STUDIES ON TETANUS PROPHYLAXIS

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Before the second world war several investigators had demonstrated the possibility of actively immunizing man against tetanus (Ramon and Zoeller, 1927; Jones and Moss, 1937; Marvell and Parish, 1940; Wolters and Dehmel, 1940). During the war this method was tested on a large scale, for in the allied armies prophylaxis against tetanus was based almost entirely on active immunization. Reports so far clearly indicate its value and its superiority to serum prophylaxis (Boyd and MacLennan, 1942; Long, 1946; Ramon, 1946).

Comparison between Active and Passive Immunity

The amount of antitoxin per ml. of serum required for protection against tetanus after active immunization of man can be estimated in at least two different ways: (1) by determining the level of antitoxin capable of protecting actively immunized animals against artificial infection; (2) by consideration of the amount of antitoxin known to be generally effective in passive prophylaxis against tetanus in man.

The first method takes no account of the difference in susceptibility to tetanus toxin between man and animal nor to the difficulties in estimating the amount and rate of toxin production in the artificial infection or its relation to that occurring in naturally sustained injuries. Since in the pathogenesis of tetanus the intoxication predominates and the infection plays a minor part, the total amount of antitoxin available as well as its concentration must be of great importance, and no direct conclusions should be drawn from experiments on small laboratory animals.

Accordingly we adopted the second method, which depends on the established fact that 3,000 I.U. of antitoxin gives effective protection against tetanus for eight to twelve days after injection.

Titration of tetanus antitoxin in serum after passive immunization have been reported by Sneath (1934) and Gold (1941). Three days after injection of 3,000 I.U. the serum antitoxin level lay between 0.2 and 0.5 I.U. per ml.; a week after injection it lay between 0.02 and 0.2 I.U. per ml.

In order to estimate the passive immunity the antitoxin titre in the serum of a number of out-patients was determined at varying intervals after the intramuscular injection of 3,000 I.U. of unrefined tetanus antitoxin, which was given as for routine prophylaxis after accidental injuries. (This work, begun in 1943, is referred to in a paper by Ericsson and others, 1944.) The titrations were carried out by a micro-method, a modification of the standard method used for routine purposes in the manufacture of the commercial products of this laboratory.

Three times the quantity of tetanus toxin, which in titration against international standard serum has been found to correspond to 0.005 I.U., is mixed with 1.5 ml. of undiluted serum or serum dilution. One third of the mixture is injected into each of two 16-g. mice. If both survive, the serum sample or dilution is considered as containing more than 0.005 I.U. per 0.5 ml.

The method thus allows the determination of a minimum concentration of 0.01 I.U. tetanus antitoxin per ml. The results for 56 bleedings are given in the Figure. On the Y axis the absolute

FIGURE.—Showing the total amount of antitoxin available in serum after the injection of 3,000 I.U.

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amount of antitoxin available in the patient's circulation is plotted, on the assumption that the patient has 40 ml. of serum per kg. of body-weight.

The values obtained vary considerably from one patient to another. During the first eight days 40 out of 43 values lie above 250 I.U.; during the following days only 3 out of 13 exceed that value. Having assumed that passive immunity is effective for eight days, we concluded that satisfactory protection against tetanus requires 250 I.U. of circulating antitoxin, corresponding to 0.1 I.U. per ml. serum in a 65-kg. man. Such a titre should offer reasonable protection against tetanus in man, and if the same titre is achieved by active immunization a broad safety margin is gained through the inherent superiority of active immunity.

There was no opportunity of titrating serum antitoxin in patients given a second injection of antitoxin. If the second injection is from an animal species different from the first, the rate of loss of antitoxin should not differ from that in the first injection. The opportunities of using serum from different animal sources are few, and consequently for second passive immunization serum from the same species often has to be used. Though the risks of disagreeable and even dangerous reactions after such a procedure have long been recognized, less attention has been paid to the reduced efficacy of the second dose. According to general rules of immunology, one would expect the rate of loss of antitoxin after the second dose of heterologous serum from the same species to be greater than that after the first dose, since a greater capacity for eliminating heterologous serum must be present at the second injection. The practical importance of this has been pointed out by Ramon and his co-workers (1939), but there are very few direct observations bearing on this question. Since the estimation of the efficacy of passive immunization is based on primary injections, the comparison between active and passive prophylaxis should be based on primary injections of antitoxic serum.

Active Immunization

The adsorbed toxoid used in these investigations is that produced regularly at the State Bacteriological Laboratory. It is prepared according to a method primarily elaborated for the production of adsorbed diphtheria toxoid. This method has already been described (Ericsson, 1946).

The method involves precipitation of toxoid with trichloroacetic acid and resolution of the precipitate in Na_2HPO_4 . The addition of AlCl_3 to this solution gives a precipitate of AlPO_4 , to which the active substance is adsorbed. No washing of this precipitate is

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done. The difficulty of obtaining accurate flocculation of the purified preparation prevents an exact determination of the amount of active substance in the final product. However, in view of the titre of 10 to 15 Lf of the raw material, the content of the final product may be estimated at 30 Lf/ml. Guinea-pigs weighing 250 to 300 g., to which 1 ml. of toxoid is given (the dose designed for human use), will give a titre of more than 1 I.U./ml. of serum in more than half the animals after six weeks.

Ninety-seven healthy men, 20 years of age, who had not received an anti-tetanus inoculation were used in the following experiment. A preliminary test of every fourth person failed to show the presence of naturally occurring tetanus antitoxin in any serum. A single injection of tetanus toxoid was given to each man, followed 6 months later by a booster dose. Table I shows the titres of antitoxin achieved.

TABLE I
SHOWING THE RESULTS ACHIEVED BY ACTIVE
IMMUNIZATION

Immunization	Number of patients with the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01-0.1	0.1-1.0	>1.0	Not tested
No immunization	25				72
1 month after single dose	16	64	17		
6 months after single dose	1	23	43		30
4 days after booster dose	1	8	68		20
8 days after booster dose	1			76	20

Bleedings one month after the injection show that it is impossible to obtain, within this space of time, an immunity comparable to that produced by passive immunization. The bleedings performed five months later disclosed a marked increase in the immunity, proving that the antigenic stimulus by adsorbed toxoid had continued for more than a month. These experiments on man confirm the findings of Faragó (1935), who, when immunizing guinea-pigs, showed that the depot of adsorbed toxoid retained its antigenic properties for thirty-eight days.

The immunity brought about by active immunization with toxoid is inferior even after one month to the best result achieved by serum prophylaxis. Therefore primary active immunization is of no value in the case of an injury unless healing is likely to be so protracted that there is a risk of tetanus even after one month.

The production of antitoxin following the booster dose was of quite a different magnitude and appeared much more rapidly than the antitoxin production after the primary injection (Glenny and Südmersen, 1921). Within four days, that is, within the probable incubation period of tetanus, the booster dose gives an immunity that equals that of the usual passive prophylaxis and, during the following days, surpasses the level of passive immunity. In a person who has already been subjected to basal active immunization a booster dose of toxoid may thus be substituted for the passive prophylaxis.

The results obtained from these studies indicate that the adsorption of the active substance on the precipitate is not so complete as to impair the effectiveness of the toxoid administered as a booster dose. These experiences seem to contradict Miller and Humber's (1943) results, according to which fluid toxoid and not an alum-precipitated one should be used for the booster dose. However, the difference may be explained by the different method employed for the production of the Swedish toxoid. The alum precipitation method usually includes a thorough washing of the precipitate, and it may be assumed that all the activity present is fixed to the precipitate. According to our method the precipitate of AlPO_4 , the formation of which seems to be the main feature of both the methods, is obtained through the addition of AlCl_3 to the solution of NaPO_4 . Since the purification takes place at an earlier stage in the procedure, and no irritating products are formed by the precipitation, no washing is necessary. Thus the method gives a certain amount of active substance in the supernatant fluid. The difficulty of obtaining accurate flocculations of the toxoid prevents, as already stated, exact determinations. In diphtheria toxoid prepared according to the same method, about one-fourth of the active substance remains in solution, and approximately the same proportion may be assumed to remain in tetanus toxoid. The fact that a certain amount of the active substance of our adsorbed toxoid remains in the solution should be regarded as an advantage, rendering it suitable for the booster dose as well as for the primary injection.

Combined Passive and Active Immunization

Since active immunization is of no immediate value to an injured individual not previously subjected to basal immunization, and as we had to take into account the possible use of serum prophylaxis in the future, it was essential to examine the possibility of attaining a basal active immunity

simultaneously with the application of serum prophylaxis.

Investigations performed by Otten and Henne-mann (1939) on guinea-pigs, and by Cooke and Jones (1943) on man, have established that passive immunization impairs the development of active immunity.

Preliminary experiments on guinea-pigs were made with our toxoid. Nineteen animals were injected simultaneously but into different sites with 1 ml. of toxoid and 3,000 I.U. of antitoxin (horse). Bleedings were taken on the fourteenth, twenty-eighth, and forty-second days. A control group of six animals was subjected to injections with toxoid only, and the animals were bled on the forty-second day. The results are shown in Table II.

TABLE II
SHOWING THE RESULTS ACHIEVED BY COMBINED
PASSIVE AND ACTIVE IMMUNIZATION OF GUINEA-PIGS*

Test animals	Number of animals with the following amount of antitoxin in serum (I.U./ml.)			
	<0.01	0.01-0.1	0.1-1.0	>1.0
<i>Test animals:</i>				
14th day		3	7	9
28th „		19		
42nd „	13	6		
<i>Control animals:</i>				
42nd day			1	5

* The test animals were given 1.0 ml. of toxoid and 3,000 I.U. of an antitoxin simultaneously. The control animals were given 1.0 ml. of toxoid only.

They clearly prove the inhibition of active immunization by the heterologous passive immunity, brought on by the simultaneous injection of antitoxic horse serum.

Clinical trials have also been carried out. Since the beginning of 1944 all the patients at the out-patients department of the surgical clinic of the Caroline Hospital, Stockholm, suffering from injuries suspected of being infected with tetanus, were given 1 ml. of toxoid subcutaneously and 3,000 I.U. of horse serum intramuscularly. Two to three years later, seventy of these patients were examined to determine their antitoxin serum titres and the effect of a booster dose of toxoid. At the first examination, bleedings were made for titration of antitoxin in serum, and at the same time a booster dose of 1.0 ml. of toxoid was administered. The effect of the booster dose was determined by further serum titrations four to nine days later. Only thirty-six patients reported for the second control examination. The results are indicated in Table III.

Out of seventy persons tested, thirty-two showed direct evidence of residual immunity as demonstrated by a measurable titre of antitoxin in serum

TABLE III

SHOWING THE REMAINING TITREABLE IMMUNITY IN PATIENTS TWO TO THREE YEARS AFTER COMBINED PASSIVE AND ACTIVE IMMUNIZATION AND THE EFFECT OF A BOOSTER DOSE

	Number of patients showing the following amount of antitoxin in serum (I.U./ml.)				
	<0.01	0.01-0.1	0.1-0.5	0.5-2.0	> 2.0
Before booster dose	38	27	1	1	3
4-5 days after booster dose	4	5	1		
6-9 days after booster dose	1	2	7	7	9

two to three years after combined active and passive immunization, but only five of them had a satisfactory immunity according to our requirements. They did not show any definite rise in antitoxin content of serum four to five days after the booster dose, but six to nine days after there was a marked rise. In only one case out of twenty-six was the response inadequate.

These investigations show that the combined active-passive immunization procedure has an effect that may still be demonstrated two to three years later by direct titration of the antitoxin content of serum or by the reaction following a booster dose. The antigenic stimulus remains, therefore, even after the disappearance of the passive immunity. In accordance with this finding a single injection of toxoid, even though this be administered together with the usual prophylactic dose of serum, produces a basal immunity, and in the event of another injury a booster dose of toxoid without antitoxin will give adequate protection. Although we do not in any way question the results of Cooke and Jones (1943), who worked with much higher doses of serum, our results show that the dose of serum generally used for prophylaxis does not notably impair the effect of our toxoid.

The basal immunity is also of direct practical importance in the prophylaxis of tetanus following minor injuries which are not treated by a physician. Experience has shown that many cases of tetanus occur after such injuries, and that certain occupations carry a heavy risk in this respect. The primary injection makes a small

amount of antitoxin available for a long period. This antitoxin neutralizes the toxin initially produced; the latter acts as a booster dose and causes a further rise in the antitoxin titre. Wolters and Dehmel (1940) showed in man that the quantity of toxin which may be produced after a naturally sustained injury actually has a considerable antigenic effect in an already actively immunized organism. In view of present knowledge a further condition must be stipulated: that there should be no passive immunity present that might interfere with the antigenic stimulus.

Practical Conclusions

Considering the results of the present investigations the following practical rules regarding prophylaxis against tetanus may be recommended.

An injury involving a risk of tetanus in a person not previously actively immunized should be treated with simultaneous active and passive immunization. Even better results would, no doubt, be achieved if the active immunization were not initiated until passive immunity had vanished. Still, since the attention of physician and patient is more easily directed to the prophylaxis against tetanus directly after an injury, simultaneous passive and active immunization is recommended. When, following a severe injury, a higher dose of serum than usual is required, the antigenic stimulus of the toxoid should be protracted so as to exceed the duration of the passive immunity. This protraction may be obtained by a second dose of toxoid or by postponing the first dose of toxoid.

In children prophylaxis against tetanus may be effected by the injection of a mixed tetanus and diphtheria toxoid. This procedure does not give rise to any disagreeable reactions and produces good results (Bigler and Werner, 1941). Any scheme of inoculation that affords good protection against diphtheria will also protect against tetanus arising from small injuries not treated by a physician. A booster dose should be given after major injuries, but serum prophylaxis should never be necessary if the child has once been actively immunized.

Experience from the second world war has served to improve the prophylaxis in military forces during wartime. As a preparatory measure tetanus toxoid may be used in the military forces in peacetime and should be administered at the beginning and at the end of the first compulsory training period, the two injections being thus about one year apart. Booster doses should be given at mobilization and to all battle casualties. Under these circumstances serum should be unnecessary.

Summary

By determining the amount of antitoxin in the serum of patients after the generally adopted dose of 3,000 I.U. of tetanus antitoxin the minimum amount of circulating antitoxin which gives protection against tetanus was estimated. The value found corresponds to 0.1 I.U. per ml. in a man weighing 65 kg.

This level may be achieved by an injection of adsorbed toxoid followed, at the time of injury, by a booster dose of the same toxoid. In man the main effect of the primary injection is not influenced by a simultaneous dose of 3,000 I.U. of antitoxin.

Conclusions are drawn as to the practical application of these experiences in prophylaxis against tetanus.

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