OBSERVATIONS BEARING ON THE QUESTION OF THE INFLUENCE WHICH IS EXERTED BY THE AGGLUTININS IN THE INFECTED ORGANISM.

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THE question as to how far the production of specific agglutinating and sedimentary substances stands in relation to the process of immunisation is a question which is manifestly of the highest moment. That there must be some relation between the production of agglutinins and the production of immunity appears certain, not only from the fact that bacteria undergo distortion and become immobilised under the influence of their corresponding agglutinins, but also from the fact that the bacteria in question are inhibited in their growth when they are transferred to a highly agglutinative culture medium. If nothing else had been elicited concerning the agglutinins, these facts would of themselves suffice to show that agglutinins are "bacteriotropic"¹ and anti-bacterial substances.

About this particular point, therefore, there would appear to be no controversy. Widely divergent views have, however, been expressed on the question as to how far the agglutinins exert an influence in warding off a bacterial invasion and in restraining the growth of such bacteria as may before the production of agglutinins have established themselves in the interior of the organism. We do not propose here to enter into consideration of all the arguments which have been advanced with the view of showing that no sensible influence in restraining the growth of bacteria is exerted by agglutinins in the organism. We propose here to confine ourselves to the consideration of the argument that the agglutinins must be inoperative *in vivo*, inasmuch as the bacillus typhosus or the micrococcus melitensis, as the case may be, continues to cultivate itself in the organism long subsequent to the appearance of agglutinins in the blood. We may begin by pointing out that the conclusion that the agglutinins are inoperative *in vivo* is forced upon us only if it can be established for a certainty that the micro-organisms which cultivate themselves in the organism are, as a matter of fact, cultivating themselves in the presence of agglutinins. Now this is a question which is capable of being resolved by a quantitative determination of the amount of agglutinins in the organs in which the micro-organisms are cultivating themselves. The observations which we have made in connexion with this question are as follows.

CASE 1.—The patient had a very severe attack of typhoid fever and died during the third week. At the necropsy, held 24 hours² after death, the typhoid bacillus was isolated in pure culture from the spleen. 12 grammes of splenic substance were extracted with an equal weight of normal saline solution. The amount of agglutinating substance in the clear filtrate which was obtained from the above was quantitatively determined in sedimentation tubes by the method which was described by one of us in the *British Medical Journal* of Feb. 5th, 1897. At the same time the agglutinating power of the heart-blood was determined in

the same manner. The results may be tabulated exactly as follows:—

—	Dilutions.					
	4-fold.	6-fold.	12-fold.	16-fold.	20-fold.	100-fold.
Serum of heart-blood ...	Complete.	Complete.	Complete.	Complete.	Complete.	Incomplete.
Serum of spleen	Trace.	Trace.	Nil.	Nil.	Nil.	Nil.

NOTE.—A slight inaccuracy has been admitted into these and all the subsequent records inasmuch as the 12 grammes of spleen which were employed for this experiment are treated here as if they had been the equivalent of 12 grammes of splenic serum. They were in reality the equivalent of only 10 grammes of splenic serum, inasmuch as the solid substances of the spleen amount to about one-sixth of its weight. The difference is manifestly one which is not worth taking into account in view of the considerable differences which are revealed in the tables of results.

CASE 2.—The patient had a typical attack of typhoid fever. He succumbed about the end of the fourth week. At the necropsy, held 24 hours after death, the typhoid bacillus was obtained in pure culture from the spleen. The agglutinins in the heart-blood and in the spleen were estimated in the same manner as in the first case. The following were the results:—

—	Dilutions.					
	10-fold.	20-fold.	40-fold.	100-fold.	150-fold.	200-fold.
Serum of heart-blood ...	Complete.	Complete.	Complete.	Complete.	Complete.	Incomplete.
Serum of spleen	Incomplete.	Trace.	Trace.	Nil.	Nil.	Nil.

CASE 3.—The patient died after a short typical attack of typhoid fever. At the necropsy, which took place 20 hours after death, Peyer's patches were found to be much swollen and congested. They were not ulcerated. The bacillus typhosus was recovered in pure culture from the spleen. The agglutinins were quantitatively estimated (by the same methods as were employed above) in the heart-blood, spleen, and in the Peyer's patches. The estimation of the agglutinins in the spleen miscarried through an accident. The results obtained on the heart-blood and in the extract from the Peyer's patches are given below:—

—	Dilutions.					
	8-fold.	16-fold.	20-fold.	50-fold.	80-fold.	100-fold.
Serum of heart-blood ...	Complete.	Complete.	Complete.	Complete.	Complete.	Incomplete.
Serum from Peyer's patches	Incomplete.	Traces.	Nil.	Nil.	Nil.	Nil.

CASE 4.—The patient suffered from a continued fever. This fever was diagnosed as typhoid during life only by the agglutination reaction. Death occurred suddenly five days after the fall of temperature. At the necropsy, which was conducted 24 hours after death, the bacillus typhosus was obtained in pure culture from the spleen, which was absolutely normal in appearance. The agglutinins in the blood and spleen were estimated as above. The following are the results:—

—	Dilutions.				
	4-fold.	8-fold.	16-fold.	20-fold.	50-fold.
Serum of heart-blood ...	Complete.	Complete.	Complete.	Complete.	Complete.
Serum of spleen ...	Trace.	Trace.	Trace.	Nil.	Nil.

—	Dilutions.			
	100-fold.	150-fold.	200-fold.	300-fold.
Serum of heart-blood ...	Complete.	Incomplete.	Incomplete.	Traces.
Serum of spleen ...	Nil.	Nil.	Nil.	Nil.

¹ The term "bacteriotropic" which is here employed is formed upon the analogy of the terms "neurotropic," &c., which have been introduced by Ehrlich. The affix "tropic" as thus used signifies the property of "turning towards" and of entering into chemical composition with.

² In view of the fact that this and all the subsequent observations were made in the winter months, when the temperature of the dead house was very low—so low, in fact, that it held in check the growth of all putrefactive micro-organisms—it would appear justifiable to assume that the results which are exhibited above cannot have been due to any post-mortem cultivation of typhoid bacilli in the spleen. Vide also in this connexion note to Observations 5 and 6.

CASE 5.—The patient succumbed to a severe attack of Malta fever which ran a comparatively short course. Death took place in the third week of the disease. A necropsy was made 12 hours after death.³ The micrococcus melitensis was obtained in pure culture both from heart-blood and spleen. It was obtained in small quantities from the former and in relatively large quantities from the latter. The estimation of the agglutinins in heart-blood and spleen was carried out in the same manner as in previous cases. The results were as under :—

	Dilutions.					
	4-fold.	10-fold.	20-fold.	50-fold.	100-fold.	200-fold.
Serum of heart-blood	Com-plete.	Com-plete.	Com-plete.	Com-plete.	Com-plete.	Incom-plete.
Serum of spleen	Traces.	Nil.	Nil.	Nil.	Nil.	Nil.

CASE 6.—The patient suffered from a severe and long-continued attack of Malta fever. Death took place five and a half months after the commencement of the disease. At the necropsy, conducted 18 hours after death, the micrococcus melitensis was obtained in pure culture from spleen and heart-blood. The bacteria in the blood were few, while they were plentiful in the spleen. The estimation of the agglutinins which was conducted as above gave the following results :—

	Dilutions.						
	5-fold.	10-fold.	20-fold.	50-fold.	100-fold.	200-fold.	400-fold.
Serum of heart-blood ...	Com-plete.	Com-plete.	Com-plete.	Com-plete.	Incom-plete.	Nil.	Nil.
Serum of spleen	Nil.	Nil.	Nil.	—	—	—	—

The above observations would appear clearly to establish that the spleen in the case of typhoid and Malta fever is much poorer in agglutinating substances than the circulating blood. So far as the spleen in typhoid fever is concerned our observations merely confirm what had previously been established by Courmont in the course of a research which he instituted with a view to determining the distribution of agglutinins in various parts of the body. The research in question showed that in nine cases of typhoid fever examined post mortem the agglutinating power of the spleen juice was in every case much less than that of the heart-blood. This fact, which was arrived at by us independently in following up an entirely different line of inquiry, seems to us to acquire a fundamental importance in view of its bearing on the question of the effect exerted by the agglutinins *in vivo*.

We may therefore proceed to consider the general bearing of the conclusion arrived at above, that the micro-organisms of typhoid and Malta fever cultivate themselves in the interior of the infected organism in a medium which is relatively poor in agglutinins. We may consider in this connexion first the case in which a relatively large number of bacteria are introduced into the subcutaneous tissue of a man or animal whose blood and lymph contain sensible quantities of agglutinins.

Before we embark upon this question we shall do well to place before ourselves two facts. First, we must note that when we introduce bacteria into an agglutinative medium we effect a corresponding reduction in the agglutinative power

of that medium inasmuch as the agglutinins enter into chemical combination with the bacteria. Secondly, we must note that when the mass effect of the agglutinins in any medium is reduced beyond a certain minimum an agglutinative effect is no longer exerted by that medium. A plasma or lymph whose agglutinative power is reduced below a certain minimum⁴ may therefore for our present purposes be regarded as a non-agglutinative fluid.

Keeping these points in view we are now in position to consider what will probably happen when a large number of bacteria are subcutaneously introduced into an organism which contains agglutinins. Here and throughout this paper we may, in considering the particular case of the agglutinins, bear in mind that we may draw inferences from what happens in the case of these to what will happen in the case of other anti-bacterial substances. It will be manifest that the bacteria when subcutaneously introduced will come in contact in the first instance only with the agglutinins which are contained in that quantum of lymph which occupies the meshes of that portion of the subcutaneous tissue which is the seat of infection. If now the bacteria have been injected in sufficient quantities to abstract from the lymph all or what for our purpose amount to all the agglutinins that lymph will be converted into a non-agglutinative medium. Within the non-agglutinative envelope which will thus have been formed such of the bacteria as have not been chemically affected by the agglutinins will be able to cultivate themselves perfectly unchecked, so far at least as any influence on the part of the blood is concerned. It may be urged that, seeing that the agglutinins which have been abstracted will be replaced either by the diffusion of others from the blood into the lymph or, failing that, by the transudation through the capillary wall of an agglutinative lymph, it will very soon come about that the bacteria in the tissue will again be exposed to the full “bacteriotropic pressure” of the blood. But consideration will show that where the bacteria have been introduced in relatively considerable quantities and where the agglutinative power of the blood is relatively low the passage of agglutinins outwards from the blood into the lymph will not under ordinary circumstances⁵ suffice to keep up the agglutinative power of the lymph. For agglutinins will be abstracted from the lymph *pari-passu* with the increase of the bacteria which will be taking place.

At this stage we may pause for a moment to consider the bearing of these considerations on the prognosis and therapeutics of local bacterial invasions. First with regard to prognosis. The prognosis will, in conformity with the above considerations, be *pro tanto* good wherever the seat of infection becomes hot. For this local rise of temperature and the bright red colouration will be indicative of an increased afflux of arterial blood and of a correspondingly increased lymph-flow. Under conditions such as these the bacteria will not readily find an opportunity of establishing round themselves a non-bacteriotropic envelope. The prognosis will, on the contrary, be serious when local temperature falls and whenever cyanosis is developed at the seat of infection. For this will be indicative of an arrested or lessened arterial supply and of a consequent stagnation of lymph in the tissue. Under these conditions the bacteria will readily be able to establish round themselves

⁴ The minimum concentration in which a lymph or serum will agglutinate may thus and *a priori* be expected to vary according as the micro-organisms which are exposed to it are virulent or attenuated bacteria. In the case of the former variety of bacteria—i.e., in the case of the variety of bacteria which are most adapted for preserving their vitality in the interior of the animal organism—we must assume that the cohesive forces which hold together the bacterial protoplasm are relatively more powerful. They are therefore more capable of resisting the disruptive force which is exerted on the bacterial protoplasm by the chemical affinity of the agglutinins or certain elements of that protoplasm. This *a priori* deduction is in conformity with the balance of experience, which is, we think, to the effect that a greater concentration of serum is required when virulent bacteria are to be agglutinated.

⁵ We may remark in passing that if this holds true of the bacteria which lie free in the meshes of the subcutaneous tissue it will *a fortiori* hold true of bacteria which have been enclosed in a collodion bag. It does not appear to have been sufficiently realised by the observers who have employed these collodion bags in their experiments that a collodion membrane must inevitably, while it holds back the white blood-corpuscles, also restrain the lymph-stream which would otherwise be passing over the bacteria. Now it appears to us quite possible that it may be this restraint of the lymph stream rather than the holding back of the white blood-corpuscle which is the operative factor in favouring the growth of the bacteria. These same considerations would apply also to the enveloping pellets of agar which were employed by Vaillard in his well-known experiments with tetanus.

³ In view of the fact that the results here and in the next case are similar in all points to the results obtained in the case of typhoid fever it is obvious that the inference which is drawn in Note 2 above is justified. For the results which are obtained here cannot be explained as being the results of a post-mortem cultivation of the bacteria in the spleen. This interpretation of the results is inadmissible in view of the fact that the post-mortem examination was here undertaken only 12 hours after death and that we are here dealing with a micro-organism which can hardly develop at all at temperatures below 37° C.

a non-bacteriotropic envelope and within this will be able to cultivate themselves unrestrained

Next with regard to therapeutic measures. The facts which have been considered above bring out very clearly that even where a sufficiency of effective anti-bacterial substances are contained in the blood the invading bacteria may none the less gain the upper hand if these anti-bacterial substances are not poured in a continuous stream through the invaded tissue so as everywhere to envelop the invading micro-organisms. This inference is in agreement, not only with the results of experiments on animals, but with the therapeutic principle which has been established by experience in the case of man, that exposure to cold exerts a prejudicial effect by increasing the susceptibility to infection, while the application of heat, either in the form of hot fomentations or in any other form, exerts a beneficial effect on local bacterial invasion. The explanation of this prejudicial influence of cold is to be found in the fact that it arrests the lymph-stream and thus permits of the bacteria cultivating themselves in a non-bacteriotropic medium. The contrary good effect of heat is explained by assuming that it increases the lymph-stream, and that it does so is shown by its effect in raising a blister.

Having briefly glanced at these questions we may now turn back and consider what is the bearing of the fact that micro-organisms cultivate themselves in the infected organism in a non-agglutinative medium, upon the phenomena which come under observation when the bacteria, which have introduced them into the subcutaneous tissue, are carried on by the lymph-stream into the blood. We may in this connexion with advantage consider what is the probable sequence of events when at the very outset of a typhoid or Malta fever attack the bacteria are carried on into the blood. At the particular stage we may assume that in a person who has not been previously immunised the agglutinins, if they are present at all, will be present only in very small quantities. Consequently we may assume that some at least of the invading bacteria will pass through the blood-stream quite unharmed. These will, in conformity with the general law which obtains when bacteria are introduced into the blood-stream, be deposited in the spleen and other internal organs. Having definitely established a lodgment there then bacteria will grow out into colonies, and each of these colonies will as it grows establish round itself a non-agglutinative envelope. As the result of this, and we have seen that the inference has been confirmed, the spleen will as a whole contain less agglutinative substances than the circulating blood. We may further assume that as the fever develops, and as the agglutinins and other anti-bacterial substances are produced in greater quantities, a period will arrive when the agglutinins will be present in the blood in sufficient concentration to permit of their penetrating and abolishing the non-agglutinative envelopes which surround individual colonies. This done the production of toxins will be arrested and the temperature will fall.

But if when this was affected there happened to remain over somewhere in the organism, shut off, for instance, in a capillary which had become blocked or in some other part which was not freely permeated by the blood-stream or the lymph-stream, a single bacterial colony, conditions would obviously exist which might afterwards lead on either to a relapse or to secondary local inflammatory process. For the bacteria, sheltered as they would be in the interior of such a non-bacteriotropic nidus, might go on cultivating themselves there until they had, as occurs before relapses in typhoid and Malta fever, modified the blood in such a manner as to render it less agglutinative. Such is the mental picture or theory of the typhoid and Malta fever process which is suggested to us by our observations. We have endeavoured to test the theory by controlling so far as opportunity offered the deductions which can be drawn from this theory by actual observations. With a view to doing this we endeavoured to determine whether the characteristic distribution of the bacillus typhosus in the infected organism (absence of the bacillus from the circulating blood and growth of the bacillus in discrete colonies in the spleen and other internal organs and in the typhoid spots) can be satisfactorily explained in conformity with the theory. The first point which attracts attention here is the absence of the bacillus typhosus in blood and the growth of that bacillus in the spleen in the form of discrete colonies. As we have seen from the comparative estimation of the agglutinins in the blood and spleen, this distribution accords well with the assumption that the agglutinins are an effective factor in checking the growth of the bacillus. It, however, appeared

desirable further to test the correctness of this hypothesis, and it appeared that light might be thrown upon the question by determining whether in those septicæmic diseases in which bacteria ordinarily run riot all over the organism the bacteria would, after the introduction of antibacterial substances, be found to survive only in the form of discrete colonies in the organs. Opportunities for determining this point presented themselves in connexion with certain experiments which were made by one of us in conjunction with Lieutenant W. Glen Liston, I.M.S., on the effects of the introduction of Roux's anti-plague serum into the organism of plague-infected guinea-pigs. It was determined in the experiments in question that in the case of animals which had received sufficient quanta of serum such plague bacilli as survived were to be found only in discrete colonies in the organs. In the untreated control guinea-pigs the bacilli were, as usual, found in countless numbers in the circulating blood and all over the body. It would appear that the hypothesis enunciated above wins support from these observations.

With regard to the question as to whether the cultivation of the bacillus typhosus in the typhoid spots was or was not, as in the case of the cultivation of this bacillus in the spleen, to be regarded as a cultivation which is taking place in a non-agglutinative medium, it seemed possible to learn something by making comparative estimations of the agglutinating power of the blood from the finger-tip and of the mixture of blood and lymph which can be obtained by puncturing and pressing the typhoid spots. We have had only three opportunities of applying this test to the theory. In each case we made a comparative estimation of the agglutinating power of (a) the serum derived from a typhoid spot, and (b) the serum derived in the ordinary way from the finger-tip of the patient in question. The result of these comparative estimations (made in capillary sedimentation-tubes by methods employed above) were as follows:—

CASE 1.

—	Dilutions.				
	12-fold.	24-fold.	48-fold.	96-fold.	192-fold.
Serum from typhoid spots	Incomplete but marked.	Incomplete but marked.	Trace.	Nil.	Nil.
Serum from finger-tip ...	Complete.	Nearly complete.	Incomplete but very well marked.	Incomplete but very well marked.	Incomplete but distinct.

CASE 2.—Child, beginning of the Second Week of Fever; Delirious; Copious Eruption of Rose Spots; Very Marked Improvement Supervened 24 Hours After.

—	Dilutions.						
	12-fold.	24-fold.	48-fold.	96-fold.	192-fold.	384-fold.	768-fold.
Serum from typhoid spots ...	Reaction marked.	Marked.	Trace.	Nil.	Nil.	Nil.	Nil.
Serum from finger-tip ...	Reaction complete.	Complete.	Complete.	Complete.	Complete.	Complete.	Trace.

CASE 3.—Child; beginning of the Second Week of Fever; Mild Attack; Copious Eruption.

—	Dilution.				
	12-fold.	24-fold.	48-fold.	96-fold.	192-fold.
Serum from typhoid spots.	Mere trace.	Mere trace.	Mere trace.	Nil.	Nil.
Serum from finger-tip ...	Complete.	Complete.	Complete.	Trace.	Nil.

It will be seen that these observations, so far as they go, confirm the correctness of the theory enunciated.