THE AGGLUTINATION OF BACTERIA IN VIVO.

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Among the factors to which bacterial immunity is ascribed the phenomenon of agglutination occupies the most subordinate place. Indeed, the general view held by immunologists is to the effect that however valuable agglutination may be in vitro in identifying bacteria or discovering specific forms of infection, yet the process plays no essential part in the protection of animals as such. The appearance of agglutinins in the sera or body fluids of infected individuals or inoculated animals is considered a sign of the existence of a greater or less degree of immunity; the agglutinins themselves being merely incidental accompaniments of the true immunity factors—lysins, bacteriotropins, and antitoxins. We have, however, made certain observations within the last few months which indicate that agglutinins play quite an important part in at least certain instances of active and passive immunity.

In reporting his classical work on the specificity and practical application of the phenomenon of agglutination, Gruber expressed the opinion that agglutinins were quite essential properties of an immune serum. He believed that the phenomenon of agglutination was due to the fact that the agglutinins (Gläbrificine) increased the viscosity of the bacterial bodies and caused them to adhere to one another. Gruber advanced the idea also that this increased viscosity aided in the englobement of the bacteria by the phagocytes, and would probably explain the accumulation of bacteria in the organs when they were injected into the circulation. Gruber's hypothesis has never been further developed or proved by himself or others.

Metchnikoff contends that agglutinins play no rôle, however small, in active or passive immunity processes. "The phenomenon of agglutination is of no great importance from the point of view of natural immunity." We have already given the arguments which render it impossible for us to attribute to the agglutinative property of the fluids of the body any rôle, however unimportant.

2 Metchnikoff, E., Immunity in Infective Diseases, Cambridge, 1905.
3 Metchnikoff, loc. cit.
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in natural immunity against micro-organisms. "4 "The part played by agglutination in this immunity (acquired) is merely accidental and subordinate."5

After extensive investigation, Salimbeni6 concluded that bacterial agglutination never takes place within the animal organism. This author worked with animals immunized to the cholera vibrio. The sera of the animals were strongly agglutinative in vitro, but he was unable to find any evidence indicative of agglutination in vivo.

Sawtschenko and Melkich7 believed that agglutinins were present and active in the plasma of cases of recurrent fever. They found small clumps of spirochaetes in blood immediately after its removal from the patients. It was suggested by them that the agglutination in the plasma was incomplete because of the rapid movement of the blood.

A glance at the literature given above readily convinces one of the slight consideration agglutinins as protective agents have received. Gruber's suggestions seem to have gone by as such and Sawtschenko's observations have not been considered sufficient proof of the functioning of agglutinins in vivo. Salimbeni has done the most direct and extensive work on this subject and his results led him to conclude that agglutination is strictly a phenomenon of the test-tube. And Metchnikoff is quite firmly convinced of the unimportance of agglutinins in both natural and acquired immunity. Our observations made in a more or less incidental manner seem to justify a different conception of agglutination as an immunity factor, for which reason they have been put together in this paper.

Pneumococci and Agglutination in Vivo.

In the course of the study of the manner of the rapid disappearance of pneumococci from the blood stream of rabbits following the injection of antipneumococcus serum the observation was made that an almost instantaneous agglutination of the pneumococci was produced in the blood by the serum introduced and, furthermore, that the clumped or massed cocci quickly accumulated in the internal organs.8

These facts in themselves are not only new, as is evident from the literature previously quoted, but they are important as bearing on our conception of the biological processes which come into play in the course of bacterial immunity. Aside from this consideration, however, the facts are of interest in connection with the following points: (1) the time required for the reaction to take place; (2) the

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4 Metchnikoff, loc. cit., p. 258.
5 Metchnikoff, loc. cit., p. 263.
7 Sawtschenko and Melkich, Ann. de l'Inst. Pasteur, 1901, xv, 497.
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degree of its specificity; (3) the concentration of serum necessary to effect agglutination; and (4) the relation of the quantity of serum employed to the course of a bacterial septicemia.

EXPERIMENTAL.

When the agglutination tests are made in test-tubes it is customary to incubate the tubes for two hours at 37° C. and then to set them aside at a low temperature for another period of several hours before the results are read.

In contradistinction to the slowness with which agglutination occurs and becomes evident in vitro is its instantaneous occurrence in vivo. To demonstrate this point we have proceeded as follows: The bacteria (pneumococci) from 50 cc. of bouillon are injected into the ear vein of a rabbit, after which a specimen of blood is taken from the heart to ascertain if sufficient bacteria are present to be easily found with the microscope and to determine the absence of clumps. The antiserum is then injected and a specimen of blood taken from the heart not later than thirty seconds afterwards. Other specimens are taken at one, two, three, five, and ten minute intervals. The sample of blood removed at twenty seconds usually contains the largest and greatest number of clumps. The second sample may also show many clumps of fair size, but the later samples show only a few small clumps. As a rule, no clumps or free bacteria whatever can be found at the expiration of five or ten minutes, particularly if from 1 to 2 cc. of serum have been injected.

Why bacteria agglutinate so much more rapidly in vivo than in vitro is not evident. The temperature conditions do not offer a satisfactory explanation, because the reaction does not occur in the test-tube immediately after body temperature is reached. The effect of the agitation caused by the circulation of the blood is not known, but would not seem to suffice to produce the great differences noted. It is quite possible that some constituent of the blood of the host aids the specific serum in producing those changes in the bacterial bodies which precede agglutination, but of this we are wholly ignorant.

The specificity of antipneumococcic agglutinins is as strict in vivo as in vitro. A heterologous serum causes no agglutination in a
concentration fifty times as great as that in which a homologous serum gives a positive reaction.

In following the reaction quantitatively in vivo we found that quantities of serum as small as 0.05 cc. per kilo of body weight of the rabbit caused the formation of small clumps, and that that quantity was the least amount that exercised any appreciable influence on the course of the septicemia or sufficed to prolong the life of the inoculated animals. Since the agglutinating titer of the antiserum employed was 1 to 50 by the macroscopic method, this result was wholly unexpected. But the result was rendered more comprehensive when it was ascertained that by merely modifying the method somewhat the titer could be increased to 1 to 500. Thus, when from 0.08 to 0.1 cc. of a bouillon culture of the pneumococci is added to 1 cc. of the diluted serum, clumps are formed which may be large enough to be seen by the naked eye, provided the fluids are clear. Stained preparations viewed with the microscope also reveal the clumps. But even so, agglutination within the body seems to take place with dilutions of the serum which are wholly ineffective in the test-tube.

**Typhoid Bacilli and Agglutination in Vivo.**

When typhoid bacilli are injected into the circulation of normal rabbits, they quickly leave the blood. The precise manner of the disappearance has not been investigated. Because the typhoid bacilli are subject to lysis it has been supposed that they are in fact disintegrated.

When, however, the blood was examined as described in connection with the pneumococci it was learned that the bacilli agglutinate within a few seconds after entering the blood stream. The rapidity with which agglutination takes place is affected by the power of the serum to cause agglutination in the test-tube. With rabbits whose blood does not possess this power a single bleeding could be performed before clumps were formed; in all others, agglutination had taken place within the brief period of thirty seconds.

The heating of typhoid bacilli to 80° C. for half an hour rendered them more readily agglutinable in vivo than unheated bacilli. While bacilli heated to from 65° to 80° C. proved inagglutinable in test-
tubes, our experience here confirmed the observation of others.\textsuperscript{9} In this respect, therefore, agglutination \textit{in vivo} differs from that \textit{in vitro}.

\textit{Dysentery Bacilli and Agglutination in Vivo.}

As a rule, normal rabbit serum does not agglutinate \textit{in vitro} in dilutions of 1 to 10 any of the varieties of dysentery bacilli. The sera of all the rabbits we tested were inactive in this dilution. We employed the following strains: Shiga, Strong, Hiss, and Flexner.

The strains of the Shiga type do not agglutinate and remained in the blood for twenty minutes distributed uniformly; while the other strains undergo immediate agglutination, clumps being found twenty seconds after the bacteria were injected. By means of this reaction it was possible to determine within two minutes whether the culture tested belonged to the Shiga or Flexner groups of dysentery bacilli.

The fate of the dysentery bacilli could also be followed. When the rabbits are killed as early as seven minutes after receiving an inoculation of one of the Flexner group of bacilli, large numbers of leucocytes carrying clumps of one hundred or more bacilli were already present in the lungs, liver, and spleen.

When an immune serum for the Shiga type of bacillus is injected into the circulation the Shiga bacilli, which otherwise do not agglutinate, become immediately clumped. The distinction, therefore, in the behavior of the Shiga and the Flexner groups depends on the presence in the normal rabbits of agglutinins for the one and not for the other group.

\textit{Bacillus influenzae and Agglutination in Vivo.}

A non-virulent strain of \textit{Bacillus influenzae} isolated from the respiratory tract agglutinated in the circulation of normal rabbits one minute after injection. A virulent strain isolated from a case of influenzal meningitis was not agglutinated and was still in the circulating blood twenty minutes after injection. The distinction of virulent and non-virulent strains is determined by the fact that the former causes a fatal septicemia in young rabbits, while the latter does not.\textsuperscript{10}

\textsuperscript{9} Porges, O., \textit{Ztschr. f. exper. Path. u. Therap.}, 1905, i, 621.
DISCUSSION.

The results described in the preceding pages emphasize the occurrence as well as the significance of agglutination of bacteria in the blood once they have gained access to the circulation.

In the first place, the power of the blood in normal animals to cause agglutination determines, apparently, in large measure whether the bacteria are to be promptly removed and septicemia avoided or to remain and to bring about without restraint that serious condition. The importance of this power as far as the normal rabbit is concerned is well illustrated by the examples afforded, on the one hand, by pneumococci, dysentery bacilli of the Shiga type, and virulent influenza bacilli; and, on the other hand, by typhoid bacilli, dysentery bacilli of the Flexner group, and non-virulent influenza bacilli.

The normal blood of the rabbit fails to agglutinate the Shiga dysentery bacilli outside (in vitro) or even inside the circulation, with the result that they are not converted into clumps in the bloodstream and hence are not promptly removed from it, and while the normal blood does not agglutinate the Flexner group of dysentery bacilli in the test-tube in a 1 to 10 dilution even after two hours’ incubation at 37° C., it does agglutinate them immediately in the circulation, whence they are quickly removed. Similarly, the circulating blood of the rabbit does not agglutinate pneumococci or the virulent form of influenza bacilli, with the result that they remain and multiply there, leading to a septicemia to which the animal succumbs; while typhoid bacilli and the non-virulent form of influenza bacilli are instantly agglutinated and promptly removed from the circulation.

In view of these facts we can discern in the phenomenon of agglutination in the blood an essential mechanism through which bacteria are so changed that they accumulate in the capillaries and sinuses of the viscera; and perceive in this mechanism the decisive act which determines whether protection is to be afforded or fatal septicemia supervene.

Indeed, the facts at hand illuminate the mechanism of protection from infection even further. It would appear that in the instances given the bacteria neither accumulate in the organs to any degree
when unagglutinated nor are they taken up by the leucocytes within
the circulation when they remain there. Moreover, those bacteria
which enter the organs before agglutination takes place remain in
the single form and escape phagocytosis. When, however, agglu-
tination has taken place the bacteria are quickly removed from the
circulation and are englobed by leucocytes in the lungs, liver, spleen,
and other organs, and, as may be inferred from the rapidity with
which they are disintegrated in the phagocytes as compared with
their persistence when free, are quickly destroyed, and possibly
finally detoxicated. In other words, in order that the bacteria may
be promptly removed from the blood stream it is requisite that they
be first agglutinated, which condition is also required in order that
they be destroyed en masse within the organs, a process achieved,
apparently, chiefly through phagocytosis.

This phenomenon of protection in the normal animal is paralleled
by what happens when an effective antiserum is employed to pre-
vent or combat a bacterial infection, as is illustrated by the ex-
amples given of serum protection in pneumococcus and Shiga bacil-
lus infection in the rabbit. It may be inferred that similar processes
occur in other infections and in other animals, including man, but
many more particular cases will need to be investigated before gen-
eral deductions are made.

**SUMMARY.**

1. Small quantities of antiserum bring about instantaneous ag-
glutination of pneumococci in the circulation of the rabbit; the re-
action is specific and occurs in every case in which sufficient serum
is given to influence the course of the septicemia or to prolong the
life of the animal.

2. The agglutinating titer of antipneumococcus serum can be
made considerably higher by adding only a small quantity of cul-
ture to the tests, thus making the test a finer differential.

3. Typhoid bacilli agglutinate spontaneously in the circulation
of the normal rabbit; the reaction is positive in vivo even in cases
in which undiluted serum gives a negative result in vitro; heating
the bacilli to 80° C. for thirty minutes renders them more agglu-
tinable in vivo.
4. Dysentery bacilli of the Shiga type do not agglutinate in the blood stream of the normal rabbit, but a small quantity of antiserum injected into the circulation causes immediate agglutination; while all strains of the Flexner group undergo spontaneous agglutination.

5. Non-virulent influenza bacilli agglutinate spontaneously in the circulation of the normal rabbit; virulent strains remain in the blood unclumped.

6. In all instances so far investigated of both passive and natural immunity, agglutination of the bacteria within the blood of the infected animal was followed by a rapid removal of the bacteria from the circulation, and by phagocytosis and destruction of the agglutinated bacteria in the capillary systems of the viscera; while those bacteria which are not agglutinated remain in the circulation and produce a progressive septicemia.

7. Hence the agglutinins seem to play the decisive part in at least certain instances of bacterial infections.