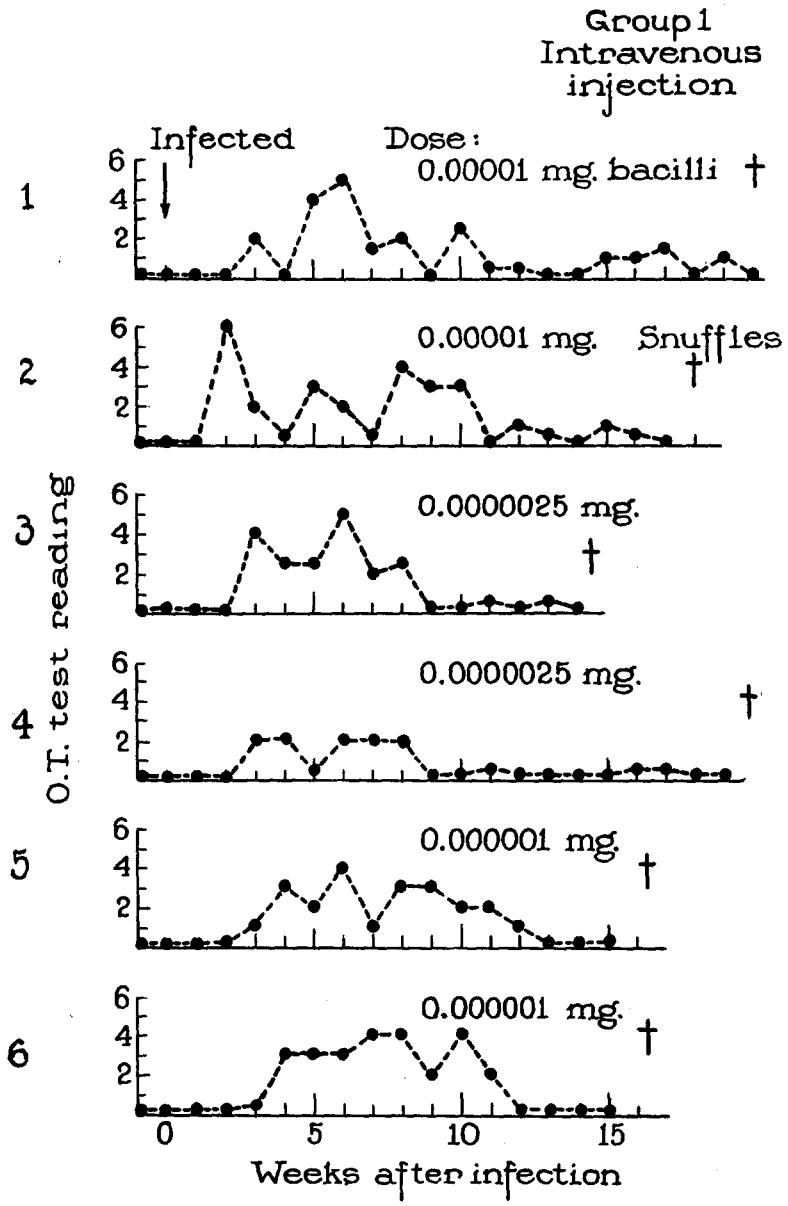

CHART 1. Old tuberculin tests.

there was a fairly intense sensitization, sometimes reaching a maximum of 4 plus or 5 plus, but with fluctuation of level. At a later period, *i.e.* from the 10th to the 15th weeks, sensitization diminished, in most instances disappeared, so that it was often almost completely absent during 5 or more weeks preceding death. In this group of rabbits serological tests were not made.

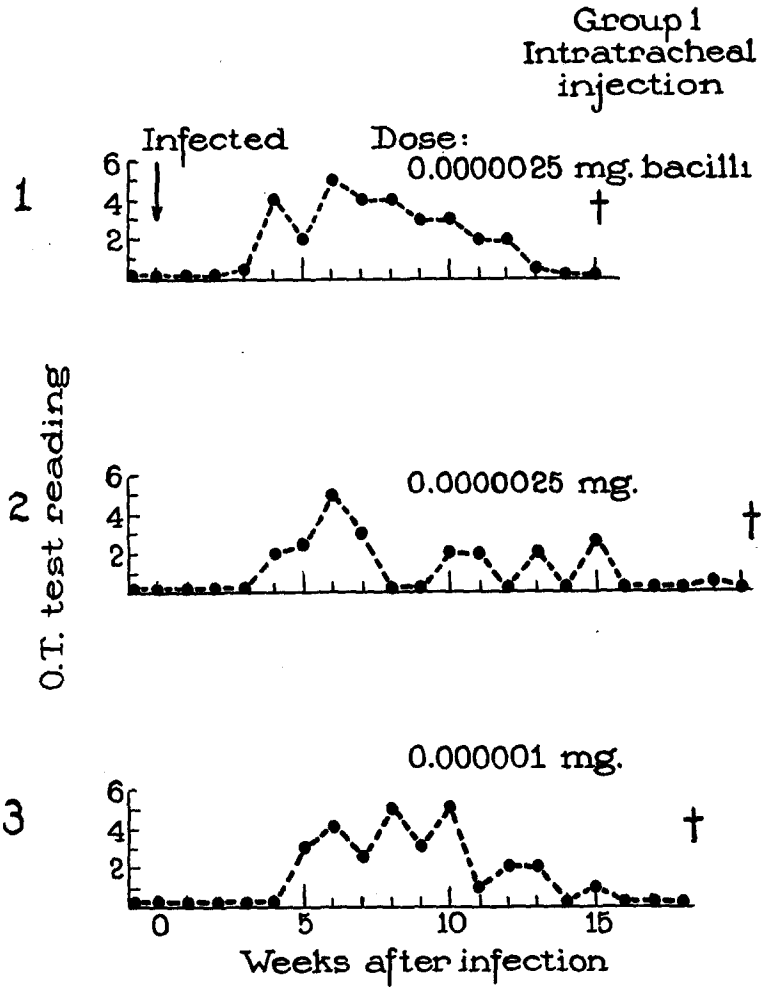

CHART 2. Old tuberculin tests.

In group 2 (Chart 3) six rabbits were infected with 0.00001 mg. tubercle bacilli and the intensity of both sensitization and antibody formation was followed at weekly intervals. The animals died after from 112 to 155 days, with extensive tuberculous lesions. The lesions found at autopsy were very similar to those in group 1. One of the rabbits, No. 1, developed snuffles during the 3rd week after infection.

Intense sensitization became evident in five of the six rabbits during the 4th or 5th week and in one during the 6th week. The sensitivity was maintained for from 3 weeks to almost 3 months. In five rabbits the intensity of the tuberculin reactions diminished gradually from week to week and in one animal rapidly (in 1 week). None of the six rabbits reacted to tuberculin for a period of 2 to 16 weeks preceding their death.

The graph that represents the antibody titers from week to week during the course of the disease is fairly uniform in five of the six rabbits and very different from the shape of the graph of the tuberculin sensitiveness. Before the infection, complement fixation reaction was obtained with 1:10 dilutions of the sera in two instances, with 1:5 dilution in two instances, and the sera of the remaining two rabbits did not react in the latter dilution. Antibodies developed and were maintained for long periods in all rabbits except in the animal with snuffles. Significant titers were observed from 3 to 7 weeks after infection. There was some fluctuation of the antibody titers; however, antibodies were present in all rabbits throughout the whole course of the disease. The persistence of antibodies was in striking contrast with the disappearance of skin sensitiveness to tuberculin.

In group 3 (Chart 4) six rabbits were infected with a relatively large dose, 0.001 mg. tubercle bacilli, and as in group 2 tuberculin sensitiveness and antibody formation were followed by weekly tests. Four of these animals were killed *in extremis* from 77 to 98 days after infection, one died of tuberculosis after 102 days, and one was killed because of snuffles and diarrhea 84 days after infection. All the rabbits had extensive tuberculosis of the lungs and kidneys.

In most of the rabbits sensitization was evident at the 3rd week and lasted from 1 to 6 weeks. One of the six rabbits, No. 6, showed a fairly strong tuberculin reaction in only one of the weekly tests, 7 weeks after the infection. In all animals sensitization disappeared several weeks before they became moribund.

Complement fixing antibodies appeared during the 4th week. In all rabbits there was a rapid and conspicuous increase in antibody titer during the course of the disease. When the antibody titers reached their maximum height they remained at that level almost until the death of the animal, with slight, if any, decrease, except in one instance (rabbit 4). In this rabbit the drop of antibody titer was considerable, *i.e.* from 1:120 to 1:30.

The comparison of the curves of hypersensitiveness and antibody formation shows the following: The development of tuberculin sensitiveness as a rule preceded the formation of antibodies and the maxima of the two curves did not

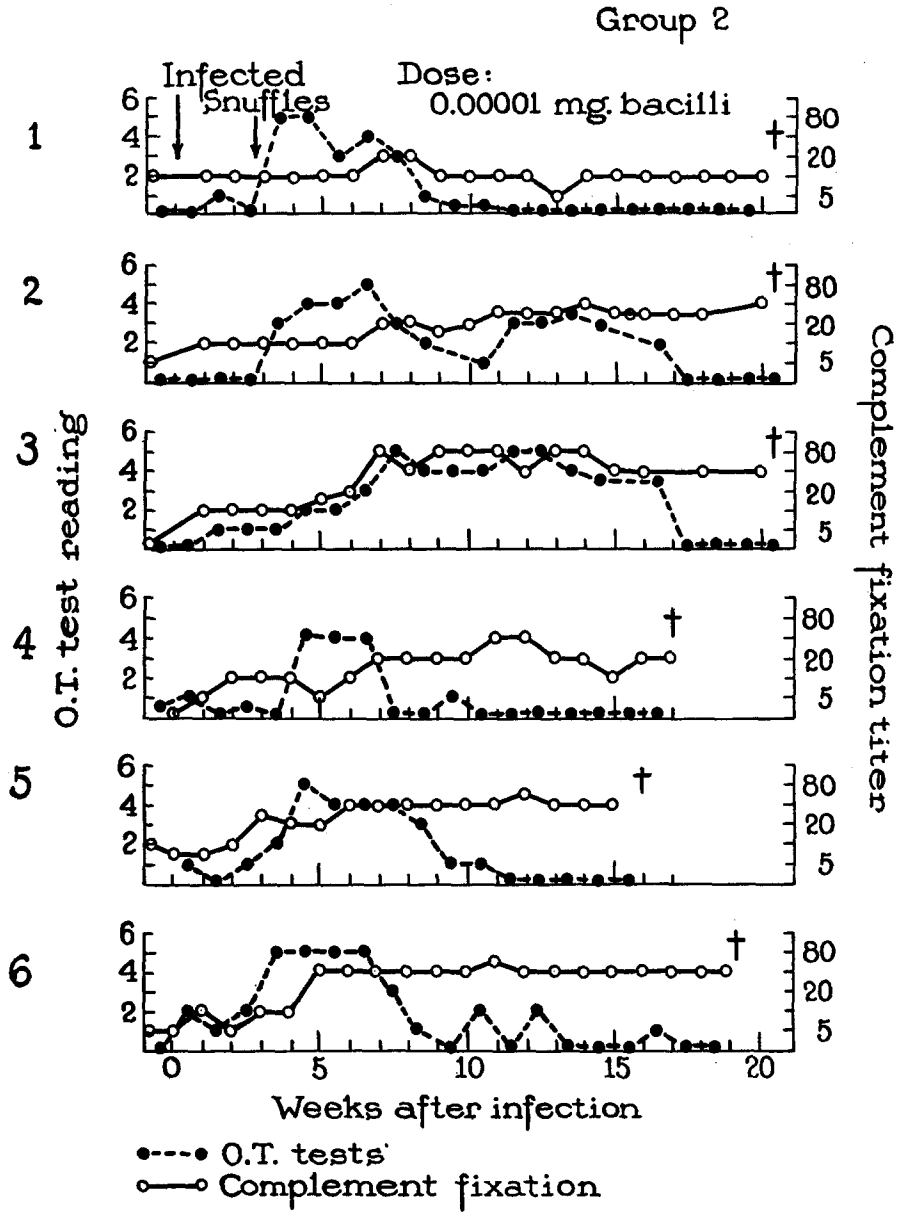

CHART 3. Old tuberculin tests and complement fixation reaction.

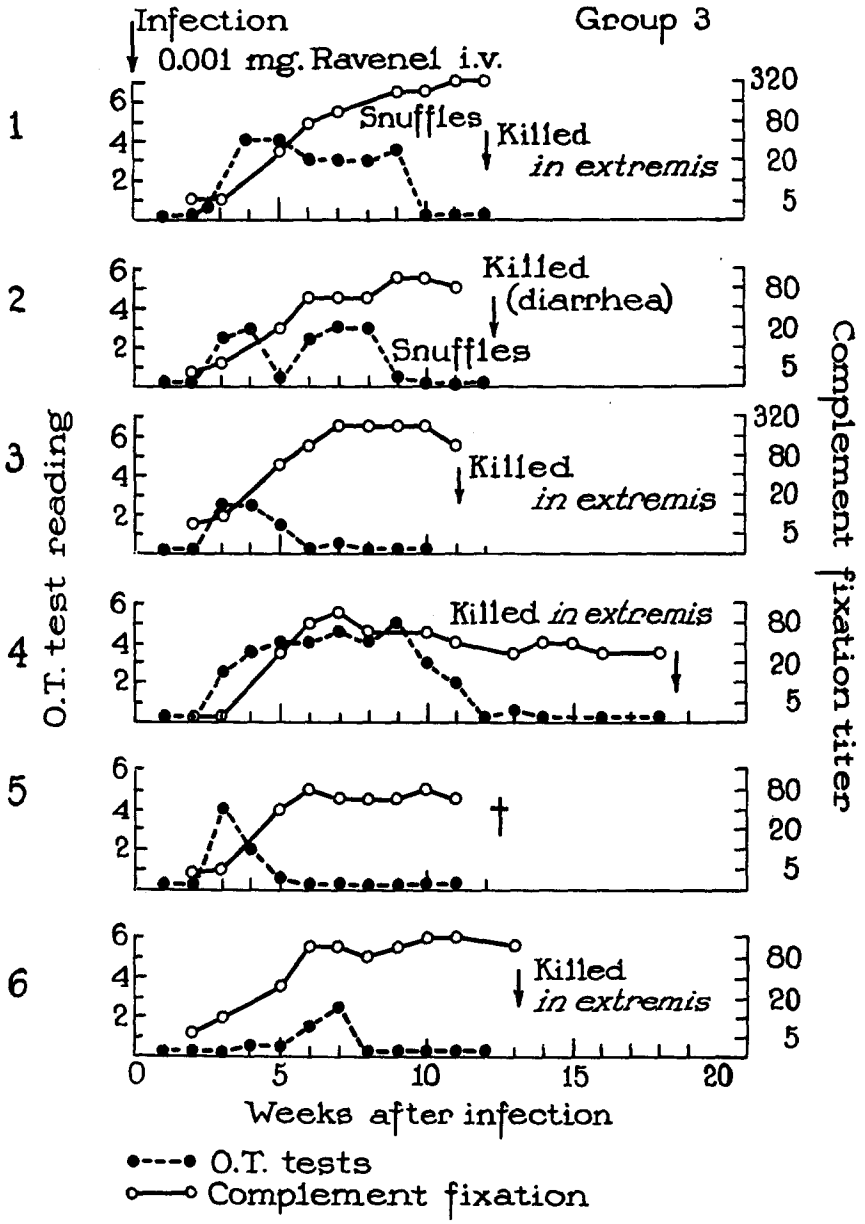

CHART 4. Old tuberculin tests and complement fixation reaction.

coincide. The tuberculin sensitiveness disappeared several weeks before death, while the antibodies persisted in the blood until the animals became moribund or died. The intensity of tuberculin reaction and antibody formation was dissimilar in the individual rabbits, although the animal (rabbit 1) that showed the most abundant antibody formation reacted most intensely.

DISCUSSION

The data presented show that all rabbits infected with various amounts of highly virulent tubercle bacilli (from 0.000001 to 0.001 mg.) developed allergic skin sensitiveness to tuberculin and antibodies demonstrable by the complement fixation test. The intensity and the duration of hypersensitiveness varied in the different animals even within the same group. In spite of this variation in all instances during the course of the disease in relation to skin reaction, three periods, a pre-allergic, an allergic, and a post-allergic period, can be recognized in all animals.

Antibody formation was evident in all rabbits that lived more than 3 weeks, save one (rabbit 1, Chart 3) that developed snuffles during the 3rd week after the injection of 0.00001 mg. of tubercle bacilli. The production of antibodies was more rapid and the titers of the sera were higher in rabbits injected with 0.001 mg. of tubercle bacilli than in rabbits that received from 0.001 to 0.000001 mg. of tubercle bacilli.

The disappearance of tuberculin sensitiveness in the presence of progressive tuberculosis is known to occur in man when miliary tuberculosis develops, and sometimes before death. Tuberculin sensitiveness often decreases or vanishes during measles or whooping cough, but it returns after recovery from these diseases. Some tuberculous guinea pigs do not react to intracutaneous injection of tuberculin for a short period of time preceding death.

An almost complete absence of the capacity of the skin to react to tuberculin was observed in young tuberculous guinea pigs, highly allergic in the systemic tuberculin test (Freund (2), Valtis (3)). The skin of tuberculous albino rats does not react to tuberculin although tuberculin death may be produced with small doses of tuberculin in some tuberculous rats (Smith (4), Freund and Hehre (5)).

In interpreting the disappearance of tuberculin skin reactions, it should be remembered that tuberculous rabbits or guinea pigs do not lose their allergic sensitiveness to the toxic effect of tuberculin in the

systemic reaction. Three of four rabbits tested 80 days after the injection of 0.0001 mg. bovine tubercle bacilli and after they had lost their skin sensitivity, died with tuberculin death following the intravenous injection of 1 cc. of tuberculin.

The question naturally arises as to the nature of the desensitization observed. The relationship of skin sensitiveness to antibodies was best studied with sensitization of rabbits to complex and single protein antigens. Opie (6) found that there is a correlation between the precipitin titers of the serum and sensitiveness in the Arthus reaction. When a complex antigen such as horse serum is used, it is not possible to desensitize rabbits completely; however, when a simple antigen, crystalline egg albumin, is used for sensitization a large amount of antigen causes a complete desensitization that is accompanied with a loss of serum precipitins. When the sensitiveness returns, antibodies reappear in the serum. The data presented in this paper show that when skin sensitiveness disappears with the progress of tuberculosis, there is no diminution of complement fixing antibodies.

In correlating the tuberculin tests with the antibody titration, it should be taken into consideration that in these two tests two different preparations were used. Tuberculin, however, acts also as antigen in complement fixation and heat-killed tubercle bacilli elicit tuberculin-like reactions in the skin of allergic rabbits. It is difficult to compare the potency of these preparations: the suspension of heat-killed tubercle bacilli and tuberculin in the two tests. Tuberculin preparations are feeble "antigens" in the complement fixation test.

CONCLUSIONS

1. Rabbits infected with bovine tubercle bacilli develop hypersensitiveness to an intracutaneous injection of tuberculin. This sensitiveness appears from the 2nd to the 6th week after infection and increases rapidly thereafter. Tests, as a rule, show fluctuation in the intensity of the sensitization. Sensitization is followed by an interval of several weeks preceding death during which the animals fail to react.

2. Rabbits infected with bovine tubercle bacilli form antibodies that fix complement in the presence of tubercle bacilli. The antibodies

appear after 2 weeks, increase during 6 to 10 weeks, and persist until the animals die.

3. In the later period of infection the skin fails to react to tuberculin at a time when the serum contains complement fixing antibodies.

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