AGGLUTININ PRODUCTION: FREQUENCY OF INJECTIONS CONSTANT, DOSAGE AND TOTAL AMOUNT INJECTED VARIED.

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In a previous paper[1] entitled "Agglutinin Production. Frequency of Injections Varied, Dosage and Total Amount Injected Constant", it was demonstrated that small doses of a foreign protein injected not too frequently over a short period of time produces agglutinins of a higher titre than similar amounts of the antigen extended over a long period of time. It will be recalled that the above mentioned procedure, is quite the opposite of that used in daily practice both in vaccinating man and lower animals against disease.

Animals, therefore, that are injected daily with 1 cc. for ten days with a typhoid vaccine develop agglutinins of a higher titre than if they were inoculated with the same dose hourly, once or twice or even thrice weekly.

These results suggested the experiment in which the converse is taken up, namely, that of injecting animals with amounts varying from a very small (0.1 cc.) to a relatively large dose (10 cc.), and consequently the total amount inoculated into each animal will likewise vary from a small (1 cc.) to a large amount (100 cc.) over a period of ten days.

These results then will indicate whether or not high titre production depends upon the amount of antigen injected at one time and its tenfold total amount, or upon the frequency of stimulation of the body cells of the animal by inoculation of small amounts of the foreign protein.

Since this was a comparative experiment the antigen used in it was similar to the one used in the previous tests. It contained one billion typhoid bacilli which were killed by heating to 58° C. for one hour. The injections were all made intravenously at daily intervals. The doses of antigen varied from 0.1 cc. to 10 cc. which were constant for each set of animals inoculated throughout the experiment. Consequently it will be noted that some animals received as high as 100 cc. of the antigen while others were inoculated with a total amount of only 1 cc.

A pair of rabbits (I and II, see Table) were injected each with 0.1 cc. for ten days, receiving a total amount of 1 cc. of the typhoid vaccine. A second set of animals (III and IV) were inoculated with 1 cc. for ten days, or an amount totaling 10 cc. While a third series of a pair of rabbits (V and VI) were vaccinated for ten days with a dose of 10 cc., receiving in all 100 cc. of the antigen. These rabbits

[1] Proc. Ind. Acad. Sci. 34: 259-260, 1926.

were bled ten days after the last injection was made and the titres of the agglutinins in their sera were determined macroscopically.

It will be observed from the data contained in the table that rabbits I and II, which received only 0.1 cc. daily for ten days, or a total of 1 cc., developed agglutinins with titres 1:5000 and 1:8500 respectively, an average of 1:6750. The highest titres, those of 1:8500 and 1:10,000, an average of 1:9250, resulted when rabbits (III and IV) were inoculated with 1 cc. daily for ten days, thus receiving a total of 10 cc. of the vaccine, while the lowest titres 1:5000 and 1:4000, respectively, developed in rabbits (V and VI), or an average of 1:4500, which received daily injections of 10 cc. each or a final amount of 100 cc. of the antigen.

Upon further observation of the results included in the table, nothing especially striking is noted relative to the weights and the physical condition of the animals. There is, though, an indication as was rather remarkedly shown in a previous paper[1] that when the animals are not in good physical condition as observed principally by their weights and appearances, that there is usually also a poor degree of agglutinin developed, whereas, good physical condition makes for high antibody production. After all, this is, of course, what one would expect since some of the body cells produce these antibodies and consequently, if the animal is not fit and the cells are in a "low tone", nothing much can be expected of them.

Rabbits VI, V, and I which yielded sera with the lowest titres (1:4500, 1:5000, and 1:5000, respectively) also showed a poorer physical condition accompanied by a greater loss of weights (290, 220, and 230 grams respectively) than the other rabbits did with one notable exception. Although rabbit III, which also lost weight (240 grams) which was about as much as was lost by the animals (VI, V, I) just mentioned, produced a serum of a high titre 1:8500.

These results indicate that an agglutinin serum of a high titre can be developed in rabbits in good physical condition by successive daily injections of small doses (1 cc. and even as small as 0.1 cc.) of a bacterial protein. They further suggest the practicability of this method not only when vaccinating lower animals, but humans as well, in cases where the individuals are inoculated with bacterial antigen (bacterines).

[1] Proc. Ind. Acad. Sci. 34: 259-260, 1926.

TABLE I. Showing the Agglutinin Titre, Weights and Physical Condition of Rabbits Injected * with Different Doses and Total Amounts at Similar Intervals.†

Number of Rabbit	Dose	Total Amount	Titre	Average Titre	Weights in Grams				Physical Condition	Remarks
					Before Injected	After Injected	Loss	Gain		
I	0.1 cc	1 cc	1:5000	1:6750	1700	1470	230	.....	Fair	Good condition two weeks later
II	0.1 cc	1 cc	1:8500		1970	1980	.....	10	Excellent	
III	1.0 cc	10 cc	1:8500	1:9250	2450	2210	240	.....	Fair	Only fair condition 4 weeks later
IV	1.0 cc	10 cc	1:10000		2450	2350	100	.....	Excellent	
V	10.0 cc	100 cc	1:5000	1:4500	2620	2400	220	.....	Fair	Poor condition 4 weeks later
VI	10.0 cc	100 cc	1:4000		2160	1870	290	.....	Fair	Died on 20th day

*Intravenous Injections. †Daily for ten days.