THE LATE APPEARANCE OF AGGLUTININS IN PARATYPHOID A FEVER.

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(*Continued from p.* 152.)

The Late Appearance of Para A Agglutinins.

Previous work on the subject gives one the impression that not only are the agglutinins of para A bacillus produced in small amount but also that they disappear very early in convalescence. Thus Walker Hall [2] makes the statement: "General evidence seems to point to the fact that in the later weeks of paratyphoid fever the paratyphoid titre falls rapidly. This higher agglutination (i.e., than the inoculation agglutinins) must be read therefore in association with the early weeks of the disease." A perusal however of Hall's own records (Tables A and B) shows that the agglutinins for both para A and para B are present in relatively large amount from the fifth to the twelfth weeks, while their persistence, to judge from those tables, is more marked than his deductions would suggest. A study of the cases recorded in the present paper in relation to the time dating from the commencement of the pyrexia, at which there is the maximum production of para A agglutinins is summarized as follows:—

TABLE III.—Analysis of the Thirty-three Cases in reference to the Date of Appearance of the Highest Para A Titre.

	Series inoculated with T.A.B.	Series inoculated with T.V.
First three weeks	Nil	Nil
Fourth and fifth weeks	6	5
Sixth and seventh weeks	8	5
Eighth week onwards	5	4

It is seen that the highest para A titre in the great majority of the cases in both the T.A.B. and T.V. series occurs between the fourth to the seventh weeks inclusive. Dreyer [1] has called attention to the fact that in his series of cases the maximum agglutination is reached about the twentieth day, although he insists that in para A cases the titre is often very low. In the present records, at this stage of the disease, the agglutinins are usually present; but when the end titre is considered and when the cases are followed up into the later weeks of convalescence a much higher agglutination titre is found to be recorded. Thus in none of the present series of cases is the maximum agglutination reached earlier than the fourth week, while from the fourth to the seventh week inclusive the titre is maintained at a high level. In the several records of individual

cases of paratyphoid infections quoted in their paper by Martin and Upjohn [4], an equally high agglutination in the fourth and fifth weeks of the fever is seen to have been obtained. These observations appear to have an important practical bearing. There are doubtless many cases of slight and transient fever, due to a para A infection, in which an agglutination test within the first fifteen to twenty days has proved negative, even in low dilutions of the serum, and which owing to the indefinite clinical manifestations have been diagnosed "non-enteric." In this way a certain proportion of potential paratyphoid carriers may be missed, through reliance being placed on laboratory examinations confined to the period of pyrexia, or early convalescence, while serological investigations, if continued into later convalescence, may prove that a paratyphoid infection is present. In this connexion, the present series of cases affords several good illustrations. One of the most marked examples is that of Case 14, Table II. Here we have a clinically mild paratyphoid infection in an uninoculated subject—temperature of ten days' duration with concomitant symptoms, rose spots, etc. Agglutination reactions on the third and fourteenth days were quite negative to *B. paratyphosus* A, the figures for *B. typhosus* being ten and five units respectively. The agglutination test was repeated on the twenty-first day, when para A agglutinins were present, viz., thirty units, and on five subsequent examinations up to the eleventh week from the commencement of the illness the agglutinins for para A remained persistently present. The same delayed appearance of the specific agglutinins is seen in cases of long continued fever. For example, Case 4, Table I, is a continued fever of twenty-six days' duration which was clinically extremely suggestive of an infection of the enteric group. Examinations on the second and fourth week were completely negative to para A agglutinins, and it was only in the light of previous experience that we were led to do a later examination, when the para A titre was found to equal 140 agglutinin units and a fortnight afterwards 325 units. The organism in this case was not isolated from either blood culture or fæces. Case 17, Table I, is another case in which no para A agglutinins were obtained until the fifth week. This patient was very ill until the sixth week, for after a typical sixteen days' fever there followed a continuously intermittent temperature from the twenty-fourth to the forty-fifth day, para A being isolated from the fæces during this relapse. Such cases as these show the futility of basing any laboratory opinion on single and casual agglutination reactions in persons inoculated with typhoid or T.A.B. vaccine, while even in a series of tests the fallacy involved in a negative report unless correlated with the clinical course of the affection is sufficiently apparent.

Moreover from a statistical point of view there is the obvious error of basing comparative figures on the incidence of typhoid and paratyphoid fevers on agglutination tests done during pyrexia or early convalescence where blood cultures or bacteriological examinations of the excreta have

either been negative or neglected. This is partially due to the increase in *B. typhosus* agglutinins which precedes the production of those of para A. In the present series of cases recorded in Tables I and II this group reaction for *B. typhosus* has been a remarkably constant feature, and further demonstrates how unsatisfactory it is to base a diagnosis of the type of enteric infection on agglutination reactions within four weeks of the commencement of pyrexia. Thus to take Case 19, Table I, the agglutinins for *B. typhosus* on the twelfth day of illness equalled 222 units—a relatively high figure—while those for para A were fourteen units. In the fourth week *B. typhosus* had fallen to 22 units, while para A had increased to 57 units, and to 288 units by the fifth week. Curves showing a similar variation may be seen in Charts A, B, and E, which are taken from Cases 1, 2, and 17 of Table I. The possibility of this statistical fallacy has repeatedly revealed itself, for quite a number of cases admitted as "typhoid fever"—the diagnosis having been based on agglutination tests carried out during the acute stage in Mesopotamia—have been found on later examination to be really cases of paratyphoid A fever.

The Extent of Production of Para A Agglutinins.

The extent of the production of Para A agglutinins is usually considered to be slight. Although it is true that the low dilutions of the serum are a considerable aid to their detection during the earlier stages of the fever, yet the neglect of the study of their variation later in convalescence has led to an erroneous idea as to their amount. Thus Firth [7] says, "Even under the most favourable circumstances the patient's serum rarely gives a positive reaction for the A variety in higher dilution than 1 in 100. Often *B. typhosus* agglutinins are marked while Para A is not agglutinated by 1 in 10 dilutions of the serum." A perusal of the present records and charts appears to show that Para A agglutinins are produced in many of the cases in relatively large amount, especially at some period between the fourth to the seventh week. Although there is a comparatively feeble production of Para A agglutinins in the early stages of the fever, yet frequently at a later period the agglutinins are quite as strong as those agglutinins of *B. typhosus* which occur in the early stage of paratyphoid fever, or in typhoid fever itself. Hence such statements as "the variable and comparatively feeble production of agglutinins in paratyphoid fever, especially Para A" [7] seem to require considerable modification.

In view of the fact that previous work on the subject has been carried out on patients who had either never been inoculated or who had received simple typhoid vaccine, it was thought that the triple vaccine might in some way contribute to this delayed but stronger production of Para A agglutinins. Support is given to this suggestion by the relative diminution of inoculation agglutinins which is found to occur in a certain number of the cases, followed by the preliminary rise of *B. typhosus* agglutinins previous to Para A asserting itself. This initial diminution in agglutinins

may be analogous to the "negative phase" phenomenon. Our ignorance of the processes on which agglutination depends prevents any dogmatic statements, but our observations on the response of inoculation and infection agglutinins suggest that inoculation may play an important rôle in this delayed appearance of Para A agglutinins. A consideration of the average time when the agglutination titre is highest in patients inoculated with T.V. and T.A.B. respectively does not show any difference either in the date of appearance or amount of agglutinins produced. The average period for the T.A.B. series is 6·4 weeks, and for the T.V. series 6·5 weeks. A comparison between the mass agglutinins during (1) the first four weeks of the illness and (2) the period from the fourth week onwards of the cases in Tables I and II is so untrustworthy that no deductions can be made with any confidence. Apart from the fact that it has not been possible to make agglutination tests with any regularity during the first three weeks, and therefore no average can be struck for the mass agglutinins during that period, there is the fallacy of attempting to draw conclusions from a comparison of those two intervals, since Para A agglutinins reach their maximum production from the fourth to the seventh week, and the mass figures will depend on whether the rising Para A titre and the preliminary increase in *B. typhosus* agglutinins fall immediately before or after the commencement of the fourth week.

The Question of the Relative Diminution or Absence of the Inoculation Agglutinins during Pyrexia.

The relative diminution of the inoculation agglutinins during pyrexia in a number of the present series of cases has already been mentioned. This observation has an additional interest in its bearing on Tidy's [8] much discussed assertion that the inoculation agglutinins of *B. typhosus* are markedly reduced or entirely disappear in the early stage of febrile conditions, and that a positive agglutination test to *B. typhosus* after the fifth day of fever has the same value in an inoculated as in an uninoculated person. An analysis of the cases which had been inoculated with T.A.B. vaccine shows that agglutination reactions during the first and second weeks of the pyrexia have been obtained in twelve cases. Five of these show complete absence of all agglutinins during the pyrexia. Three others show a marked diminution, while of the remaining four the figures are either doubtful or suggest no diminution. The average interval that has elapsed since T.A.B. inoculation in the case of those showing complete absence of agglutinins is twenty weeks, as compared with an average of twenty-three weeks for the others. These figures certainly suggest that that there is a considerable reduction and occasional disappearance of the inoculation agglutinins in a proportion of the cases, small though the numbers be. It is to be noted that this reduction is immediately followed by an increase, usually considerable, in the *B. typhosus* agglutinins as a

result of the stimulation produced by the paratyphoid infection. An analogous phenomenon, we find, is mentioned by Ainley Walker [9] as occurring in experimental work in animals. If an animal which has been immunized some weeks or months before with a particular micro-organism be inoculated with a non-lethal dose of a vaccine prepared from some other micro-organism, its agglutination titre for the first organism exhibits a new rapid rise of greater or less extent and pursues a curve similar to the ordinary inoculation curve. "Immediately upon the second inoculation there may occur in a proportion of cases a moderate and purely temporary fall in titre probably somewhat similar in character to the negative phase of the opsonic index and passing off within a few days to be followed by the rapid rise just mentioned." Walker further notes that if a longer period has elapsed, e.g., five months before heterologous inoculation is given, there may be no change of any kind in the inoculation titre, though if any change does take place its main feature is a rise in titre.

Apart from this possible interaction between the inoculation and the Para A infection agglutinins, nothing in the clinical condition can be suggested as accounting for this irregular disappearance or reduction of agglutinins. No evidence from any of the records of the present investigation, however, can be obtained to support Tidy's assertion that a positive agglutination reaction to *B. typhosus* after the fifth day of pyrexia has the same value in an inoculated as in an uninoculated subject; moreover, numerous febrile cases of non-enteric origin showed no diminution of the inoculation agglutinins resulting from previous T.A.B. inoculation in tests carried out during the acute stage as compared with the results obtained during the afebrile period; while a study of the agglutination reactions in a series of malarial patients, who had previously had T.A.B. vaccine, revealed no difference between the agglutination titre of the serum taken during the febrile paroxysm, and of that obtained during the apyrexia of convalescence. It is to be noted that in Table I no reduction in the agglutinin values is apparent after the second week. Indeed only the cases in which investigations were made during the first two weeks have been considered in the present connexion. Hence it may have been that in some of the previous work on this subject, a diminution or absence of agglutinins has been missed through the serological test not having been carried out at a sufficiently early stage of the affection.

The Agglutination Curve of B. Typhosus in Paratyphoid Infections.

Little need be said regarding the rise in the *B. typhosus* agglutinin curve which precedes or is synchronous with the increase in the production of Para A agglutinins in most of the present cases. This was first pointed out in 1911 by Grattan and Wood [3], and has been confirmed by subsequent workers. Its chief interest appears to be its relation to the reduction in the agglutinin content which immediately precedes the rise, and to the

possible light sheds on the interaction between inoculation and infection agglutinins. But what is also to be noted from the protocols is the irregularity in the agglutinin curve of *B. typhosus* which appears to be largely due to a second rise which occurs very often subsequent to the agglutinin titre for Para A reaching a low level and which tends to give the whole curve a "saddle-back" appearance. This rebound is difficult of explanation, although all the evidence points to the inoculation agglutinins being the factor thus influencing the curve. In this connexion attention may again be drawn to the first appearance of para B agglutinins very late in convalescence (twelfth to sixteenth weeks) in a number of the cases which had been inoculated with T.A.B. and which had never shown any trace of those agglutinins at an earlier period of their investigation.

It has, of course, to be remembered that certain of these variations are "mean variations" in that they represent the next dilution above or below, and no attention need be paid to those "one tube differences." Yet some of the alterations in agglutinin content are most perplexing when the behaviour of the curve is being studied. In several of the cases in which the diagnosis of para A has been confirmed by isolation of the organism, sudden drops in the amount of para A agglutinins occur, to be followed by an equally sudden and unexpected rise for which no explanation can be assigned from a consideration of either temperature chart or clinical condition. For example, in the case of No. 5, Table I, in which para A was isolated from the fæces, the figures for para A in standard agglutinin units are :—

	Week : 7th	9th	11th	12th	13th	15th
Paratyphoid A agglutinins	.. 510	.. 510	.. 145	.. 250	.. 580	.. 58

The history and clinical condition of these cases were always carefully considered, while the occurrence of all such divergences led to the repetition and careful controlling of the agglutination tests. Incidentally it may be mentioned that in connexion with an investigation at present in progress we have repeatedly examined the agglutination reactions of forty of the men of our own personnel, the date of whose inoculation with T.A.B. vaccine was accurately known. As many as five tests in certain instances have been carried out over a period of nine months. A gradual diminution in the amount of the inoculation agglutinins in any individual case was perfectly apparent, but the very marked differences and striking contrasts between the figures obtained for men inoculated at the same time, and from the same bottle of vaccine, has convinced us that the average figure for the agglutinins obtained from such divergent results cannot be taken as representing the probable agglutination titre of any individual's serum at any given period after inoculation.

The Relation of Pyrexia and of Relapses to the Production of Agglutinins.

The elucidation of some of the irregularities of the agglutinin curves has been attempted by a study of the relation of the end of the fever and of the occurrence of relapses to the agglutinin content. A consideration

of the records with regard to the relation between the pyrexial period and the date of the maximum agglutination titre of the serum shows that the period varies from a few days to seven weeks, the average being approximately three weeks. The relation, however, is more evident in cases of long-continued pyrexia. In these instances the para A agglutinins are correspondingly late in their appearance and serological diagnosis is for a considerable period indefinite in spite of a series of agglutination tests. Of this, Case 17, Table I, is probably the best example. The patient ran a continuous temperature for over six weeks. Examination during the first and third weeks gave a figure of 110 and 220 units for *B. typhosus* agglutinins, while those for para A and para B were absent. The patient had been inoculated with T.A.B. vaccine forty weeks before. By the fifth week the titre for para A had reached a considerable height, viz., 285 units, and this increased until the record for the eleventh week was 500 units, after which there was a gradual fall, 37 being the figure for the fifteenth week. It may be noted that in this case the para A bacillus was obtained from the fæces during convalescence.

Relapses occurred during the convalescence of five of the cases, viz., Nos. 3, 5, 6, 11, of Table I, and No. 1 of Table II. The exact periods of the return of pyrexia in these cases have been compared with the date of the maximum production of agglutinins as well as with the agglutinin curves as a whole. No connexion can be detected between the relapse and either an increase or decrease in agglutinins. Indeed, in Case 1 of Table II, the relapse is synchronous with the highest figure recorded for para A, as it occurs from the thirty-second to the thirty-ninth day, while the maximum agglutinin production takes place during the fifth and sixth weeks. However, if one considers the usually accepted theory of the etiology of relapses that they are due to an infection with a different but closely allied organism, then, unless there be a production of co-agglutinins, no inter-relation between the pyrexial relapse and the agglutinin curve would be expected to exist.

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Conclusions.

(1) The present study of the behaviour of the agglutination curve is based on a series of thirty-three acute cases, mostly paratyphoid A fevers, from fourteen of which the infective organism has been isolated either by means of blood culture or from the fæces. The agglutination reactions have been carried out at intervals from the first to the twentieth week

dating from the commencement of the fever, while in every instance the agglutinin titre has been worked out to its end-point.

(2) The agglutinins for *B. paratyphosus* A are in a proportion of cases of paratyphoid A fever very late in their appearance. In the present series of thirty-three cases the highest agglutinin-titre has never been recorded before the fourth week from the commencement of pyrexia, while in twenty-two of the cases the maximum agglutinin content did not occur before the sixth week.

(3) Although the presence of para A agglutinins may occasionally be revealed by agglutination tests carried out in low dilutions of the sera, yet this is by no means constant, for those agglutinins appear to be sometimes altogether absent until a comparatively late period of convalescence when they may be found present in large amount. It would appear as if the reason why the maximum agglutination titre for para A has always been considered to be relatively low, is that its estimation has not been followed into convalescence.

(4) A certain proportion of potential paratyphoid carriers may thus be missed through reliance being placed on negative laboratory tests confined to the period of pyrexia or early convalescence, whereas serological investigation, if continued into later convalescence, may prove the presence of a paratyphoid infection.

(5) In two-thirds of the present cases in which agglutination tests have been carried out during the first and second weeks of the pyrexia, a marked diminution and occasional disappearance of the inoculation agglutinins has been found to occur. In cases of malaria and other acute non-enteric febrile conditions no such diminution of the agglutinins resulting from previous T.V. or T.A.B. inoculation has been detected when compared with the agglutination reactions obtained during the afebrile state.

(6) A marked ascent in the *B. typhosus* agglutination curve usually precedes or is synchronous with the appearance or increased production of para A agglutinins. This group reaction for *B. typhosus* has been a remarkably constant feature in both the T.V. and T.A.B. series of cases.

(7) No relation is to be detected either between the occurrence of relapses and an alteration in the agglutination curve or between the end of the pyrexia and the date of record of the maximum agglutinin content.

(8) The increase in *B. typhosus* agglutinins which seems invariably to precede the ascent in the para A curve, coupled with the frequent late appearance of para A agglutinins, shows the futility of basing any statistics on the relative incidence of typhoid and paratyphoid fevers diagnosed from single and casual agglutination tests carried out during either pyrexia or early convalescence in cases where blood cultures have either proved negative or been neglected. This conclusion applies to persons inoculated either with typhoid or triple vaccine; while even after the performance of a series of agglutination reactions there may be a fallacy involved in a negative report unless correlated with the clinical course of the infection.

(9) These observations suggest that in suspected cases of fever of the "enterica" group in subjects inoculated with T.A.B. vaccine, the value of the agglutination reaction as an aid to the rapid and accurate diagnosis of the specific infective organism is for practical purposes nil. If, however, it be possible to plot out the agglutination curve obtained by the performance of a series of tests repeated at short and regular intervals and continued well into convalescence the scientific interest of the reaction may be considerable in the study of the inter-relationship of inoculation and infection agglutinins, and of the processes on which the agglutination phenomenon depends.

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