THE EFFECT OF THE INJECTION OF KILLED STREPTOCOCCI ON THE STREPTOCOCCO-OPSONIC INDEX OF NORMAL RABBITS.*

J. P. SIMONDS.

(From the Memorial Institute for Infectious Diseases, Chicago, Ill.)

WITH the hope of arriving at a better understanding of the effects of the injection of killed bacteria on the opsonic index in cases in infection, it was thought wise to determine the effect of such injections on the opsonic index in normal animals. A so-called vaccine was accordingly prepared by Dr. Weaver from a streptococcus isolated from the tonsil of a patient with scarlet fever. Twenty-hour growths on ascites-agar were suspended in NaCl solution and heated to 65° C. for two hours. Before heating, smears showed only streptococci; and after heating cultures made from the suspension remained sterile. Immediately before heating it was estimated by Wright's method and by plates that each c.mm. of suspension contained 350 millions streptococci. Dilutions of the suspension were used in the experiments to be described.

Normal rabbits were injected and with three chief objects in view: (1) To determine the effect on the opsonic index of different quantities of killed streptococci injected at intervals of several days or after such cycle of events as might follow each injection had been completed; (2) to observe any differences in results produced by intravenous and subcutaneous injections; (3) to determine the effect of very frequent or daily injections on the opsonic index with especial reference to possibility of producing a persistent negative phase.

As a preliminary to making these experiments the normal streptococco-opsonic index of the rabbits to be used was determined every second day, five estimations being made for each animal. The sera of three other normal rabbits were used as a pool. An average of 15 estimations gave an index of 1.05. The indices varied as a rule between 0.80 and 1.20, the lowest was 0.76, the highest 1.40. This high index was observed in normal rabbits but once, 1.27 being next highest.

* Received for publication October 1, 1907.

The technique used was that generally employed in estimations of opsonic indices. The pooled serum consisted of equal parts of sera from three normal rabbits. Both human and rabbit washed leucocytes were used as phagocytes at first, but the results being practically the same in both cases, during the remainder of the experiments, only human leucocytes were used. The results given are those obtained with human leucocytes.

The experiments directed toward the determination of the effect of intravenous and subcutaneous injections of dead streptococci

CHART 1.—Streptococco-opsonic Index of Rabbits Injected with Dead Streptococci.

Solid Line=subcutaneous injection.

Broken " =intravenous "

upon the opsonic index to the homologous streptococcus extended over a period of 30 days. Charts 1 and 2 show the dates of injections, the amount injected and the time of taking the blood for estimation of the opsonic index. Each animal charted received three injections. Seven days after receiving the third dose of vaccine, one of the rabbits (III) died (Chart 1). At autopsy no gross changes could be detected

and cultures from the heart's blood and bile remained sterile. It is to be noted, however, that the streptococco-opsonic index had been below 1.00 for five days. No other perceptible deleterious effects from the use of the vaccines could be determined. The temperature of the animals, taken for several days after the first injection, showed some fluctuation but did not vary much above or below the maximum and minimum temperatures of six normal rabbits. There was no significant loss of weight.

The results of these experiments seem to indicate, (1) that small doses subcutaneously produce the greatest rise in the opsonic index

CHART 2.—Streptococco-opsonic Index of Rabbits Injected with Dead Streptococci.

(Chart 1). In these experiments the highest index, 2.50, was obtained after injection of 100,000 dead streptococci subcutaneously. As each succeeding dose was double the preceding one, it is to be noted that the best results really were obtained with the first and smallest dose. After the second injection it is true that the index reached a point 0.13 higher than after the first injection, but it remained above normal only two days and the rise was preceded by a much more marked depression than the first injection. (2) That as a rule there is a definite cycle of events after each injection, a "negative phase," a

“positive phase,” and a return to normal. In several instances there occurred in these experiments a preliminary rise followed by a fall in the index, and later by a more marked rise, i. e., the positive phase proper; this is especially well shown in Chart 1. A curious feature is illustrated in Charts 1 and 2, namely, that after the second and third injections a longer time was required for the index to reach the maximum than after the preceding injection. Thus in Chart 1, the highest point after subcutaneous injection was reached four, seven, and eight days after the first, second, and third injections respectively. The cycle of events after intravenous injections is more irregular and atypical than after the subcutaneous.

CHART 3.—Streptococcus opsonic Index of Two Rabbits Receiving Daily Injection of Dead Streptococci.

The experiments to determine the effect on the opsonic index of very frequent and increasing doses of dead streptococci were made along much the same lines as the preceding and were continued over a period of 56 days (Chart 3). Two rabbits were injected, at first every second day, later every day, with increasing doses of dead streptococci. To begin with, doses containing 50,000 dead streptococci were used, the doses being gradually increased until 9,000,000 dead streptococci were given subcutaneously each day. An effort was made to inject each time into the same place. The opsonic index was estimated daily at first, later at less frequent intervals.

For the first 20 days there was a slow, but steady loss of weight by both animals, one losing 180 grams, the other 230 grams. During the last 37 days of the experiment both rabbits regained much of their lost weight without quite reaching the standard shown before the injections were begun. The loss of weight could hardly have been due to the diet. During about the first two weeks of the experiment the animals were fed on carrots and oats, and on this feed both lost in weight. After this time only oats were fed and on this diet both rabbits at first lost, and later gained in weight. It seems likely, therefore, that the loss of weight was due to the effect of vaccine.

Chart 3 shows the effect of these injections on the opsonic index. These curves seem to indicate that, while daily injections of even large doses of dead streptococci do not keep the opsonic index permanently below normal, they do appear to have a restraining effect so that the index in these experiments was below the minimum normal limits a greater proportion of the time than in the experiments illustrated in Charts 1 and 2; nor did the index rise as high above normal nor remain up for so long a time as in these experiments. There seems to be a distinct tendency for the index to rise above normal after the injections have been continued for some time.

Five days after the last dose was given, one of these rabbits was killed because it was developing "snuffles." Post-mortem showed nothing abnormal except some scar tissue at the site of the numerous injections. On the same day the other rabbit was given a dose of live avirulent streptococci from two 24-hour agar slants. Single 24-hour agar slant growths of the same streptococcus did not kill normal rabbits. The double dose appeared to make the injected animal slightly sick but did not kill it. It would seem, therefore, that the resisting power of this rabbit was either not (markedly) reduced below normal or that the normal resistance was very quickly regained after discontinuance of the injections.

From these experiments the following conclusions seem permissible:

1. That single injections of 50,000 to 2,500,000 or more of dead streptococci in normal animals cause first a fall and later a marked rise in the streptococco-opsonic index.

2. That relatively small doses may cause greater rise in the index than larger doses, and that subcutaneous injections may be followed by more pronounced rise in the index than intravenous.

3. That daily injections of increasing doses of dead streptococci do not produce a permanent subnormal opsonic index in normal rabbits, but do have a distinctly restraining influence on the index as compared with the effect of single injections.