THE EFFECT OF DIPHTHERIA TOXOID ON EXPERIMENTAL TUBERCULOSIS *

By

WILLIAM H. FELDMAN, D.V.M., M.S.

and

HAROLD E. MOSES, D.V.M., M.S.

Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota

In 1940 interest in the therapy of the mycobacterial diseases was stimulated by the reports of Collier and McKean (1) and by Oberdoerffer (2) concerning the use of diphtheria antitoxin and toxoid in the treatment of leprosy. Although this form of therapy must naturally arouse scepticism, a disinterested observer reported that "the impression was gained very definitely that in many of the cases there had been obtained results more favorable than would have been expected from ordinary forms of treatment." Wade (3), after examining the hypotheses advanced in support of the reasoning that led to the therapeutic use of diphtheria antitoxin and toxoid, and after appraising the evidence that diphtheria toxoid had a favorable effect on at least certain forms of leprosy, took the position that pending proper confirmation the question regarding the alleged effect of toxoid should be considered as unsettled. It was Wade's opinion that the introduction of the use of toxoid "may very well prove to be a material step forward" in the therapeutics of leprosy, but he insisted that it would be premature to hold that the problem of leprosy therapy has been solved. Wade emphasized that if toxoid is to be employed therapeutically in leprosy it should be on no other than an experimental basis.

The phylogenetic relationship of *Mycobacterium leprae* and tubercle bacilli would perhaps justify the assumption that should an agent be found that was effective therapeutically against the organism of leprosy the same agent might also be effective against the organism of tuberculosis. It is admitted that such a position might be difficult to defend successfully since not only are the etiologic agents unlike in certain important respects, but the pathologic characteristics of tuberculosis and of leprosy are distinctly dissimilar. Furthermore, the inability to establish experimentally an infection with *Mycobacterium leprae* makes it impossible to compare certain fundamental aspects of the pathogenesis of leprosy and of tuberculosis. In testing the effects of therapeutic agents on infections associated with or caused by *Mycobacterium leprae,* affected

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human beings must be utilized, while the effects of similar agents on infections induced by the bacteria of tuberculosis can be studied in experimentally infected animals.

From the reports mentioned previously on the favorable effects of diphtheria antitoxin and especially of toxoid in treating certain forms of leprosy, we believed that a limited study of the effect of toxoid on experimentally induced tuberculosis would be justified.*

Methods**

Animals. For experiments 1, 2 and 3, guinea-pigs weighing between 400 and 450 gm. were used. For experiment 4, adult rabbits were utilized.

Infective agents. Three different strains of tubercle bacilli were used. One was strain H37RV, another was a recently isolated "sputum strain" from a case of pulmonary tuberculosis in a human being, and the third was obtained from the tuberculous tissues of a bovine animal. This last mentioned strain was "typed" by tests of pathogenicity and found to be typically "bovine" in character.

Guinea-pigs in all instances were inoculated subcutaneously, while rabbits received the infective inoculum intracutaneously. All animals were caged in pairs.

Toxoid. The diphtheria toxoid was that manufactured by Eli Lilly & Co., the label carrying the numeral V913. Several vials of the product were used during the seven months required to complete the study. In all instances the toxoid was purchased in the open market. The diphtheria toxoid was administered by intramuscular injection.

Respective experiments. For experiment 1, thirty guinea-pigs were each inoculated subcutaneously with 0.0005 mg. of H37RV. The animals were separated into three groups of ten guinea-pigs each. Each animal in group A also received intramuscularly 0.1 c.c. of diphtheria toxoid on the same day that it received the inoculation with tubercle bacilli. Subsequently diphtheria toxoid was administered every two weeks, each succeeding dose being 0.05 c.c. larger than the preceding one until a total of eight doses was given. After the eighth dose subsequent doses were increased by 0.1 c.c. over the preceding one. The animals in group B did not receive their first dose of diphtheria toxoid until four weeks after the guinea-pigs had been inoculated with tubercle bacilli. After treatment with toxoid was started it was continued at two-week intervals, the succeeding schedule of doses being the same as for group 1. The animals in group C were not treated and served as controls.

For experiment 2, twenty guinea-pigs divided into two groups of ten each were used. One month before being inoculated with tubercle bacilli each of the ten guinea-pigs in one group was injected intramuscularly with 0.1 c.c. diphtheria toxoid. Four weeks later each of the ten animals received a second injection of 0.1 c.c. of diphtheria toxoid, and at the same time 0.0005 mg. of tubercle bacilli strain H37RV was injected. No additional toxoid was administered to the animals in this group. The second group of ten guinea-pigs in this experiment

* Early in 1942 Faget and Johansen (4) made a preliminary report on the use of diphtheria toxoid in the treatment of patients suffering from leprosy at the United States Marine Hospital in Carville, Louisiana. Although the study had not been completed when the report was written it was the opinion of the authors that no evidence had been obtained from their study that would indicate that toxoid had had a beneficial effect.

** The dose schedule of toxoid followed in the respective experiments was arrived at through consultation with Dr. D. R. Collier. Dr. Collier readily consented to our request to make the study, and his co-operative attitude in the planning of the experiment was very helpful and much appreciated.

were inoculated with a similar amount of the same bacterial suspension used to inoculate the animals in the first group. These did not receive diphtheria toxoid and served as controls.

For experiment 3, two groups of ten guinea-pigs each were used. Each animal was inoculated subcutaneously with 0.0001 mg. of a recently isolated "sputum strain" of human tubercle bacilli. On the day the animals were inoculated with tubercle bacilli, the ten guinea-pigs in one group also received intramuscularly 0.1 c.c. diphtheria toxoid. The animals in this group received additional injections of toxoid at intervals of two weeks, each succeeding dose being 0.05 c.c. larger than the preceding one until a total of eight doses had been given. After the eight doses subsequent doses of toxoid were successively increased by 0.1 c.c. at two-week intervals.

For experiment 4, ten rabbits were used. These were each inoculated intracutaneously with 0.0001 mg. of bovine tubercle bacilli. The animals were divided into two groups, one of which received toxoid and the other group served as a control. The toxoid-treated rabbits received the first dose of toxoid amounting to 0.2 c.c. on the same day they were inoculated with tubercle bacilli. Subsequent doses were administered at intervals of every two weeks, each succeeding dose being increased by 0.1 c.c.

Duration of experiments. The respective experiments were permitted to continue until all of the animals in the nine groups had died. The earliest that any one group of ten animals were all dead was 167 days and the longest period required for any group of ten animals to die was 247 days.

Necropsies. Each guinea-pig and rabbit was subjected to necropsy as soon after death as possible. The amount and character of tuberculosis present in the organs of predilection were recorded and tissues preserved from the liver, spleen, and lungs of all guinea-pigs, and from the liver, spleen, lungs, and kidneys of the rabbits. Although the presence or absence of tuberculosis was definitely evident in most instances from the gross appearance of the tissues, histologic examination was resorted to whenever necessary to establish the presence or absence of tuberculous changes.

Computing degree of infection. In drawing conclusions as to the relative amount of tuberculosis present in two different guinea-pigs or in two or more different groups of guinea-pigs previously inoculated with tubercle bacilli, it is of much assistance to be able to express the degree of infection in terms of a common denominator. Experience has indicated the practical usefulness of the following scheme, which was followed in recording the amount of tuberculosis present in the guinea-pigs utilized in this study. The figure 10 is accepted as representing the maximal severity of tuberculosis possible for a given animal. Tuberculosis of the spleen, lungs, and liver, respectively, is graded 1 to 3 depending on whether the character of the disease is minimal, moderate, or severe. If lesions are present in the region of inoculation, even though contiguous lymph nodes may or may not be involved, the index of infection for this region is considered as 1. This scheme has not been found applicable in recording the severity of tuberculous involvement of rabbits.

Results

To determine the therapeutic effect of any agent against an experimental tuberculous infection, certain important factors should be kept in mind. Of first importance is the pathogenicity of the tubercle bacilli used to induce the infection. The organisms should have at least standard virulence for guinea-pigs. By this it is implied that following the parenteral inoculation of a known amount of the bacilli there eventually is produced in every animal a progressive tuberculous disease

that inevitably results in the death of the animal. The disease-producing propensities of a given strain of tubercle bacilli should be clearly apparent from the necropsy findings of untreated or control groups of animals. Indications of therapeutic efficacy would be revealed by 1) a longer survival of the animals that were treated compared with the survival time of the animals that were not treated, and 2) more importantly, the treated animals should definitely have less tuberculosis than the controls as determined by the severity of the tuberculous involvement and the anatomic distribution of the lesions. If the use of a therapeutic agent results in the treated animals' living considerably longer than the controls, and if the treated animals have definitely less tuberculosis than those that were not treated, one may conclude that the therapeutic agent had a deterrent effect.

Judged on the criteria just set forth, the results of the experiments constituting the basis of this report fail to reveal that diphtheria toxoid under the conditions imposed had any significant effect on the expected course of tuberculosis in experimentally infected guinea-pigs and rabbits.

As may be noted in Table 1, the results in the three experiments with guinea-

TABLE 1. *Summary of results of several experiments to determine the effect of diphtheria toxoid on experimental tuberculosis in guinea-pigs: ten animals in each group.*

Study	Survival time in days		Index of infection*
	First death	Last death	
Experiment 1			
Group A (Treated) (1)	85	241	7.6
Group B (Treated) (2)	43	247	8.7
Group C (Controls) (3)	91	233	9.3
Experiment 2			
Group A (Treated) (4)	81	206	8.4
Group B (Controls) (5)	86	203	8.8
Experiment 3			
Group A (Treated) (6)	86	218	9.7
Group B (Controls) (7)	82	167	9.1

* Based on the arbitrary figure of 10 as representing the maximal degree of tuberculous involvement.

Information concerning respective groups:

1. Infected with strain H37RV. Treatment with toxoid started day of infection.
2. Infected with strain H37RV. Treatment with toxoid started four weeks after infection.
3. Infected with strain H37RV. Not treated.
4. Received two inoculations of diphtheria toxoid four weeks apart before being infected with H37RV.
5. Infected with H37RV. Not treated.
6. Infected with "sputum" strain. Treatment with toxoid started day of infection.
7. Infected with "sputum" strain. Not treated.

pigs were satisfactorily consistent. In practically all instances the disease was present in the liver, spleen, and lungs and was sufficiently severe to justify the conclusion that every animal had died as a consequence of tuberculosis. The guinea-pigs in the groups that were treated with diphtheria toxoid failed to show a prolongation of life in excess of the controls, and the similarity of the tuberculous involvement in all groups as indicated by the "index of infection" permits of only one conclusion—diphtheria toxoid did not mitigate in any recognizable degree the course and ultimate effects of the infective process.

The results of experiment 4, in which rabbits were infected intracutaneously with bovine tubercle bacilli, were quite comparable to those obtained from the tuberculous guinea-pigs. All of the rabbits died of tuberculosis and at necropsy lesions of the Villemin type predominated in the control as in the treated group. As was true in the experiment with guinea-pigs, diphtheria toxoid did not alleviate or modify in any way the expected course of the infection.

Whether or not the results following the administration of diphtheria toxoid might have been more favorable had larger amounts of toxoid been given is problematic. Each guinea-pig received during the period of treatment a total of between 8 and 10 c.c. of toxoid, while each rabbit received a total of 10 to 12 c.c. of this product.

Sensitivity to tuberculin. Guinea-pigs and rabbits that were living approximately eight weeks after having been inoculated with tubercle bacilli were tested for sensitivity to tuberculin. A 1:100 dilution of old tuberculin was injected intracutaneously in 0.02 to 0.03 c.c. amounts. The results were recorded after forty-eight hours. Of sixty-four guinea-pigs tested all except six showed definitely positive tuberculin reactions. In five of the latter the reaction was indefinite, while in the sixth the reaction was negative. Nine of the ten rabbits were definitely positive to the test, and in one a negative result was recorded. Although tuberculin failed to elicit a characteristically positive reaction in the seven animals previously mentioned, lesions of tuberculosis were strikingly evident in all at the time of necropsy. In only one instance could the failure of tuberculin to provoke a typically positive reaction be attributed to anergy frequently associated with the terminal phase of fatal tuberculosis. In this instance the animal died of extensive tuberculosis five days after the injection of tuberculin. The other animals that failed to show a typically positive reaction to tuberculin lived for periods of from six weeks to as long as twenty weeks after being tested with tuberculin before dying of tuberculosis.

Comment

After the experiments constituting this report had been started there appeared a report by Schain and Petroff (5) on the inhibitory action of diphtheria antitoxin and toxoid on the growth of the tubercle bacillus. These workers approached the problem by *in vitro* and by *in vivo* studies. It was found that diphtheria antitoxin when added to glycerinated beef broth medium had a definite inhibitory action on the growth of tubercle bacilli. The action was thought to be bacteriostatic and not bactericidal. Although the data obtained from the *in vivo* experiments were insufficient to justify definite conclusions, it appeared to the authors "that diphtheria bacilli and their products in one form or another, when injected into animals exert some favorable influence

on the progress of tuberculosis." The most impressive results occurred in guinea-pigs that were inoculated intracutaneously with viable diphtheria bacilli and 500 units of diphtheria antitoxin subcutaneously before being inoculated with tubercle bacilli.

In one experiment in which the procedure was somewhat comparable to that followed in our experiment 2 in that guinea-pigs received diphtheria toxoid prior to infection with tubercle bacilli, Schain and Petroff's results were essentially similar to ours. While the animals that received the toxoid lived on the average somewhat longer than the untreated controls, there was no significant difference in the amount of tuberculosis in the two groups of guinea-pigs.

Bacteriologic or immunologic bases for the observed effects of diphtheria toxoid in the treatment of leprosy or for expecting similar effects in tuberculosis are necessarily related to fragmentary data. Unlike the accumulated information on the pathogenesis of diphtheria and related corynebacterial infections and of tuberculosis, that on the pathogenesis of leprosy is much less abundant and informative. The same general order may be stated for the information on immunity. The ability to demonstrate acid-fast bacilli in lepromatous tissues is the sole practicable feature by which causal significance is attributed to these bacteria. The importance of the acid-fast and of weakly or transiently acid-fast bacilli and of the non-acid-fast bacilli, or diphtheroids, which have been cultivated from lepromatus tissues is an unsettled question. The possible immunologic significance of diphtheria toxoid treatment of leprosy would seem to be more closely related to the combating of any diphtheroids than of any acid-fast micro-organisms. However, Krah and Witebsky (6) have reported the presence of an antigenic substance that is common to the bacillary bodies of some diphtheroids, the diphtheria bacillus, and the tubercle bacillus. Also, common to the genera Mycobacterium and Corynebacterium are the features that have given them a familial relationship.

While the results of our experiments failed to provide evidence that would lead one to believe that diphtheria toxoid would be capable of exerting a significant deterrent effect on the course of experimental tuberculosis in guinea-pigs, the results do not necessarily imply that the use of this product would be of no value in the treatment of certain types of cases of leprosy. As mentioned previously, tuberculosis and leprosy are distinct entities, and it is superfluous to point out that experimental tuberculosis in guinea-pigs and in rabbits is even more remote from leprosy than is tuberculosis in human beings. Our results should be interpreted as applying specifically to tuberculosis as the disease develops in highly susceptible animals following experimental inoculation.

Summary and Conclusions

Using guinea-pigs infected experimentally with human tubercle bacilli and rabbits infected experimentally with bovine tubercle bacilli, a series of studies was made to ascertain whether or not diphtheria toxoid would influence the expected course of the resultant tuberculosis. Seventy guinea-pigs and ten rabbits were used. The administration of toxoid in relation to the inoculation of the animals with the infective agent varied in the different studies. In two groups of guinea-pigs, treatment was started the same day that the animals were infected. In another group treatment was delayed for four weeks after the animals had been infected, and in another group the guinea-pigs had received two doses of toxoid one month apart before being infected. The rabbits received the first dose of toxoid simultaneously with the infective organism. The initial dose of toxoid for the guinea-pigs was 0.1 cc. Subsequent doses were administered every two weeks, with each succeeding dose 0.05 cc. greater than the preceding one until a total of eight doses was given. After the eighth dose subsequent doses were increased by 0.1 cc. over the preceding dose. The initial dose for rabbits was 0.2 cc. This was increased by 0.1 cc. at each succeeding injection, which was at two week intervals. The toxoid. was injected intramuscularly. The experiments continued until all of the animals had died, which was somewhat in excess of seven months.

All of the animals died of tuberculosis, and there were no significant differences between the treated and the untreated groups. The results indicate quite convincingly that under the conditions of the experiments diphtheria toxoid failed to exert any significant deterrent effect on tuberculosis experimentally induced in guinea-pigs and in rabbits.

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