THE ACTION OF SODIUM SALICYLATE UPON THE FORMATION OF IMMUNE BODIES.

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INTRODUCTION.

This study is one of a series to determine the action of the salicylates upon some of the manifestations of infection in the animal body. From a pharmacological view-point it is well established that the salicylates have an antipyretic and analgesic action in patients when administered in therapeutic doses. The analgesic action varies in different individuals and is apparently more powerful with some of the salicylate compounds than with others. This effect is obtained in many conditions, both in the general toxic state seen in most infections, and also in neuralgias and myalgias when indefinite pain is the chief symptom, and fever and other general manifestations of infectious intoxication are usually absent.

The antipyretic action is due to an increased loss of body heat and, as shown by Barbour (1), is evidenced almost exclusively by individuals who are suffering from infection; normal persons show little if any antipyretic effect after taking fairly large doses of the drug. Afebrile patients suffering from infections, on the other hand, show an increased heat loss after receiving comparatively small doses of the drug. It seems as though the heat-regulating center of this class of individuals is in a different state of sensitiveness to salicylates than is that of normal persons.

A third action of salicylates demonstrated almost exclusively in patients with the polyarthritis of rheumatic fever is that of an antiphlogistic agent. Following the administration of the drug in amounts just under the toxic dose, a most striking decrease of local heat, redness, and swelling, as well as of pain and tenderness in the
involved joints is usually observed; and, in addition, the tendency for
the inflammation to spread to other joints is arrested. Patients
suffering from gonorrheal arthritis or other joint infections in which
bacteria can be demonstrated in the arthritic fluid are not benefited in
this striking manner by salicylates. So often has this observation
been repeated that the therapeutic test is frequently applied to
differentiate the acute polyarthritis of rheumatic fever from that due
to other causes. It seems fairly well established, on the other hand,
that the duration of the general infection in rheumatic fever is not
materially shortened by the drug, and that cardiac complications
often arise even though the patients are taking amounts sufficient to
keep their temperatures almost normal and to prevent arthritis
recurring. From clinical observation alone it would seem, therefore,
that the drug either lowered the infectivity of the etiologic agent of
rheumatic fever by a direct action upon the virus or increased the
ability of the body to react against the infection.

It is well known that salicylic acid and sodium salicylate have
an antiseptic action in vitro; this action is much stronger with free
salicylic acid than with its salts. It is, moreover, impossible for the
free acid to occur in the tissues or body fluids in sufficient concentra-
tion to exert any marked bactericidal action. A bacteriostatic action
must be considered; the drug may exist in sufficient concentration or
in a state to inhibit the growth of the infectious agent, even though
the virus is not completely killed. A study of this problem is now
under way.

It is conceivable that the salicylates may increase the resisting
power of the animal body by stimulating the formation of immune
bodies; but in so far as we are able to determine very little attention
has been given to this possibility.

Jacoby and Schütze (2) studied the opsonin and bacteriotropin content of the
serum of normal and immune rabbits; the serum was obtained both before and
8 hours after the animals received a single dose of 2 gm. of sodium salicylate by
stomach tube. Staphylococci, B. coli, and B. typhosus were used in the tests.
The authors state that five out of twelve rabbits showed a distinct increase of
immune bodies following salicylate treatment. Unfortunately, the details of
the experiments are not given, so it is impossible to tell whether the opsonins
or the bacteriotropins were increased the more.
EXPERIMENTAL.

In view of the fact that no definite etiologic agent has been demonstrated for rheumatic fever, it was thought advisable in our studies to use various members of the coccus group of bacteria as antigens to stimulate the formation of antibodies in rabbits, and to compare the amount of antibody formation in immunized animals receiving sodium salicylate with that of animals similarly immunized but untreated with salicylates. In some of the experiments living bacteria were inoculated into the rabbits, in others killed bacteria in the form of vaccines were employed. In all instances the antigens were injected intravenously. In addition, three experiments were performed to determine the effect of salicylates upon hemolysin formation.

The rabbits received the sodium salicylate in the form of a 2.5 or 5 per cent solution twice daily by stomach tube. In the earlier experiments this was combined with an equal amount of sodium bicarbonate; but later it was found that the salicylate was well tolerated without the bicarbonate so the latter was omitted. In most instances the daily dose of sodium salicylate was from 0.16 to 0.2 gm. per kilo of body weight; the weight of the animals was determined from an average computed by repeated weighings for several days before the actual start of the experiments. Subsequent change in the weight of the rabbits was not used as an indication for altering the dose.

The rabbits were bled before beginning inoculation and twice a week subsequently. The separated serum was kept in the ice box, and the sera obtained at several different dates were tested at one time against the homologous antigens. This precaution was taken so that the results might be as comparable as possible. In all except the first two or three experiments a single standardized pipette was used to dilute all of the sera in one series of tests; this pipette was washed five or six times with saline solution after making each dilution. In doing comparative work with dilutions of different sera this precaution was necessary, for the ordinary pipettes were found to vary from 10 to 20 per cent. After recording the strength of reaction in the ordinary way by a series of plus marks the antibody content was computed from an interpolation table and curve. In this way the strength of reaction can be recorded by means of curves or figures and the comparison made easier than when the reactions are merely recorded by a certain number of pluses.

In some of the earlier experiments the temperature of the rabbits was charted twice daily; subsequently this procedure was discontinued as it was found that the information obtained was of little value in interpreting the immunity results.
Influence of Salicylates on the Formation of Complement-Fixing Antibodies.

In the first three experiments the formation of complement-fixing antibodies against the homologous streptococci was followed.

The antigens used in the tests were prepared by dissolving dried bacteria in antiformin and titrating this solution back to neutral according to the method described by Kinsella and Swift (3). The reactions were carried out in the usual manner, with a period of 1 hour for incubation of antigen, complement, and antibody; after which sensitized sheep red blood cells were added and the tubes returned to the water bath at 37°C. for 2 hours. Immediate readings were then made. The antigens were used in one-half the anticomplementary dose, and both the complement and hemolytic amboceptor in double the hemolytic dose.

Experiment 1.—Four rabbits of practically equal weight were chosen and weighed daily for a week; their temperatures were taken morning and evening as a control of the subsequent period. The animals were divided into two groups of two each. All received intravenous injections of living cultures of Streptococcus viridans A49 twice a week in amounts shown in Text-fig. 1. Rabbits 50 and 51 received daily sodium salicylate, 0.16 gm. per kilo of body weight. All of the sera were tested for complement-fixing antibodies on the same day with the same complement and antigen. The general results of the experiment are shown in Text-fig. 1.

It is evident that prolonged administration of sodium salicylate by stomach tube in the dose used had no deleterious effect on the rabbits. A loss of weight in all of the animals can be attributed to other factors than salicylates, such as the repeated inoculation with streptococci, or snuffles. Two control rabbits receiving the same amount of sodium salicylate, but not inoculated with streptococci, showed no loss in weight and little variation in the temperature curve.

Apparently, the salicylates had little or no antipyretic effect, for the rabbits, whether receiving the drug or not, showed a similar fever within a few hours after intravenous inoculation of living streptococci. Rabbit 52 showed less effect following inoculation than did any of the others. The complement-fixing antibody is indicated in units. The first antibodies were detected in all of the rabbits on the 11th day following the first inoculation, when the two salicylated animals and one control showed equal titers; the other con-
control had more antibodies. On the 15th day the combined non-
salicylated controls yielded sera with stronger titer than did the
salicylated animals. The experiment was terminated at this point
because of the death of one of the controls.

Text-Fig. 1. Comparison of temperature curves and complement-binding
antibody units in salicylated and control rabbits inoculated twice weekly with
living Streptococcus viridans A49. The cross-hatching indicates periods of sodium
salicylate administration.
Experiment 2.—Of six rabbits, two served as controls; the other four received sodium salicylate by stomach tube, two doses a day, as follows: No. 152, 0.4 gm. per kilo of body weight a day; No. 154, 0.3 gm.; Nos. 153 and 157, 0.2 gm. All of the animals were inoculated twice a week with increasing amounts of living Streptococcus viridans A49, as indicated in Text-fig. 2. Blood was obtained before each inoculation; the separated sera were stored until the termination of the experiment, and all were tested simultaneously against the homologous streptococcus for complement-fixing antibodies. The experiment was terminated after the 25th day; the results are shown in Text-fig. 2.
It is evident that there is no striking difference in the antibody content of the rabbits that received different amounts of sodium salicylate; the variation is no greater than might be seen in four different untreated rabbits. It is noteworthy that the two non-salicylated controls showed higher antibody curves than did any of the salicyl-treated animals.

All of the animals were weighed, and all showed similar losses of from 100 to 200 gm.; therefore, in so far as this feature is concerned, neither deleterious nor beneficial effects could be attributed to the salicylates.

**Experiment 3.**—The same general plan was followed as in Experiment 1, with the following exception. The rabbits were immunized with a stock vaccine prepared by suspending in normal saline solution the centrifugate of a 24 hour broth culture of hemolytic Streptococcus K, and killing the bacteria by heating at 56°C. for ½ hour. The animals were immunized by injecting intravenously increasing amounts of this vaccine. Temperature and weight curves of the rabbits were followed; only the temperature curves are reproduced, as the weights of three of the animals increased during the experiment, and that of the fourth was stationary. Blood was withdrawn before each inoculation; the sera were tested at two different periods, after the 18th and 32nd days, respectively. The number of complement-fixing units is recorded in Text-fig. 3.

It is evident that there was little if any difference in the antibody content of the two groups of animals; the difference is no greater than might be expected in such small groups. The fever normally following the intravenous injections of bacterial vaccines was uninfluenced by the administration of salicylates in the same manner as was the fever following the intravenous inoculation of living Streptococcus viridans.

A similar experiment was conducted in which the rabbits were inoculated with living cultures of hemolytic Streptococcus K. These rabbits died from generalized streptococcus infection before sufficient time had elapsed to obtain comparable results. Here, again, no distinct antipyretic effect was seen in the salicyl-treated animals as compared with untreated controls. All four of the rabbits inoculated with living hemolytic streptococci had multiple purulent arthritis from which pure cultures of hemolytic streptococci were recovered. It is therefore evident that the salicylates had no influence in preventing the development of arthritis.
Text-FIG. 3. Comparison of temperature curves and complement-binding units in salicylated and control rabbits immunized twice weekly with vaccines of *Streptococcus hemolyticus* K. The cross-hatching indicates periods of sodium salicylate administration.
A summary of all of the experiments showing the influence of salicylates upon the formation of complement-fixing antibodies is given in Text-fig. 4. Here the averages derived from a larger group of animals than was used in any individual experiment indicates that salicylates in general had a depressing influence upon the formation of this type of immune bodies.

Text-Fig. 4. Comparison of average complement-binding antibody formation in the foregoing series of salicylated and control rabbits. The number of animals tested each day is indicated in parenthesis.

**Influence of Salicylates on the Formation of Agglutinins.**

In the next four experiments the rate of agglutinin formation was determined.

In all experiments the sera were tested against killed cultures of the homologous bacteria prepared as follows: A 24 hour broth culture was heated at 56°C. ½ hour, and standardized by Gates' (4) method so that the density of the suspensions was similar. The reaction of the suspensions was brought to a pH 7.3 or 7.4 to avoid acid agglutination. In order to insure that the suspending medium was the same in all of the tubes the sera were diluted with broth, instead of salt solution. The tubes were incubated at 56°C. for 2 hours, removed from the incubator, and allowed to cool for 15 minutes; then the strength of reaction was recorded.

**Experiment 4.**—Six brown rabbits weighing from 1,400 to 1,600 gm. were immunized with a pneumococcus of Type I by giving daily intravenous injections of vaccines prepared by suspending the centrifugate of 24 hour broth cultures in normal saline solution and heating at 56°C. for ½ hour. An amount of vaccine representing 2 cc. of culture was given to each rabbit daily for 6 days. Three of the rabbits (Nos. B-21, B-22, and B-23) received sodium salicylate daily for
14 days; the dose was 0.16 gm. per kilo of body weight. The other three rabbits served as controls. The sera of all of the animals were tested for agglutinin content against the same suspension of homologous pneumococci. The results are given in Text-fig. 5.

In this experiment the amount of immunizing antigen was small and the period of immunization was short in comparison with the previous experiments. It is difficult to obtain in rabbits an agglutinating serum of high titer against pneumococci. In this series, therefore, the curves are low; the salicylated rabbits, nevertheless, had uniformly less antibodies than did the controls.

Experiment 5.—Ten brown rabbits weighing between 1,700 and 1,800 gm. each were divided into two lots of five. One lot was given sodium salicylate, 0.16 gm. per kilo of body weight, daily for 20 days. On the 2nd day all of the animals received intravenously the centrifugalized sediment of 5 cc. of broth culture of Streptococcus viridans 38D; on the 6th day a similar amount of the same organism; and on the 14th day 10 cc. Blood was obtained from the animals before the beginning of treatment and on the 6th, 10th, 14th, 17th, and 21st days. Three of the salicylate-treated animals and one of the control group died during the course of the experiment; all of the surviving animals were killed and examined for lesions of the joints and viscera on the 20th or 21st day. At the termination of the experiment all of the sera were titrated for agglutinins. The final interpolated titers of the various sera are given in Table I.
### TABLE I.

Comparison of Agglutinin Formation in Salicylated and Control Rabbits Inoculated with Living Streptococcus viridans 38D.

<table>
<thead>
<tr>
<th>Date</th>
<th>Insoculation</th>
<th>Salicylated rabbits.</th>
<th>Controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1921</td>
<td>Apr. 2 2</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>7 2</td>
<td>5 cc. of Streptococcus 38D.</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>11 6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>11 6</td>
<td>5 cc. of Streptococcus 38D.</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>15 10</td>
<td>10 cc. of Streptococcus 38D.</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>18 10</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>22 17</td>
<td>26</td>
<td>21</td>
</tr>
</tbody>
</table>
Although the usual variation in the agglutination titer in the serum of different animals is seen in both groups, most of the rabbits in the control group consistently yielded stronger agglutinating serum than did the salicyl-treated rabbits; the average curve of the control group was uniformly higher than that of the salicyl group, and on the 14th, 17th, and 21st days it was nearly twice as high.

In another experiment carried out in a manner similar to No. 5, part of the salicylated rabbits succumbed to intravenous inoculations of Streptococcus A49; on the 8th day the three surviving rabbits in this group gave an average agglutinin content of 46 units; and six controls gave an average content of 57 units. On the 16th day only a single salicylated animal was living, while five controls still survived; at this time both groups had an average agglutinin content of 90 units. Because of the difference in the number of animals in the two groups this experiment is not given in detail. It merely shows that a single salicylated animal may have as high an antibody curve as some untreated controls; and emphasizes the desirability of having more than one animal in each group.

In still another experiment in which only two salicylated rabbits and four controls lived until the 6th day, the average agglutinin curve on this day was 735 for the first and 450 for the second group. These animals were immunized with a single intravenous injection of 35 cc. of Streptococcus viridans A135. The difference in the number of animals in the two groups also decreases the value of the results in this experiment. It is possible that the large amount of immunizing antigen may have been a factor in eliminating the difference in the two groups of animals.

These two experiments were the only ones in which the animals receiving salicylates yielded serum with as high or higher average antibody content than that of control animals.

Experiment 6.—This experiment was designed to determine whether salicylate-treated rabbits and non-salicylated controls, all previously inoculated into the knee joints with killed cultures of green streptococci, would show any difference in the agglutinating power of their sera after intravenous inoculation with living streptococci.

Each of six brown rabbits weighing between 1,600 and 1,900 gm. was inoculated into the right knee with the sediment of 0.5 cc. of broth culture of Streptococcus viridans 38D killed by heating for ½ hour at 56°C. and into the left knee with 0.5 cc. of Streptococcus viridans A49 similarly treated. The knees were measured and otherwise observed until they had reached their original dimensions or were stationary in size. Serum was separated from blood obtained on the 3rd, 20th, and 28th days following intraarticular inoculation. The animals were then divided into two groups of three each, and the members of one group given
sodium salicylate daily in doses of 0.2 gm. per kilo of body weight; this treatment was started on the 27th day after the intraarticular inoculation. The next day each of the animals received an intravenous injection of the sediment of living streptococci from 9.3 cc. of an 18 hour culture of Streptococcus 38D. Serum was obtained on the 3rd and 9th days following this intravenous inoculation, and on the 9th day all of the animals were autopsied to determine the extent of articular and visceral lesions.

In the immunity studies several points may be noted: (1) the possibility of detecting agglutinins in the blood following the intraarticular inoculation of small amounts of two different strains of killed streptococci; (2) the effect of subsequent intravenous inoculation of one of the two strains—whether it stimulates antibodies to one or both; and (3) the difference in the salicylate-treated and control groups. The results are summarized in Table II.

Although the antibody curve was not followed at frequent intervals after the intraarticular injection of killed streptococci, it is evident that a single injection of Strain A49 stimulated the formation of agglutinins much more than did a simultaneous injection of Strain 38D. It is impossible to state whether the agglutinins detected on the 28th day were stimulated by the intraarticular injection or by the intravenous inoculation because the blood was withdrawn 8 hours after the intravenous inoculation. The increase in agglutinins for Strain A49 on the 9th day after the intravenous injection of Strain 38D must have been due to the stimulating effect of the latter strain in animals already slightly immune to No. A49. The rise in antibody curve at this time was noted for both strains. The two strains are distinct antigenically; the serum of many animals immunized alone with Strain 38D has never agglutinated No. A49, and vice versa. The increase in antibodies for No. A49 on the last day must, therefore, have been a concomitant phenomenon, and did not seem to have been influenced by salicylates. The lower agglutinin content for Streptococcus 38D in the salicylated animals is similar to that observed in other experiments.

Experiment 7.—The object of this experiment was to determine in addition whether streptococci treated in vitro with salicylates were less active antigenically than untreated streptococci. Three groups of animals were chosen. Group 1, consisting of Rabbits B-29, B-30, and B-31, received sodium salicylate by stomach
**TABLE II.**

Comparison of Agglutinin Formation in Salicylated and Control Rabbits Inoculated with *Streptococcus viridans 38D*, but Previously Injected into the Knee Joints with *Streptococcus viridans 38D* and A49.

<table>
<thead>
<tr>
<th>Date</th>
<th>Day.</th>
<th>Inoculation</th>
<th>Salicylated rabbits</th>
<th>Controls</th>
<th>Salicylated rabbits</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr. 21</td>
<td>1</td>
<td>Right knee 0.5 cc. of killed <em>Streptococcus 38D</em>. Left knee 0.5 cc. of killed <em>Streptococcus A49</em>.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>May 10</td>
<td>20</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>9.3 cc. of living <em>Streptococcus 38D</em> intravenously.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td></td>
<td>480</td>
<td>140</td>
<td>480</td>
<td>366</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td></td>
<td>480</td>
<td>140</td>
<td>480</td>
<td>366</td>
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<tr>
<td></td>
<td>26</td>
<td></td>
<td>480</td>
<td>140</td>
<td>480</td>
<td>366</td>
</tr>
</tbody>
</table>
tube, 0.2 gm. per kilo of body weight, and daily injections of vaccine prepared from broth cultures of \textit{Streptococcus viridans} 38D. The vaccine was given for 5 days. Group 2, consisting of Rabbits B-32, B-33, and B-34, received daily intravenous injections of salicylated vaccine. This vaccine was prepared as follows: An aliquot portion of the vaccine used in immunizing Group 1 was mixed with an amount of sodium salicylate solution that would have been used had the rabbit received the drug by stomach tube. The vaccine and salicylate were incubated at 37°C. for 4 hours, then centrifugalized, and the centrifugate was taken up in saline solution and injected intravenously. This vaccine was given for 5 days. Group 3, the control group, consisting of Rabbits B-35, B-36, and B-38, received daily injections of untreated vaccine. All of the rabbits received each day vaccines in an amount equal to 2 cc. of broth culture per kilo of body weight. The rabbits were bled before the experiment and on other days indicated in Text-fig. 6. All of the sera were tested for agglutinin content on the same day. The results are shown in Text-fig. 6.

Here, as in previous experiments, the control group had a higher agglutinin content in their sera than did the rabbits treated with sodium salicylate. The second group, namely those that received salicylated vaccine, had the lowest antibody content. In this experi-

\begin{figure}
\centering
\includegraphics[width=\textwidth]{Text-Fig. 6}
\caption{Comparison of agglutinin formation in salicylated and control rabbits immunized with vaccines of \textit{Streptococcus viridans} 38D, and rabbits immunized with salicylated vaccines.}
\end{figure}
ment it seemed, therefore, that the action of the salicylate might be directly upon the antigen, causing it to be a less active stimulator of antibody production in the animal body.

It was thought advisable to determine whether streptococci treated with salicylate might act differently towards an agglutinating anti-

![Text-Fig. 7. Comparison of average agglutinin formation in the complete series of salicylated and control rabbits. The number of animals tested each day is indicated in parenthesis.](image)

serum than untreated streptococci. The following experiment was therefore performed.

A 24 hour broth culture of Streptococcus 38D was killed by heating ½ hour to 56°C. The reaction of the medium at this time was pH 7.4. The culture was divided into two parts: X was kept in the original state; Y was treated as follows: It was centrifugated and the supernatant serum broth retained. The separated bacterial sediment was mixed with 400 cc. of 5 per cent sodium salicylate and incubated for 4 hours at 37°C. It was then centrifugated to clearness, the supernatant sodium salicylate solution discarded, and the bacterial sediment added to the original broth. The resuspension of the cocci in the medium in which they were grown insures that Suspensions X and Y were the same except that Suspension Y contained salicylated streptococci. These two suspensions were
then tested against the sera of Rabbits B-29, B-31, B-32, B-33, and B-34. With each serum the agglutination reached the same point in both suspensions; the differences in the agglutinating power of the serum of the rabbits of Groups 1 and 2 cannot, therefore, be attributed to the possibility that Group 2 rabbits were immunized with less agglutinable streptococci.

The average agglutinin content of the serum of all of the rabbits used in the foregoing experiments is shown in Text-fig. 7. The irregular course of the curves is due to the difference in the number of animals tested at different periods. At each period, however, the number of controls is equal to or greater than the number of salicylated animals. This summary shows that the average agglutinin formation in the entire group of salicylated animals was lower than that of the controls, and is in accord with the findings of the animals tested for complement-binding antibodies.

**Influence of Salicylates on the Formation of Hemolysin.**

In the remaining experiments the antibody tested was an hemolysin against sheep red blood cells.

All of the sera were inactivated by heating at 56°C. for ½ hour. The diluted serum was then mixed with guinea pig serum so diluted that 0.5 cc. equaled one unit of complement previously tested with a constant standard hemolytic serum. To the rabbit serum and guinea pig complement was added 0.5 cc. of a 5 per cent suspension of sheep red blood cells. The mixtures were incubated in the water bath at 37°C. for 2 hours and read immediately. The dilution at which hemolysis was complete was considered as containing the charted unit of antibody. Because of the result obtained in Experiment 7 the experiments with hemolysins were all carried out with three groups of animals: Group 1, in which the animals received washed sheep red blood cells intravenously and sodium salicylate by stomach tube; Group 2, in which the animals received intravenous injections of salicylated sheep red blood cells, prepared by incubating together washed erythrocytes and a 2.5 per cent sodium salicylate solution, then centrifuging the mixture and retaining the cells alone for inoculation; Group 3, in which the animals received simply intravenous injections of washed sheep red blood cells.

*Experiment 8.*—Seven rabbits about 6 weeks old, all brown, of the same litter, were chosen for this experiment. Inactivated serum obtained from a preliminary bleeding was tested for hemolysin content against sheep red blood cells. The rabbits were then divided into three groups according to their weight and the native hemolysin content of their serum. This precaution was taken because certain investigators claim that the amount of antibody formation following
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Text Fig. 8. Comparison of hemolysin formation in salicylated and control rabbits immunized with sheep erythrocytes, and rabbits immunized with salicylated erythrocytes. Salicylate and erythrocytes given daily for 3 days, 1 day intermission, then for 3 more days.
immunization is more or less in direct proportion to the amount of antibody the serum of the animal contained before immunization. Group 1, consisting of Rabbits B-43, B-45, and B-46, received injections of sheep cells daily for 3 days followed by an interval of 1 day, and then for 3 more days, and at the same time sodium salicylate by stomach tube—a total daily amount of 0.16 gm. per kilo of body weight per day. Group 2, consisting of Rabbits B-44 and B-49, received intravenous injections on the same days as did rabbits of Group 1. They received sheep red blood cells that had been incubated for 3 hours with sodium salicylate, the supernatant salicylate solution discarded, and the salicylated cells resuspended in saline solution. Group 3, controls, consisting of Rabbits B-47 and B-48, was injected with washed sheep blood cells. The antibody curves of these rabbits are shown in Text-fig. 8.

In all three groups some of the rabbits showed hemolysin titers as high as 1,000 to 2,000. Comparing the average curve for each group, however, the highest and most prolonged antibody content was found in Group 3, the next in Group 1, and the lowest in Group 2, the group immunized with salicylated cells.

Experiment 9.—This experiment was carried out in essentially the same manner as Experiment 8, with the following exceptions. Three groups of larger rabbits, consisting of three animals each, were used; injections of erythrocytes were given only 3 days; and the sodium salicylate by mouth was given for a similar period. The influence of the salicylate was, therefore, correspondingly less than in animals receiving the drug over a longer period. The results are shown in Text-fig. 9.

In this experiment the differences between the various groups were less marked than in previous experiments although the average curve in the control group (Group 3) was higher than in Groups 1 and 2. This high average is due to the fact that the serum of Rabbit H-36 had a much higher antibody content than that of any of the others. The other two rabbits in this group had a lower antibody content than any of the rabbits in the other two groups. As in Experiment 8 the animals receiving salicylate by stomach tube had a higher average curve than did those receiving salicylated red blood cells intravenously.

Experiment 10.—A comparison of the results in Experiments 8 and 9 seems to indicate that a long or short period of administration of the salicylates might be a factor in determining the degree of antibody formation. Experiment 10 was made to test the influence of more prolonged administration of the drug. The rabbits used in this experiment were all brown, full grown, and of approximately
Text-Fig. 9. Comparison of hemolysin formation in salicylated and control rabbits immunized with sheep erythrocytes, and rabbits immunized with salicylated erythrocytes. Salicylate and erythrocytes given daily for 3 days.
TEXT-Fig. 10. Comparison of hemolysin formation in salicylated and control rabbits immunized with sheep erythrocytes and rabbits immunized with salicylated erythrocytes. Salicylate given for 12 days; erythrocytes injected on the 1st, 5th, and 9th days.
Three intravenous injections of 25 per cent sheep red blood cells, 4 cc. per kilo of body weight, were given to each rabbit on the 1st, 5th, and 9th days, respectively. In Groups 1 and 3 the cells were not salicylated; in Group 2 the cells were salicylated by incubating them with 2.5 per cent sodium salicylate solution for 15 hours, the suspension centrifugated, the supernatant solution discarded, and the cells resuspended in saline solution. Group 1 animals received sodium salicylate in a total daily amount of 0.2 gm. per kilo of body weight. The animals received three doses before the first immunizing cells were given and were treated 4 days after the last intravenous injection of erythrocytes. The results are shown in Text-fig. 10.

Text-Fig. 11. Comparison of average hemolysin formation in the salicylated and control rabbits used in Experiments 8 to 10. The number of rabbits tested each day is indicated in parenthesis.

Here, as in previous experiments, the control group had a higher total as well as higher average antibody content than did the other two groups. Group 1, in which the rabbits received salicylate for 12 days, showed the lowest antibody content. Group 2, treated with salicylated cells, showed almost as great an antibody content as did Group 3. The discrepancy between the animals in Group 2 in this experiment and in the three previous experiments cannot be explained.

If the average hemolysin content of the sera of all of the animals used in Experiments 8 to 10 are plotted (Text-fig. 11), it is seen that
the control animals not receiving salicylate yielded sera with consistently higher titer than did animals receiving salicylate by mouth. This is in complete accord with the results obtained for agglutinating and complement-binding antibodies.

DISCUSSION.

Aside from the two incomplete experiments in which the number of animals in the salicylated group was small, the controls all had a higher antibody content than did animals similarly immunized but treated with sodium salicylate. The dose of salicylate given the rabbits each day was comparable with the largest amount that may be safely administered to patients for short periods, and much larger than can be tolerated over a long period by patients with rheumatic fever. While some rabbits receiving 0.4 gm. per kilo each day seemed to suffer no deleterious effects, others receiving this amount of the drug succumbed more quickly to intravenous inoculation of living streptococci than did unsalicylated controls.

Fantus, Simmonds, and Moore (5) showed that sodium salicylate, when used in a dose which they state was comparatively harmless to animal controls, is decidedly detrimental and liable to be fatal to rabbits infected with hemolytic streptococci. In their experiments from 0.9 to 1 gm. of sodium salicylate and 1.2 gm. of acetylsalicylic acid were given by mouth each day; in another series 0.3 gm. of sodium salicylate was given hypodermically. These are much larger doses than may be given with safety to patients. Our experiments indicate that 0.2 gm. is as large a daily dose as can be safely administered to rabbits over long periods.

Rabbits receiving the drug daily by stomach tube at first refused to take much food, but after 3 or 4 days they again resumed their normal feeding habits. Malnutrition in this group of animals cannot, therefore, be considered as the cause of a lowered antibody formation.

It is possible that animals with less febrile response following the intravenous injection of antigens might show a correspondingly lower antibody formation. As the salicylates have a marked antipyretic effect in patients this argument might be used theoretically to explain our results. In the first and third experiments it was shown, however, that salicylates in the doses employed did not have an anti-
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pyretic effect in inoculated rabbits. A hypothetical correlation between fever and antibody formation must, therefore, be abandoned in explaining the results.

Only a few drugs or chemicals have been tested to determine their action upon the formation of immune bodies.

With the introduction of salvarsan several workers (6; 7) claimed that one of the marked actions of arsenicals in general was to increase antibodies. Others (8, 9) have claimed that this effect was less marked and less constant than found by the earlier experimenters. Toyama and Kolmer (9) state that large doses of both salvarsan and mercury bichloride appear to depress hemolysin and agglutinin production in immunized rabbits; while smaller doses of both drugs tend to increase the production of hemagglutinins, but not of hemolysins.

Müller (10) found that animals treated with aleuronat or cinnamic acid while undergoing immunization yielded more antibodies than did controls. He thought that because these two substances stimulated leucocytosis the two effects were to be attributed to a common action. Similarly the depression in immune bodies in animals under the influence of benzene led Hektoen (11) to believe that substances exerting a destructive action on the blood-forming organs had a depressing effect on the tissues from which immune bodies arise. But his later studies concerning the effect of toluene (12) and thorium (13) on immune body production indicate that variations in the antibody content of the blood serum and leucocytosis or leucopenia need not necessarily go hand in hand.

Abbott and Bergey (14), Müller (10), and Friedberger (15) have all demonstrated conclusively that alcohol in repeated doses has a very depressing influence on the capacity of immunized animals to form antibodies. Melnikowa and Wersilowawa (16), and Müller (10) have shown that phlorhizin has a similar effect. Hektoen and Carper (17) have shown that mustard gas (dichloroethylsulfide) depresses immune body formation.

The foregoing review of previous experiments fails to furnish any explanation that can be brought to bear upon our studies.

Cook (18) has shown that the rate of antibody formation is dependent upon the rate of absorption of the antigen. She reviews and makes use of the work of Loeb, Lillie, and Osterhout, all of whom have demonstrated that the permeability of cells is increased by increasing the sodium ions and decreased by increasing the calcium ions. In her experiments, rabbits treated with sodium citrate formed antibodies more intensely than did controls, and those treated with calcium chloride less intensely than controls. In all of our experiments the rabbits receiving salicylates were at the same time subject to an
increased amount of sodium from the sodium salicylate. From Cook’s experiments one would expect an increased antibody output if the sodium ion is the important element in the compound. Whether the salicyl ion has an antagonistic effect by decreasing the absorption of antigen is a question that must be submitted to experimental study. Reasoning simply by similarity of effect it seems possible that the decreased antibody production in rabbits treated with sodium salicylate may be due to the decreased power of these animals to absorb antigen.

It must be emphasized in this connection that there is nothing in our experimental results to indicate that the salicylates are deleterious in infection simply because they decrease immune body formation. In none of the experiments was this decrease extremely marked, and all of the salicylated animals produced immune bodies in fair amounts. It is probable that immune bodies in the circulating fluids are not the only factors concerned in the process of recovery. The same mechanism that makes an infectious agent less antigenic might at the same time make it less pathogenic. It is possible that this effect may be more marked in rheumatic fever than in other infections; hence the more striking effect of the drug seen in this disease.

CONCLUSION.

1. Rabbits treated with sodium salicylate in daily doses of from 0.16 to 0.2 gm. per kilo of body weight and at the same time immunized with intravenous injections of Streptococcus viridans, both living and in the form of vaccines, and also with washed sheep red blood cells, showed diminished complement-fixing antibodies, agglutinins, and hemolysins when compared with controls similarly immunized.

2. If the antigens were treated with sodium salicylate in vitro and subsequently injected intravenously into rabbits, the animals usually showed lower antibody curves than did rabbits that received the untreated antigen intravenously and sodium salicylate by stomach tube.

3. The beneficial effect of sodium salicylate in rheumatic fever patients probably cannot be attributed to an increased production
of circulating immune bodies against the infectious agent. This is, however, no contraindication to the administration of salicylates to patients suffering from infectious diseases.

BIBLIOGRAPHY.