A METHOD OF SERUM TREATMENT OF PNEUMOCOCCIC SEPTICEMIA IN RABBITS.

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PLATE 53.
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In a separate paper I have reported experiments which indicate that the removal of pneumococci from the circulation in rabbits and their destruction are determined by three main factors: (1) the agglutination of the bacteria in the blood stream; (2) the assembling of the clumps in the internal organs; and (3) the inclusion of the masses by and digestion within polymorphonuclear phagocytes. In order that these several processes may occur it has been found necessary, in the case of virulent pneumococci, to employ a suitable immune serum.

In this paper I propose to describe still other experiments which bear upon the above conception of the manner of interaction of immune serum and the animal body. It has hitherto been ascertained that the activity of an immune antipneumococcic serum is quite strictly limited. Beyond a given infecting dose of the pneumococci any practicable quantity of immune serum ceases to be protective. Indeed, as the dose of serum is increased, especially when it comes from a foreign species of animal, a point is reached at which the serum becomes deleterious rather than helpful. The precise conditions upon which this change of action depends are not known; it is surmised that a heterologous serum, in small experimental animals especially, may exert a toxic action which becomes noticeable in effect when the dose is relatively large. In any case a striking disparity exists between the unlimited neutralizing action of the antitoxic sera, diphtheria and tetanus sera, for instance, and the strictly limited anti-infectious action of the antibacterial sera, among which the antipneumococcus serum is to be classed.

Although the literature on the anti-infectious power of antipneumococcic serum is voluminous, the chief recent important contributions to the subject are those of Neufeld and Haendel\(^2\) and of Cole\(^2\). The former investigators established the fact of the existence of specific types of pneumococci which are subject to influence only by the corresponding immune sera; and the latter, together with his coworkers, besides extending the knowledge of the types of pneumococci and their specific antisera, observed also that up to a certain degree of infection the protective dose of serum is parallel to the quantity of culture inoculated, after which the quantity of serum required to save life is proportionally large, and finally, a degree of infection may be reached against which no amount of serum will protect. For example, 0.2 cc. of serum of Type I will regularly protect a mouse against 0.1 cc. of a Type I culture, no matter how high its virulence. That is the largest dose of culture, however, against which the immune serum will protect, no matter how much serum is employed. 1 or even 2 cc. are no more effective than 0.2 cc. (Cole).

In view of these data, it has been long believed that the animal body supplied a necessary substance which cooperated with the immune serum in overcoming bacterial infections. The nature of this substance has been conjectured merely; there exists no actual knowledge of its nature. The experiments reported in the previous paper referred to\(^4\) seem to indicate quite definitely that the part which the rabbit's body itself plays in overcoming pneumococcus and some other bacterial infections is supplied by the phagocytes. The experiments to be related here bear on this conception of the protective mechanism.

**EXPERIMENTAL.**

As has just been stated, experiments seemed to indicate that the phagocytic cells function as the chief defensive agents against infection. If this is true, then the limit of the effective action of an immune serum may possibly be extended by giving the phagocytes assistance and providing them sufficient time in which to do their work. We observed that when a rabbit suffering from septicemia is given a


\(^4\) Bull, *loc. cit.*
large injection of immune serum intravenously, the bacteria are agglutinated into massive clumps (Figs. 1 and 2) most of which accumulate in the lungs where they come imperfectly under the influence of the phagocytes and may even, through extensive capillary obstruction, interfere with the circulation. The experiments to follow were performed on rabbits with a strain of pneumococcus of Type I maintained at high virulence by continuous passage through these animals. The corresponding immune serum was prepared in the horse and kindly supplied by the Hospital of The Rockefeller Institute.

The virulence of the culture was not accurately titrated. It was such, however, that one drop of the blood of a rabbit succumbing to infection sufficed to kill in twelve hours a rabbit weighing two kilos. The pneumococci were grown in beef infusion broth and were eighteen to twenty-four hours old when used. When large quantities were to be injected the bacteria were thrown out of the culture in the centrifuge, the greater part of the fluid was poured off, and the remainder suspended for injection. The bacteria were not washed. The injections both of bacteria and serum were made intravenously.

**Experiment 1. Effect of Large Serum Injection in Rabbits Suffering from Severe Pneumococcus Septicemia.**

**Rabbit A.**—Weight 1,800 gm. 0.1 cc. of culture given. 8 hours later the blood contained large numbers of pneumococci. 4 cc. of immune serum injected. The animal died within 1 minute of respiratory failure.

**Autopsy.**—Large clumps of pneumococci in the heart's blood; the lungs were distended, and many clumps of bacteria were in the vessels; clumps of bacteria also in the vessels of the choroid plexus (Figs. 1 and 2).

**Rabbit B.**—Weight 1,600 gm. 0.1 cc. of heart's blood of a rabbit just dead of pneumococcus septicemia given. 6 hours later the blood revealed the existence of a severe septicemia with the diplococci uniformly scattered throughout. 2 cc. of immune serum were injected and films from the heart's blood were made every minute for 3 minutes.

The immediate effect of the serum was to produce collapse of the animal attended by labored respiration and urination. The 1 minute film showed many large clumps of bacteria, the 2 minute fewer clumps, and the 3 minute few small clumps. The rabbit survived, temporarily recovered, but died 6 hours later. The blood and organs contained many pneumococci.

**Rabbit C.**—Weight 2,200 gm. The sediment from 50 cc. of a bouillon culture injected. 2 minutes later 10 cc. of immune serum given in opposite ear vein. The blood was quickly cleared of the pneumococci. Death occurred after 12 hours.
Autopsy.—Lungs edematous, emphysematous, and hemorrhagic. Small hemorrhages in surface of the kidneys and peritoneal surface of the intestines. The blood, lungs, spleen, and liver contained very great numbers of pneumococci.

Rabbit D.—Weight 2,300 gm. The sediment from 50 cc. of bouillon culture injected. No further treatment. Death in 4½ hours. The blood and organs contained very many pneumococci.

The experiments indicate that when the blood of the rabbit contains a very large number of pneumococci the intravenous injection of a large amount of immune serum causes certain definite effects. In the first place, the effect may be to cause almost immediate death. This accident results from the rapid agglutination in large clumps of the pneumococci in the circulation and the massing of the clumps in the lungs and brain where, acting as emboli, they produce respiratory failure and death. In the second place, the blood may be temporarily cleared of the bacteria and life be prolonged. Finally, however, the life of the animal is not spared, as the bacteria reinvade the blood and cause death.

From the foregoing it appears that the formation of large clumps is a disadvantage to the animal, since they tend to be held back chiefly in the lungs, the circulation of which they obstruct; and, besides, the large bacterial masses are not readily phagocytized.

These disadvantages can be avoided by the injection of small quantities of immune serum which produce clumps containing twenty to thirty pneumococci and to their quite regular distribution in the lungs, liver, and spleen, where they come under the influence of phagocytes under favorable conditions. Once removed from the circulation and lodged in the organs, the effect of a larger quantity of serum was studied.

Experiment 2. Effect of First Small and Later Larger Doses of Immune Serum in Pneumococcus Septicemia.

Rabbit D.—Weight 1,800 gm. The sediment from 75 cc. of a bouillon culture given. Immediately 0.5 cc. of immune serum injected into opposite ear vein. At the examination 25 minutes later there were no bacteria in the blood. 5 cc. of immune serum injected. Animal showed no symptoms for 2 hours, after which symptoms appeared, and death occurred at end of 5 hours.

Autopsy.—The lungs were edematous and hemorrhagic. Hemorrhages in surface of kidneys and peritoneal coat of intestine. The blood was free of diplococci and very few were found in the spleen, lungs, and liver. Almost all had been destroyed.

Treatment of Pneumococcal Septicemia in Rabbits.

The experiment recorded under Rabbit A was repeated several times but without changing the end result. In spite of the practical destruction of the pneumococci, death quickly resulted. The appearance of the organs, and especially the hemorrhages, were taken to indicate a severe grade of intoxication, and this effect was provisionally related to the large second dose of serum leading to too rapid disintegration of the diplococci. Hence it was decided to bring about a more gradual disintegration of the bacteria if possible.

Experiment 3. Effect of Repeated Small Doses of Immune Serum in Pneumococcal Septicemia.

Rabbit C.—Weight 2,000 gm. The sediment from 150 cc. of a bouillon culture given. Two minutes later 0.5 cc. of immune serum injected. At the end of 30 minutes the bacteria had left the blood and 1 cc. of the serum was administered. From now on 1 cc. of the serum was administered every 2d hour. Although symptoms appeared at the end of the first 2 hours they disappeared and the rabbit was in good condition until the 22d hour, when restlessness and rapid respiration followed by rigidity of hind leg developed. Later opisthotonos supervened. A lumbar puncture yielded cerebrospinal fluid containing many diplococci. Death occurred in the 28th hour.

Autopsy.—Blood, spleen, liver, and lungs were free of bacteria. The meninges were inflamed and contained very large numbers of pneumococci. Death obviously resulted from meningitis.

Rabbit D.—The sediment from 100 cc. of bouillon culture was given. No treatment. Died in 4 hours. The blood and organs contained very large numbers of diplococci.

The experiment described in Rabbit C was made several times but always with the same final result. The success of the experiment was frustrated by the intervention of the meningitis. The possibility is not excluded that this complication may itself be prevented by bringing a proper dose of the immune serum into the meninges which are not reached from the circulating blood. The effect of reducing the infecting dose of bacteria was next tried. The sediments from 75 cc. and from 50 cc. of bouillon culture only delayed the onset of meningitis. The sediments from 35 cc. of culture gave partially successful results.


Experiment 4.

4 rabbits weighing from 1,900 to 2,200 gm. were given the sediment from 35 cc. of bouillon cultures. 2 minutes later 0.5 cc. of immune serum were injected into the opposite ear vein. 30 minutes later and every 2d hour thereafter 1 cc. of the serum was given. This was continued up to the 56th hour. The final results are shown in Table I.

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td>Right hind leg paralyzed</td>
</tr>
<tr>
<td>83</td>
<td>Both hind legs completely paralyzed; severe opisthotonos; very restless and excitable</td>
</tr>
<tr>
<td>96</td>
<td>Comatose; lying on side; breathing labored</td>
</tr>
<tr>
<td>122</td>
<td>Died</td>
</tr>
</tbody>
</table>

Autopsy.—Brain and cord intensely injected. Smears from surface of brain and lateral ventricles showed very many pneumococci. No pleurisy, no pericarditis, Blood, lungs, liver, and spleen free of bacteria

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Diarrhea</td>
</tr>
<tr>
<td>24</td>
<td>Diarrhea improved. Rabbit showed no further symptoms</td>
</tr>
<tr>
<td>48</td>
<td>Serum agglutinates pneumococci in a dilution of 1:150</td>
</tr>
<tr>
<td>3</td>
<td>Agglutinating titer of serum, 1:125</td>
</tr>
<tr>
<td>6</td>
<td>Living and in perfect condition</td>
</tr>
<tr>
<td>50</td>
<td>Both forelegs paralyzed.</td>
</tr>
<tr>
<td>53</td>
<td>Severe opisthotonos, restless, excitable, breathing labored.</td>
</tr>
<tr>
<td>53</td>
<td>Comatose; lying on side.</td>
</tr>
<tr>
<td>60</td>
<td>Died.</td>
</tr>
<tr>
<td>80</td>
<td>Autopsy.—Same as Rabbit 1.</td>
</tr>
</tbody>
</table>

Experiment 4 shows that it is possible by employing properly graduated doses of an immune serum to cure rabbits of a massive pneumococcus infection. The experiment indicates also that it is only the chance diplococci excluded from the influence of the circulating immune serum which escape destruction and multiply. It is of interest to know that the probability of the pneumococci escaping the destructive action of the serum is reduced by one-half by diminishing the infecting dose to a point which is, however, still massive according to the grade of virulence of the strain of pneumococcus employed.

Experiment 5. A Comparison of the Three Methods of Treatment Employed.

2 rabbits were used for each method. The infecting dose was the sediment from 20 cc. of bouillon cultures. 2 rabbits were treated, as in Experiment 4,
with frequent small doses of serum; both recovered. 2 were treated first with a small dose of serum and then with a large dose. They died. 2 were given one large dose of serum immediately after the infection; both died of septicemia.

DISCUSSION.

The experimental data presented in the foregoing pages should be considered from several points of view: from their bearing on experimental pneumococcus infection in the rabbit, and from their bearing on the theory of the anti-infectious action of immune serum in general.

The most striking fact regarding the experimental pneumococcus infection is the one that a single large dose of the immune serum given at the beginning of the infection is far less effective in overthrowing the infection than small repeated doses of which the maximal amount may not equal the single large quantity unsuccessfully employed.

The reason for this disparity has also been made clear in large part. The serum does not protect by a process of neutralization of a true toxin as antitubercular serum does; or if any neutralization occurs it is a minor, and not the decisive process. The anti-infectious serum performs two things: it brings about an agglutination of the bacteria, and it prepares them for phagocytosis in the organs. Hence, whatever favors this process will be beneficial, and whatever hinders it will be detrimental. A certain concentration of the serum likewise promotes the assembling of the leucocytes in the organs. On the other hand, higher concentration causes the formation of such large clumps as to escape phagocytosis. The free bacteria then quickly multiply, escape into the blood, and cause fatal infection. An excess of serum acts disadvantageously in another way not yet explained. Even when, through a small dose of serum, the small clumps have been formed in the blood and removed by the organs, a large following dose of the serum brings about a fatal issue. In this instance, the bacteria do not begin to multiply and invade the organs. The blood and the organs may be quite or nearly sterile. Death appears to result from intoxication. But just how the serum acts in producing the intoxication has not been determined.

When the pneumococci have all been destroyed in the organs

\footnote{Bull, loc. cit.}
through the operation of the small doses of the serum repeated at intervals, only part of the rabbits are saved. The effect of the serum thus administered is clearly to provoke the destruction of the bacteria under conditions which avoid intoxication. But only those pneumococci are destroyed and rendered harmless that come under the direct influence of the serum which can reach all essential parts of the body except one; namely, the subdural space. When, under any circumstances, the pneumococci reach the subdural space they are not restrained by the treatment but develop rapidly and in great numbers and thus cause a fatal meningitis. The conditions leading to the meningitis are two: (a) large dosage and (b) survival for a sufficiently long period of time. The dose must be larger than the small doses of serum capable of destroying in a certain time period, and the animal must survive even the large doses about 20 hours in order that the meninges may become infected.

But the quantity of virulent pneumococci which rabbits can be made to support under the influence of the method of repeated serum injections is still so very large that the question may be raised whether the limits of activity of this anti-infectious serum have not been greatly underestimated. It remains, of course, to be determined whether still other anti-infectious sera are capable of having their powers enhanced by a similar method of administration. In any case, the subject is one that calls for restudy and perhaps revision.

On the other hand, the experiments affirm nothing as to the efficacy of the method in the serum treatment of lobar pneumonia in man. It may be supposed that so far as the pneumococci in the circulating blood in lobar pneumonia are concerned, small doses of the serum would suffice to bring about their removal. What effect the small doses might have upon the pneumococci in the lungs cannot be predicted. But since the method is one that is readily carried out in man it will doubtless receive attention in due time.

**SUMMARY.**

The treatment of pneumococcal septicemia in the rabbits by large doses of immune serum is detrimental, since the serum causes the
formation of large clumps of bacteria in the blood which are taken out chiefly by the vessels of the lungs in which they accumulate and impede the circulation.

The large doses of serum are also detrimental when they follow upon small ones through which the small clumps formed are deposited in the spleen, liver, and other organs. In this instance, the large amount of serum leads to the destruction of the pneumococci under conditions which promote an intoxication. The precise mechanism of this action is not known.

The treatment of pneumococcic septicemia in rabbits by small repeated doses of immune serum can be successfully carried out. The number of pneumococci capable of being brought to destruction through phagocytosis in the organs in this way is very great.

Not all the rabbits treated with small repeated doses of the serum survive. Those that succumb do so not to a general infection but to a pneumococcus meningitis. The explanation of this phenomenon is simple. When the number of pneumococci originally inoculated is very great a small number penetrate into the subdural space. Those in this space do not come under the influence of the serum, hence they are not agglutinated and prepared for phagocytosis, whence they multiply and set up a fatal meningitis.

The activity of the immune serum administered in this way against virulent pneumococci is so great that a revision of our notions in the limit of powers of the anti-infectious sera seems necessary. It is patent that the problem is not simply a relation between quantity of immune bodies and number of bacteria. It is more complex than that conception indicates. The factor of the leucocytes and the degree of their possible activities under the conditions of the experiment come into play. Hereafter, in defining the mode and power of action of anti-infectious sera the condition of cooperation of the body-forces will have to be more strictly considered.

EXPLANATION OF PLATE 53.

Fig. 1. A large mass of pneumococci in a blood vessel. Tissue was taken from the region of the choroid plexus of a rabbit dying 1 minute after receiving 3 cc. of immune serum. The rabbit had a severe pneumococcus septicemia when the serum was given.

Fig. 2. A large clump of pneumococci. Preparation from the lung of the rabbit described in Fig. 1.
(Bull: Treatment of Pneumococcal Septicemia in Rabbits.)