

MICROBIOLOGY AND IMMUNITY

THE INFLUENCE OF THE MODE OF ADMINISTRATION OF ADSORBED TOXOIDS ON THE RATE OF DEVELOPMENT OF IMMUNITY TO TETANUS AND GAS GANGRENE

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Several investigations, among them work carried out at the Laboratory of Infectious Pathology in 1957-1958, have shown the importance of the physical state of the antigen and of its dosage to the rate of development of immunity against tetanus and gas gangrene when only one injection is given. It has been established, in particular [1], that tetanus and gas gangrene toxoids produce signs of immunity earlier in an adsorbed form than as fluid preparations, whether given by subcutaneous or intramuscular injection. A single injection of adsorbed toxoids produces a greater immunizing effect than does the repeated injection of fractional doses of fluid preparations, in accordance with the so-called rapid method of immunization.

On the basis of these findings, and also paying attention to the fact that tetanus toxoid in liquid form, when injected fractionally at definite intervals in the same place, causes signs of immunity to appear earlier than when the total dose is given in the form of a single injection [6, 2, 1], we studied, in the present investigation, the possibility of securing the development of immunity even earlier by means of the fractional injection of adsorbed toxoids.

EXPERIMENTAL METHOD

Experiments were carried out on white rats weighing 120-140 g. For immunization we used concentrated and purified tetanus toxoids of different series, and Clostridium welchii toxoid of series No. 640, obtained from the anaerobic division of the N. F. Gamaleya Institute of Epidemiology and Microbiology of the AMN SSSR. The toxoids were adsorbed on aluminum hydroxide (5 mg Al_2O_3 /ml)*. The dose of toxoid was constant in all the experiments: tetanus – 200 units of combination (CU), gas gangrene – 20 CU. The antigens were injected intramuscularly or subcutaneously into the thigh, in a volume of 1 ml. as a single dose, or as 9 fractional doses in the same place at intervals of 3 hours (first injection – 0.2 ml, remainder – 0.1 ml each). The intensity of the animals' immunity was determined on the 7th day after immunization by means of the intramuscular injection of the corresponding toxin (3.5 MLD of tetanus or 2.0 MLD of gas gangrene), symmetrically opposite to the place of injection of the toxoid into the limb. Normal, unimmunized animals were used as controls. In some experiments, the blood antitoxin level of the experimental animals was estimated. As earlier experiments had shown, the resistance on the 7th day after the primary injection of toxoid was still too low to ensure survival of all the experimental animals, but commencing on the 8th day, even comparatively small doses of toxoids ensured

*The exception was tetanus toxoid of series No. 47-1, which was obtained in the adsorbed form, ready for use.

practically complete protection against the doses of toxin mentioned. For this reason, it was desirable to investigate the differences in the effectiveness of these methods of immunization on the 7th day after injection of the antigens. The mortality rate of the animals and the duration of life of the rats which died (the latter in the tetanus experiments) were taken into consideration.

EXPERIMENTAL RESULTS

The first part of the work was carried out with Cl. welchii toxoid. The toxoid was injected subcutaneously. The results of the two separate experiments are shown in Table 1, from which it can be seen that there were no essential differences in the effectiveness of the above-mentioned modes of administration of the toxoid.

In the second part of the experiments, we used tetanus toxoids of different series with a different antigen content (in units of combination), in the original preparations. In the first experiments series No. 157 toxoid was injected subcutaneously. The results of these experiments are shown in Table 2.

It follows from Table 2 that the fractional administration of toxoid was more effective. Statistical treatment showed that the difference between the survival rates of the animals in the experimental groups was significant, if the collective results of both experiments shown in this table were considered (with a single injection, 28 (13 + 15) of the 30 (15 + 15) animals died, and with fractional injections, 19 (10 + 9) of the 30 (15 + 15) animals died.

Estimation of the antitoxin titer of the blood of the animals on the 7th, 8th, 10th and 12th days after immunization failed, however, to show any essential difference in the effectiveness of the fractional and single methods of administration of adsorbed tetanus toxoid of series No. 157. This can be seen from Table 3.

Later experiments, carried out with tetanus toxoids of other series, also injected subcutaneously, did not confirm the results of the previous experiment with series No. 157 toxoid. The results of these experiments are given in Table 4.

The experimental results in Table 4 show that the effectiveness of both the single and fractional subcutaneous injection of the toxoids were practically identical.

In the final series of experiments, adsorbed tetanus toxoids of series Nos. 160b2 and 47-1 were injected intramuscularly, instead of subcutaneously, and singly and fractionally (Table 5).

In this series, also, no significant difference was observed in the effectiveness of the single and fractional methods of administration of toxoid. Supplementary experiments showed that on the 8th day after intramuscular injection of 200 CU of adsorbed tetanus toxoid (series No. 60b2), all the animals were alive, irrespective of the mode of injection of toxoid used for immunization (the intensity of immunity was tested by injecting 3.5 MLD of tetanus toxin).

The results thus showed that, in the majority of experiments with tetanus toxoid and in the experiments with Cl. welchii toxoid used in the adsorbed form, the intensity of immunity in early stages after immunization (7th day) was the same whether the single or fractional methods of injection were used. These results differed from those obtained from a comparison of the effectiveness of the fractional and single methods of injection of liquid tetanus toxoid [6, 2, 1]; in these experiments the fractional method was more effective. These differences may be understood, however, if the explanation, first put forward by Ramon [6], of the increased effectiveness of the fractional administration of the liquid toxoid in the same place, is borne in mind. According to this explanation, as a result of the frequent injections of toxoid in the same place, inflammatory changes develop in the tissues, leading to a slowing of absorption of antigen from the site of injection and, at the same time, to the better utilization of the antigen by the cells than after a single injection. This view was supported by numerous investigations by Ramon and his co-workers.

The single injection of adsorbed toxoids also ensures delayed absorption of antigen and the formation of an inflammatory focus at the site of injection. The first is due to adsorption of the toxoid and to its slow elution, the second, to the injection of an insoluble residue of aluminum hydroxide. It is therefore obvious that the mode of injection of adsorbed toxoid cannot essentially influence the effect of immunization, since the principle of action of adsorbed antigens is to achieve a fractional method of administration of liquid antigens.

TABLE 1

Comparative Effectiveness of the Single and Fractional Methods of Injection of Adsorbed *Cl. welchii* Toxoid (subcutaneous injection)

Experiment No.	Mode of administration of toxoid	Number of animals	Number dying
1	Single	10	3
	Fractional	10	4
	Control	10	10
2	Single	10	5
	Fractional	10	7
	Control	10	10

TABLE 2

Comparative Effectiveness of the Single and Fractional Methods of Injection of Adsorbed Tetanus Toxoid (series No.157, subcutaneous injection)

Experiment No.	Mode of administration of toxoid	Number of animals	Number dying	Mean duration of survival of animals which died (in days)
1	Single	15	13	5.7
	Fractional	15	10	6.2
	Control	15	15	2.6
2	Single	15	15	4.8
	Fractional	15	9	4.9
	Control	15	15	2.3

If we agree with the findings of Eisler and Eibl [3, 4] and of Prigge [5] and others, that a fundamental part of the mechanism of action of adsorbed preparations is a change in the physicochemical state of the antigen, the "extension" of its molecule as a result of adsorption, bringing about a higher immunizing effect than is possible with liquid antigens, then the results of our own experiments also are in complete agreement with this hypothesis. It is quite clear that the mode of administration of adsorbed toxoid – singly or fractionally in the same place – cannot change its physicochemical structure. The exception was the experiments with adsorbed tetanus toxoid of series No. 157, in which the fractional injection was more effective than the single (according to survival of animals after receiving injections of toxin, but not according to antitoxin titer of the blood; see Tables 2 and 3).

Attention is drawn, however, to the fact that the mortality among the animals immunized by a single injection of adsorbed tetanus toxoid of series No. 157 was much higher than that among those immunized by a single injection of the other series of toxoids, both subcutaneously and intramuscularly (see Tables 2, 4 and 5).

TABLE 3

Antitoxin Titer of the Blood of Rats Immunized by Single or Fractional Injection of Adsorbed Tetanus Toxoid of Series No. 157 (subcutaneous injection)

Mode of injection of toxoid	Animal No.	Day after immunization on which antitoxin was estimated			
		7th	8th	10th	12th
		Antitoxin titer, in antitoxin units			
Single	1	<0.002	<0.002	0.06	>0.075<0.1
	2		<0.002	>0.01 0.03	>0.15 <0.2
	3		>0.004<0.006	>0.002<0.005	>0.075<0.1
	4		<0.002	>0.01 <0.03	0.1
	5		<0.002	>0.01 <0.03	>0.075<0.1
Fractional	1	<0.002	<0.002	>0.01 <0.03	>0.1 <0.15
	2		<0.002	>0.05 <0.06	>0.075<0.1
	3		0.004	>0.01 <0.03	>0.075<0.1
	4		<0.002	>0.01 <0.03	>0.075<0.1
	5		<0.002	0.05	>0.075<0.1

TABLE 4

Comparative Effectiveness of the Single and Fractional Modes of Injection of Adsorbed Tetanus Toxoids of Series Nos. 60b2 and 47-1 (subcutaneous injection)

Series of toxoid	Experiment No.	Mode of injection of toxoid	No. of animals	Number dying	Mean duration of survival of animals which died (in days)
No. 60b2	1	Single	10	5	5.0
		Fractional	10	6	5.8
		Control	10	10	2.7
	2	Single	15	2	6.5
		Fractional	15	5	6.0
		Control	10	10	2.4
No. 47-1	3	Single	15	4	5.5
		Fractional	15	6	5.5
		Control	9	9	2.6

Since all these experiments were carried out under identical conditions – the dose of toxoid was constant – and adsorption – as checked by estimation of the toxoid in the supernatant fluid – was complete, the cause of the low effectiveness of the preparation must evidently be sought in the special features of the antigen itself or in the properties of aluminum hydroxide. It seems to us a likely hypothesis that the low effectiveness of series No. 157 adsorbed tetanus toxoid, when given as a single injection, is due to the instability of the adsorption bond

TABLE 5

Comparative Effectiveness of the Single and Fractional Methods of Injection of Adsorbed Tetanus Toxoids of Series Nos. 60b2 and 47-1 (intramuscular injection)

Series of toxoid	Experiment No.	Mode of injection of toxoid	No. of animals	Number dying	Mean duration of survival of animals which died (in days)
No. 60b2	1	Single	15	4	8.2
		Fractional	15	2	7.5
		Control	10	10	2.4
	2	Single	10	5	6.6
		Fractional	10	5	5.8
		Control	10	10	2.7
No. 47-1	3	Single	15	3	7.1
		Fractional	15	8	5.0
		Control	15	15	2.9

between the antigen and the particles of aluminum hydroxide, since we know from the literature [3, 4] that the effectiveness of an adsorbed preparation is determined by the degree of adsorption as well as by its stability. Injection of toxoid which, for some reason or other was loosely connected with the adjuvant, resembles the administration of a mixture of adsorbed and liquid antigens, for the least firmly adsorbed part of the antigen behaves like a liquid toxoid as a result of its rapid elution. This also explains the low immunizing effect of such a preparation, since the liquid toxoid is a weak immunizing agent. It is obvious that the fractional administration of such a preparation will have an advantage over the single method, for with the fractional method, conditions are created for the better utilization of the liquid component. This was confirmed by our experiments with the concentrated and purified tetanus toxoid [1], which showed that fractional injection of this toxoid in the unadsorbed state is far more effective than single injection. If this hypothesis is valid, the results of the experiments with adsorbed toxoid of series No. 157 do not, in fact, contradict those obtained with adsorbed tetanus toxoid of the other series and with *Cl. welchii* toxoid, since when the series No. 157 toxoid was used it showed features characteristic not only of an adsorbed but also of a liquid toxoid.

In conclusion, it must also be stated that the subcutaneous and intramuscular injection of adsorbed tetanus toxoids are equally effective in both single and fractional methods of administration, as we have previously pointed out [1].

SUMMARY

It was demonstrated in experiments on white rats that the rate of immunity development in primary immunization with sorbed tetanus and gangrenous toxoids is the same, irrespective of whether these toxoids be injected in a single dose or in 9 fractions at the same site and with an interval of 3 hours. These data differ from the results obtained with liquid tetanus toxoids, the fractional administration of which causes an earlier appearance of immunity than a single injection. The efficacy of the sorbed toxoids appeared to be the same in subcutaneous and intramuscular injection. Several experiments in which fractional administration of sorbed tetanus toxoid appeared to be more effective than a single one are discussed from the standpoint of modern conceptions on the mechanism of antigen action.

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* Original Russian pagination. See C.B. Translation.