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

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In the identification of rather closely allied pathogenic microorganisms, such as the bacteria of the Colon-Typhoid group, reliance is placed chiefly upon the agglutination test because different strains of the same organism give inconsistent results upon the various media.

The agglutination phenomenon is the clumping together of bacteria in suspension by a specific immune serum. The substances which bring this phenomenon about are called agglutinins. They are produced in animals by the injection of antigens, or to be more specific, agglutinogens. The ordinary method of producing agglutinins is to inject animals with relatively large amounts of the agglutinogen one to three times a week until five to ten or more inoculations have been made.

Of the many phases that present themselves in a problem such as this, it was decided to note the development of the agglutinin in the serum of rabbits when injected with comparatively small amounts of the agglutinogen at varying intervals, while the total amount inoculated remained the same.

(Experiments are now under way in which the converse is being taken up; that is the frequency of the injections are constant while the dosage and the total amounts inoculated vary.)

It will be noted that this is quite the opposite of that which is ordinarily done; that is, large doses which are injected at definite intervals and consequently the total amount is also great.

The antigen used in these experiments contained one billion typhoid bacilli which were killed by heating to 58°C for one hour. All injections were made intravenously. The amount inoculated was one cubic centimeter at intervals varying from once every hour; once a day; to once a week while the total amount introduced into the animal was ten cubic centimeters in all cases.

A pair of rabbits (I and II, see table I) received 1 cc of the antigen every hour for ten hours. Another pair (III and IV) were injected with the same amount (1 c. cm.) for a period of ten days while a third set of rabbits (V and VI) were inoculated once a week for ten weeks. Ten days after the last injection, these rabbits were bled and the titre of their sera was determined, as shown in table I.

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TABLE I—The Agglutinin Production and Weights of Rabbits Inocculated* at Different Intervals, Dosage† and Total Amounts‡ Injected Being Constant.

Number of Rabbit	Interval of Injection	Length of Injection	Titre	Average Titre	Weights in Grams		Condition of Rabbits
					Before Injection	After Injection	
I.	Hourly	10 Hours	750	535	2750	2600	Good
II.	Hourly	10 Hours	320		2826	2450	Poor
III.	Daily	10 Days	2500	3250	2810	2795	Good
IV.	Daily	10 Days	4000		2925	3000	Excellent
V.	Weekly	10 Weeks	1800	2000	2676	2880	Excellent
VI.	Weekly	10 Weeks	2200		2729	2892	Good

* Intravenous injections.
† Dosage 1 cc.
‡ Total amount 10 cc.

It will be noted from the results given in the table that rabbits I and II which were inoculated every hour developed in their sera agglutinins with titres of 1:750 and 1:320 respectively, an average titre of 535.

Rabbits V and VI receiving weekly injections produced sera with titres of 1800 and 2200, an average of 2000. While the animals III and IV receiving daily inoculations gave the highest titred sera; 1:2500 and 1:4000 respectively or an average of 1:3250.

These results indicate then that a serum of comparatively high titre can be built up in rabbits that are stimulated over a rather short period of time, though not too frequently, with fairly small doses. It will be recalled that this is quite the opposite from the usual method of procedure where the amount injected at any one time as well as the total amount is large.

It will be further observed that when the animals are in good physical condition and suffering apparently only slightly as a result of the introduction of the foreign protein as judged mainly by their weights and appearances, sera showing a high titre of antibody may be expected.

This is especially brought to light in the case of rabbit IV with a titre of 1:4000 where the animal was in excellent condition and showed a gain in weight after receiving the injections as compared to rabbit II with only a titre of 1:320 and accompanied by a marked loss in weight. This too is in line with other results obtained over a long period of time, which quite conclusively show the relationship between physical strain and development of antibody.