ON THE NON-SPECIFIC STIMULATION OF AGGLUTININS. WITH ESPECIAL REFERENCE TO THE ENTERIC FEVERS AND TYPHUS FEVER.

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INTRODUCTION.

It is well known that the problem of the non-specific stimulation of agglutinins was the subject of lively controversies during the war and in the following years. The preventive inoculation against typhoid fever, which was widely used since the beginning of the war, in all European countries, soon brought the greatest confusion into the sero-diagnosis of enteric fevers. The Widal reaction which had for so many years been considered to be one of the most valuable methods of diagnosis in practical medicine, lost its value as such when it ceased to be a simple reaction and became a complicated serological problem. It became an urgent problem both for pathologists and clinicians, to distinguish between the inoculation agglutinins conferred upon millions of those involved in the war in all countries, and the infection agglutinins developed in the course of active enteric infection. In spite of the general endeavour directed to this end the problem was not solved anywhere during the war.

It is not proposed here to give a comprehensive survey of the literature which has reached very considerable proportions during the course of the intervening years. An attempt will be made to describe very briefly the various directions in which this controversy developed both on the Continent (in the German literature) and in this country in so far as it is necessary to elucidate the observations brought forward in this paper.

https://doi.org/10.1017/S0022172400009712 Published online by Cambridge University Press

1. REVIEW OF THE GERMAN LITERATURE.

When the disturbing effect on the Widal test was recognised shortly after the introduction of the monovalent typhoid vaccine (Dünner, 1915, 1) it was at first hoped that the difficulty could be overcome by introducing a "minimum titre," *i.e.* a limit of agglutination titre in inoculated individuals (Stursberg and Klose, 1915; Klemperer, Oettinger and Rosenthal, 1915). It was assumed that, as a result of preventive inoculation, agglutinins would be produced only up to a certain relatively low titre and that they would go down again in the course of a short time (several months) to the level of normal agglutinins. The view of a "minimum titre" of inoculation agglutinins had soon to be abandoned, as it was shown (Dünner, 1915, 2) that independently of qualitative and quantitative differences in the vaccines used, there exists the widest range of individual variation as regards the strength and the persistence of agglutination in the inoculated. The next attempt to save the Widal test for routine diagnosis was based on the assumption that inoculation agglutinins do not exhibit—in individuals suffering from some non-typhoidal disease—any marked fluctuation in the agglutination titre, similar to the antibody curve characteristic of the Widal in typhoid fever. Thus it was believed that a differentiation between inoculation and infection agglutinins could be made by means of repeated quantitative estimations (Hirschbruch, 1915). But it was soon established beyond any doubt that this was an erroneous conception.

In the elucidation of this problem, typhus fever played an important rôle. This disease, which was unfamiliar to most medical men in the war areas, offered the greatest difficulty in clinical diagnosis, and was, therefore, both before and after the introduction of the Weil-Felix reaction the subject of very numerous and very careful serological investigations. The confusion caused in the diagnosis of typhus by the presence of T.-inoculation agglutinins was even greater than in the diagnosis of enteric infections and led a number of experienced workers to completely erroneous theoretical conclusions as regards the mutual relationships between these diseases. Weil and Felix (1916) established on the basis of a large number of typhus cases in Poland that in the course of typhus fever in the inoculated, the inoculation agglutinins undergo a great stimulation, resulting in a considerable percentage of cases in such a significant rise and fall of the T.-agglutination titre as was hitherto known only in active typhoid infection. It was found to be a matter of indifference whether inoculation agglutinins were still present at the onset of fever to a more or less high titre or had already fallen below the limit of normal agglutination, placed by Weil and Felix in the dilution of 1 : 75. On the other hand, no positive Widal test was found in non-inoculated typhus patients, if a previous enteric infection could with certainty be excluded.

These observations were fully confirmed by numerous workers in different countries: Meinicke (1916) in Poland, Cancik (1916) in Serbia, Felix (1917) in Constantinople, Mühlens and Stojanoff (1917) in Bulgaria, Zlocisti (1918) in

Constantinople, and others. It was further proved by Weil and Felix (1916) that the non-specific stimulation of inoculation agglutinins was not indeed a peculiarity of typhus fever, but could in exactly the same way be observed in the course of various febrile diseases, other than enteric fever.

Non-specific stimulation of specific immune bodies has been known from experimental results on animals by various workers: Salomonson and Madsen (1898, pilocarpine), Obermeier and Pick (1904, peptone), Rostoski (1905, pilocarpine), Dieudonné (1906, hetol), Dreyer and Walker (1909, heterologous bacteria). In human beings these results seemed at first to be unexpected because the opportunity had never before presented itself in any comparable degree for the investigation of the serological conditions obtaining in inoculated persons. The non-specific stimulation of agglutinins in man was also shown experimentally by Fleckseder (1916) as the result of injections of deuteroalbumose and sodium nucleinicum. For these serum reactions the designation "anamnestic reaction," appropriately suggested by Conradi and Bieling (1916), was generally accepted.

A last attempt to restore in part the former significance of the Widal test in the diagnosis of typhoid fever, was made by Braun (1918) and Liess (1918). They proposed to use *B. paratyphosus* A. for the determination of group-agglutinins which they found to be present in the serum of typhoid cases and absent from the serum of inoculated persons suffering from non-typhoidal diseases. It is true that the observation underlying this proposal is correct, but it was not applicable in practical diagnosis. Apart from the fact that group agglutinins (for A. or B.) are formed only in a certain proportion of typhoid cases and then usually in the later course of the disease—such a method was *a priori* unsuitable after the introduction of the mixed T.A.B. vaccine and received therefore no attention.

Thus the pessimistic opinion has gradually developed, that in previously inoculated individuals the Widal test does not afford the clinician a reliable means for the diagnosis of typhoid. Kolle and Hetsch, for example, make the following statement in the latest edition (1922) of their well-known text-book (p. 330): "As typhoid agglutinins appear in the blood in consequence of inoculation, the Gruber-Widal test has lost its diagnostic value in obscure diseases." And in another place (p. 335): "In patients, who have previously undergone preventive inoculation against typhoid—and, of course, especially when the vaccine contained also a paratyphoid quota—these conditions become entirely incomprehensible. One can no longer reach, in such cases, any diagnostic results whatever from the agglutinating action of the sera of patients."

2. Review of the English literature.

In the English literature the question under discussion has been the subject of not less controversy than on the Continent. But the direction in which this discussion developed and the conclusions apparently arrived at (as shown, for instance, in official documents) differ very much from those dealt with above.

Here, too, an attempt was made at the beginning to fix a limit of agglutination titre for T.-inoculated individuals and cases which showed higher titres were diagnosed as cases of active typhoid infection. Dreyer, Ainley Walker and Gibson (1915) first emphasised that greater importance in the diagnosis has to be attached to marked changes than to the absolute degree of agglutination. In spite of the considerable amount of evidence accumulated by various workers who showed the extremely wide range of variation which occurs in the amount of inoculation agglutinins in different individuals, the original assumption of a limit of maximal titres attained after inoculation seems not to have been completely abandoned. As late as 1920, Topley, Platts and Imrie still considered "unusually high titres" as "notable departures from the normal" and attached to them diagnostic significance both with reference to the time which had elapsed since the last inoculation, and to the titres for the other two organisms (in T.A.B. inoculated).

But the main thesis in this controversy was the statement by Dreyer and his co-workers that the validity of agglutination tests is in no way impaired by T. inoculation and even not by triple inoculation (with T.A.B.)—provided that their directions for the technique and interpretation of the tests are strictly carried out.

There is no doubt that the principle underlying Dreyer's technique (Dreyer, 1906 and 1909), namely, the standardising of the agglutinability of the bacterial suspensions is of the greatest importance in the technique of the agglutination reaction. The question as to whether Dreyer's method of standardising the sensitiveness of formalinised broth cultures is the only reliable method by which to guarantee accurate quantitative measurements, "whose results on successive occasions are strictly comparable, *inter se*" (Dreyer and Walker, 1916) has been dealt with by certain workers, but it is not proposed to consider this question here. It must, however, be emphasised that the great importance which has always been attached by Dreyer and his co-workers in the course of this discussion to the necessity of employing quantitative methods is fully justified.

While certain details of Dreyer's technique have withstood the test of criticism and its adoption was therefore advocated by the Medical Research Committee, the interpretation of the results in inoculated individuals as advocated by Dreyer and his co-workers, cannot be said to be equally justified. Their method of diagnosing an active enteric infection in an inoculated patient is built up on the following assumptions:

(1) That there exists in enteric infections in non-inoculated individuals one general form of the curve of agglutinin production, showing the maximum between the 16th and the 24th day of the disease, and most frequently from the 18th to the 20th day.

(2) That the same rule is valid in enteric infections in inoculated individuals as regards the curve of "specific" agglutinin formation (agglutinins for the bacillus concerned).

(3) That as regards the heterologous agglutinins, due to previous inoculations with T. or T.A.B. vaccine, no appreciable rise in the titres or a relatively slight rise, or a marked rise, may occur approximately synchronously with or somewhat antecedent to the rise in agglutinin titre for the infecting bacillus, and subsequently followed by the usual fall.

(4) That the apparent difficulty caused by the latter phenomenon ("synchronous and sympathetic rise and fall of inoculation agglutinins") can be overcome thanks to the following circumstances:

(*a*) that at its maximum the titre for the infecting micro-organism will usually exceed the titre of inoculation agglutinins even though these may be greatly raised as a result of the infection;

(*b*) that as the fall in the titre proceeds, the curve of infection agglutinins falls more rapidly than the curve of inoculation agglutinins, so that the latter is cut by the former at some point in the fall.

(5) That equally marked fluctuations in the agglutinin titre do not occur in inoculated persons, either in health or in the course of other diseases, provided that not less than two months (according to Dreyer and his co-workers) or not less than 10 weeks (according to Topley, Platts and Imrie, 1920) have elapsed since any single prophylactic inoculation.

When the data published in support of this method for diagnosing an active enteric infection in an inoculated individual are thoroughly analysed, the surprising fact is revealed, that the sum total of evidence accumulated is exceedingly small. It is obvious that only agglutination curves from cases controlled by successful blood culture—as first insisted upon by Garrow (1916)—ought to be considered as valid evidence of the applicability of the method. But even if it were admitted that isolations from stool or urine are as valid as blood cultures in the diagnosis of enteric fevers, then the total number of such curves published (so far as the present writer has been able to establish) is represented by six cases of B. infection and two cases of A. infection in T.-inoculated persons (Dreyer and Inman, 1917, Charts 1–9) and three cases of B. infection in T.A.B.-inoculated (Perry, 1918, Cases 8, 9 and 10).

The agglutination data given for the further eight cases of Perry (1918), which were confirmed by the isolation of the infecting organism, are in no way sufficient for the determination of any curve of agglutination in these cases. Ainley Walker's paper (1916) which also brings forward agglutination curves, contains no case controlled by the isolation of the organism.

It would seem that many bacteriologists at that time were so strongly impressed with the reliability of Dreyer's method that its application to the routine examination of cases of pyrexia in inoculated persons was considered to be justified on the basis of the evidence published. Topley, Platts and Imrie (1920) went even further in applying Dreyer's technique of repeated agglutination tests as a diagnostic method in convalescent and inoculated persons.

The number of authors who were not believers in the diagnostic value of agglutination tests in inoculated men appears to be very limited.

Martin and Upjohn (1916) appear to have been the first who concluded that since the introduction of triple vaccine, "the interpretation of observations upon the agglutination of enteric organisms will...be too difficult to possess any practical value, and the isolation of the infecting organisms must be resorted to for diagnosis." This conclusion was drawn from clinical observations on T.-inoculated patients suffering from A. or B. infections and from experimental observations on T.-inoculated healthy persons who were subsequently inoculated with A. and B. vaccines.

Garrow (1916, 1917, 1920) considered agglutinins produced by inoculation as "indistinguishable qualitatively and quantitatively from those produced by disease" and "atypical" enteric fever as a myth, in the creation of which lack of co-operation between clinician and pathologist had been chiefly concerned.

Ledingham (1920, 1921), in Mesopotamia, recognised two classes only of enterica, viz. (1) confirmed enterica (isolation of T., A. or B.), and (2) clinical enteric group. In triply inoculated men diagnosis by agglutination methods was not accepted and was not practised. Ledingham doubted the correctness of all those conclusions with regard to the symptomatology of enteric fever in the inoculated, which have been drawn from cases, diagnosed by agglutination methods only.

The evidence published by these opponents of agglutination methods do not seem to have influenced the opinion of the Committee upon Pathological Methods (1920) acting on behalf of the Medical Research Council, who accepted in their Report (pp. 105, 106) most of the views of Dreyer and his co-workers.

The latest publication dealing with this matter is that by Leishman (1924), giving for the first time statistical data on the use of Dreyer's method in the British Armies in France. An attempt will be made in a later section of this paper to see how far these data can be interpreted in favour of the method or otherwise.

In concluding this review of the English literature one paper must be especially mentioned describing observations which, in the writer's opinion, were potentially capable of leading to the real solution of the problem. It is the proposal made by Dawson (1915) to use a peculiar strain of *B. enteritidis* Gärtner (Delépine 7160) for the differentiation of T.-inoculation and T.-infection agglutinins. Exactly the same suggestion was made some time later in Germany by Seiffert (1915). It is interesting to note that both of these proposals were completely neglected. Only O'Farrell (1916) studied the properties of *B. enteritidis* Gärtner (Delépine 7160). He confirmed Dawson's statement that antityphoid inoculation bears no relation to the agglutinating power of a serum for this organism; but the behaviour of the serum of typhoid patients was, unfortunately, not investigated. The importance of the suggestion made by Dawson and Seiffert will be shown in the next section.

3. Summary of the qualitative serum analysis in the Diagnosis of Enteric Fevers.

Working in Palestine since 1921 the writer was soon again confronted with the difficulty arising in routine sero-diagnosis from anti-enteric inoculation. There the Government Department of Health introduced in December, 1921, inoculation of immigrants with T.A.B. as a general routine, and since that time a steadily increasing number of inoculated patients suffering from various febrile diseases came under observation.

As all the methods hitherto employed in quantitative serum analysis had left unsolved, in the writer's opinion, the problem of differentiation between inoculation and infection agglutinins, a solution was sought by the use of the qualitative method. The facts ascertained, by means of rabbit immune sera, by Weil and Felix (1920) as regards the receptor apparatus of *B. typhosus*, *B. enteritidis* Gärtner, *B. paratyphosus* A. and B. form the theoretical basis of this qualitative method. According to the findings of these workers the labile H and the stable O antigen existing in these four species show the following relations:

(1) The labile H antigen is specific for each one of these four species.

(2) The stable O antigen consists of main receptors, which are specific for each of these species, and of group receptors, which may show close relationship among themselves.

(3) According to this scheme group agglutination between *B. typhosus* and *B. paratyphosus* A. and B. is as a rule caused by small-flaking O group agglutinins.

(4) *B. typhosus* and *B. enteritidis* Gärtner possess identical stable O antigen; these two species differ from each other only in their labile H antigen.

(5) In *B. typhosus* important differences of sensibility of the stable O antigen could be found in various strains.

When these facts were systematically applied to the Widal test the surprising result was obtained, that the differential diagnosis so long needed in obscure cases of disease among inoculated persons, could be given to the clinician, on the basis of the agglutinating action of the serum. The conclusions drawn from the examination of 1200 blood specimens from non-inoculated and inoculated patients, some 500 specimens of which came from about 250 cases of typhoid and paratyphoid, as published some years ago (Felix, 1924, 1 and 2), may be summarised here.

I. *In typhoid and paratyphoid infections in individuals not previously sensibilised by prophylactic inoculation or by a previous enteric infection.*

(*a*) Large-flaked (= labilotropic) H agglutination indicates the homologous agglutinin. The differential diagnosis between typhoid and the paratyphoids can thus be determined in most cases according to the mere appearance of agglutination in one single serum dilution. The quantitative titration of the serum becomes thereby superfluous.

(*b*) Small-flaked (= stabilotropic) O agglutinins are, in addition, formed in the large bulk of cases. They are absent only in the most severe cases and in a certain proportion of the slightest cases.

(*c*) Group, or co-agglutinins, belong to the small-flaking O type.

(*d*) When large-flaking H agglutinins are absent—a condition occurring in a minority of all cases—differential diagnosis between typhoid, paratyphoid A. and paratyphoid B. is not successful by this qualitative method, nor is it by the usual quantitative methods. The agglutination diagnosis in such cases can only be enteric group, type unable to be differentiated serologically.

II. *In inoculated individuals.*

(*a*) Inoculation agglutinins persistent in healthy and sick individuals and reappearing and markedly fluctuating in titre under the non-specific stimulus of various febrile diseases ("anamnestic reaction") are always of the large-flaking H type.

(*b*) No diagnostic significance whatsoever can, therefore, be ascribed to the presence or behaviour of large-flaking H agglutinins in cases of enteric infection in the inoculated.

(*c*) In these cases sero-diagnosis must rely exclusively upon the presence of small-flaking O agglutinins, which is decisive for the diagnosis of an enteric infection; the negative result which has to be given in the absence of O agglutinins (see above I (*b*)) is, of course, of the same significance only as that of a negative Widal test in the non-inoculated.

(*d*) According to the statement under I (*d*) the limitations of agglutination diagnosis of enteric infections in inoculated individuals are clearly fixed:

(i) If T.A.B. vaccine has been used it is only possible to diagnose "Enteric Group," without being able to differentiate T. from A. or from B.

(ii) If T. vaccine has been used then A. or B. infection can be differentiated but not T.

It may be stated here, that in the development of this method an important rôle was played by *B. enteritidis* Gärtner, which, according to the findings of Weil and Felix (1920), could serve as a controlling reagent for the isolated detection of the small-flaking typhoid agglutinins, as it does react with them like a strain of *B. typhosus* but does not react with the large-flaking typhoid agglutinins. The above-mentioned statement of Dawson (1915) and Seiffert (1915), which at first appeared to be incredible, owing to the ignorance of the real antigenic relationship between these two organisms, finds thereby its full confirmation and explanation.

It may further be mentioned that Burnet (1924), working on typhoid patients in Australia and basing himself on the knowledge of the double type of antigens and antibodies, observed independently the greater part of the facts underlying this qualitative method. But the smallness of his clinical material did not permit him to reach such definite conclusions as those formulated above.

So far as I am aware, the method proposed has hitherto not been subjected anywhere to a serious critical test; for certain authors the pre-conception based upon the accepted text-book opinion was sufficient to cause them to reject the method without further trial, while others simply neglected it.

4. Discussion of Sections 1–3.

It seems suitable in this place to subject some of the most controversial points raised in the course of the discussion under review to a searching criticism. The main direction of the criticism will be to review the findings of Weil and Felix (1916) and Felix (1917, 1) and (1924, 1 and 2) in the light of the objections which will be raised by those relying on the method of Dreyer and his co-workers. These objections will, in part, be of a technical nature, and can be subjected to the test of repetition as the published data in the papers just mentioned give the technique in adequate detail, while most of the accounts of the other authors quoted in the previous sections do not contain any mention of the technique of the agglutination reaction used.

As a result of the above-summarised investigation by means of the qualitative method, it is now known that the serum of patients ill with typhoid or paratyphoid contains (or may contain) both O and H agglutinins, whereas agglutinins persisting after inoculation belong to the H type only. In order to compare the results of agglutination tests obtained by different methods, it is, therefore, first of all necessary to establish what the behaviour of these two different kinds of agglutinins is with the different techniques of the agglutination test. In a recent paper (Felix and Olitzki, 1928) it has been proved for *B. typhosus* and the *Salmonella* group that phenol and formaldehyde, in the concentrations usually employed in preserved bacterial suspensions, produce in a selective way a transient or permanent inhibition of the O agglutination, while the H agglutination is unaltered in velocity and degree. This fact makes it obvious that all published data on agglutination tests performed with such preserved suspensions, while no doubt giving a true picture of the behaviour of the H agglutinins, give no information whatsoever concerning the O agglutinins.

Now the observations on the non-specific stimulation of inoculation agglutinins published (from Poland) by Weil and Felix (1916) and (from Asia Minor) by Felix (1917, 1) were made by invariably employing Ficker's diagnosticum (Merck) (see Felix, 1917, 1, p. 611). These phenolised suspensions have been tested on a very large scale by Felix and Olitzki (1928). They were uniformly found, for labilotropic H agglutination, of a sensitiveness equal to that of living suspensions of known laboratory strains of the best agglutinability; but in the stabilotropic O agglutination, a complete or partial inhibition was always observed.

As far as Dreyer's technique is concerned it was at an early date stated by Dreyer (1915) that notable group agglutination is not met with in the enteric group. "The reason is not apparent, but the fact is attested by all who have

worked with this method, and has been found advantageous in avoiding the confusion in diagnosis which group agglutination sometimes causes" (Committee upon Pathological Methods, 1920). The reason has now become apparent; it is the inhibitive action of the formaldehyde on the O agglutination to which type group agglutination in the enteric fevers exclusively belongs (Weil and Felix, 1920; Felix, 1924, 1 and 2).

Thus the fundamentally important fact seems to be evident that all investigations made with Dreyer's technique, as also those carried out with Ficker's diagnosticum, were concerned in the demonstration of the labilotropic H agglutination only, and are, therefore, justly comparable with each other.

(*a*) *The accuracy of quantitative agglutination tests on successive occasions.*

Dreyer and his co-workers always rightly emphasised that an accurately quantitative character of the method in use, as guaranteed by their standardised agglutinable suspensions, is a necessary condition for the demonstration of a fluctuating agglutination titre. Was this requirement met with by Weil and Felix (1916) and Felix (1917, 1 and 1924, 1 and 2)?

It is true that the individual batches of Ficker's diagnosticum (Merck) used in the first two investigations quoted, were not standardised in a way similar to that used by Dreyer, namely, by a reduction factor. But the diagnosticum was manufactured on such a large scale that the individual batches were used for a much longer period than that extending over the most protracted observations of individual cases. This time varied from a few days to several weeks, and did in no case exceed two months. Thus the observations tabulated for the individual cases may be considered as obtained with the same batch of diagnosticum, *i.e.* with a suspension of constant agglutinability.

In the later series of Felix (1924, 1 and 2), investigated in Palestine by means of the qualitative method, fresh suspensions of living bacteria were employed. In full recognition of the importance of possible variations in agglutinability—due to variability in the development of the labile H antigen —the closest observation of certain conditions laid down for the growth of the cultures was practised as was also insisted upon in these two publications (p. 135 and p. 125 respectively). The constancy of the sensitiveness of both the H and the O components of the actual cultures used was regularly controlled by pure standard H and O rabbit immune sera. As the labilotropic H agglutinins are much more resistant to various influences than the stabilotropic O agglutinins (Weil and Felix, 1917 and 1920) such controls are particularly reliable in the case of the H antigen, even if the same immune serum has been stored for a considerable time. As an additional control very often the following procedure was practised: samples of sera, after having been tested, were stored in the ice box until several sera taken on successive occasions from the same patient were collected; the whole series was then simultaneously titrated with fresh suspensions of living bacteria. Again,

particular weight can be attached in the case of H agglutinins to repetitions performed in such a way, owing to the above-mentioned resistance of these immune bodies.

It seems clear that both with the Ficker diagnosticum and with fresh suspensions of living bacteria, the precautions taken guaranteed the accuracy of the quantitative measurements on successive occasions. The marked fluctuations in inoculation agglutinins in the course of typhus fever, malaria, pneumonia, influenza, and other diseases, as tabulated in the papers quoted above, can, therefore, in no way be explained by mere technical errors. In many of these cases the fluctuations—as can be seen from the tabulated data—were so marked and showed such regularity in the rise and fall in agglutination titre, that the resulting curves indeed were indistinguishable from those known from true specific immune reactions.

As already mentioned (in Section 1) these observations of Weil and Felix were entirely confirmed by numerous workers. Their agglutination technique, mostly not specified in the published data, may or may not be open to criticism as regards the constancy in agglutinability of the suspensions used. But it is inconceivable that this factor can have been the only one responsible for the fluctuations observed and finally plotted into agglutination curves, where such investigations were performed by experienced bacteriologists on a greater number of patients simultaneously. Either the rising or the falling part of the curves built up from the data of simultaneously observed cases would have shown a distortion sufficiently marked to have destroyed the character of the curve if any considerable variation in the agglutinability of the suspension had occurred.

Analogous results were also obtained by means of Dreyer's technique. The *Report on Trench Fever*, 1918, published by the American Red Cross Research Committee, describes very thoroughly observed fluctuations in inoculation agglutinins in cases of spontaneous and experimentally produced Trench Fever. Topley, Platts and Imrie (1920), in discussing this *Report*, wrote (p. 31): "If the conclusions of the American Committee are supported by further investigation, then we must clearly discard the method of repeated agglutination tests in attempting to establish a diagnosis of enteric infection in an inoculated individual...." "We have seen no answer to this highly destructive criticism, by Dreyer or his co-workers."

(*b*) *The time relation of the maximum of the agglutination curve.*

This supposed criterion of specific (= homologous) agglutinin formation accompanying enteric infection in man, to which Dreyer and his co-workers attached so much importance, was not especially considered by Weil and Felix (1916) and Felix (1917, 1). Nevertheless, a certain number of cases are described in the first publication, showing the maximum of agglutination clearly between the 16th and 24th day of disease, and in the second paper, the proportion of such cases is still greater.

But is there sufficient evidence to attach such a far-reaching significance to this supposed time relation ("period of expectation for enteric fever" according to Ainley Walker, 1916)?

I am not aware of the published evidence in support of the first of Dreyer's assumptions (see Section 2), that there exists in enteric infections one type only of the curve of agglutinin production. The older work of Widal and Sicard (1897), Courmont (1897 and 1900), Iversen (1905) and others, clearly indicates a variety of forms in agglutinin curves in typhoid patients, a fact which led Courmont to propose to make use, though unsuccessfully, of the Widal test for seroprognosis in typhoid fever. A more recent investigation by Reichenstein and Silbiger (1917) showed that there is no consistency in the form of curve as regards the date of the maximum. They established that the maximum of the agglutination curve is usually obtained approximately at the beginning of defervescence. If this finding were correct, it would indicate that the date of maximum agglutination may show a range of variation as wide as that of the clinical course of the disease. How wide this is is indeed sufficiently well known.

I am unable to offer exact observations on this point from my experience with the qualitative method. When the possibility of seroprognosis in typhoid fever was recognised as being based on the regular and close relationship between O agglutination and the severity of disease, but independent of the H agglutination, the chief attention was directed to the O rather than to the H agglutination. It remains for future investigation to ascertain what are the exact curves for both types of agglutinins in non-inoculated patients, in cases containing one type of agglutinins only and also in cases where they are present simultaneously.

The second assumption underlying Dreyer's method is that independently of the presence of homologous inoculation agglutinins in the blood serum, the curve of specific (= homologous) agglutinin formation is governed by the same time relation in the inoculated as in the non-inoculated patient (maximum at days 16–24). This assumption certainly can still less be justified than the former, as additional experimental evidence to the contrary has to be taken into consideration since the early work of Cole (1904).

Indeed, remarkably wide limits have been accepted in this respect in the practice of Dreyer's method. Dreyer and Walker (1916), for example, state (p. 423): "If it is clear that the maximum falls markedly outside the limits given above (day 16–24) a diagnosis of typhoid or paratyphoid fever should not be based on a rise in titre of only moderate extent—*i.e.* a 100 or 200 per cent. increase in agglutination titre." This means implicitly that a greater increase in titre may well be considered as significant. In Ainley Walker's (1916) series of illustrative charts out of the total of 12 cases of enteric infections published, 4 cases are described where the time relation is expressed in "day of observation" instead of "day of disease" as apparently the date of onset was doubtful. It is obvious that in these cases the author neglected

the determination of the exact time relation of the maximum to the stage of the disease. In Perry's 1918 paper, this omission is still more conspicuous: 25 cases of enteric infections, proved by agglutination, are published there; but in 15 of these cases the tabulated data do not at all indicate a true maximum of agglutination as the last figure determined and considered to be the maximum is not followed by any further determination.

From the above observations the conclusion seems to be justified that the curves of non-specifically stimulated inoculation agglutinins, described by Weil and Felix (1916) and Felix (1917, 1), are indistinguishable from those obtained by means of Dreyer's method and stated to be due to specific stimulation in the course of enteric infection.

(*c*) *Mixed infection as probable source of error.*

In the course of the criticism to which the similar observations of Martin and Upjohn (1916), made on a series of cases of paratyphoid A. and B. in fections from Gallipoli, were subjected, the further objection was raised, that "these cases were probably mixed paratyphoid A. and paratyphoid B. infections." "If agglutinins for both these organisms become evident and exhibit the usual rise and fall, the only justifiable conclusion is that the patient is suffering from a dual infection." "The fact that bacteriological examination was successful in isolating one of these organisms only does not in any way negative this probability." These statements made by Perry (1918), whose experience of Dreyer's method was admittedly exceptionally wide, render it necessary to consider the objection which is likely to be raised, that the cases now under discussion were mixed infections of typhus and typhoid fever.

The cases tabulated in the paper of Weil and Felix (1916) were observed in Poland in 1915–1916, and were a part of those cases on which the serology of typhus fever has been worked out by these investigators. Every possible effort to exclude mixed infections was made. Blood cultures were made by enrichment methods with each blood sample which is recorded in these agglutination tests; urines and stools were examined in each case daily during the whole course of fever and for a long period during convalescence, not only with the view to exclude *B. typhosus*, but also to detect *B. proteus* X. In cases of typhoid fever in inoculated or non-inoculated patients, this procedure hardly ever failed to detect the *B. typhosus* on one or several occasions. But in these typhus cases, *B. typhosus* was never found. Thorough clinical observations made on these patients were published by Falk and Siebenrock (1916) and Berger (1917). No suspicion whatsoever as to the possibility of mixed infections can be traced in their clinical observations.

The cases tabulated in the paper of Felix (1917, 1) were observed in Constantinople in 1916–1917 and still more exhaustively examined bacteriologically. The results of the cultural examinations have been published (Felix, 1917, 2). Extremely detailed clinical observations derived from these patients have been published by Zlocisti (1917 and 1918). His second paper, devoted

especially to a comparison of the Gruber-Widal test and the Weil-Felix test in typhus fever, contains a great deal of clinical, bacteriological and epidemiological data, especially directed to exclude completely any suspicion as to the possibility of mixed infections.

Still the objection could be raised that these cases might have been mixed infections, but were not correctly recognised owing to the "atypical" clinical course and to the supposed difficulty of isolating the causative organism in cases of enteric fever in inoculated individuals. These latter views, as is well known, have been held by many bacteriologists, but Ledingham (1921) and other workers have proved that there is no evidence in support of such views. In a previous paper (Felix, 1924, 2) I gave some arguments supporting the view that bacteriaemia in typhoid and paratyphoid infections in inoculated persons is neither shorter in duration nor less in degree than in non-inoculated. Further experience on some hundreds of cases of enteric fever, observed in Palestine during the years 1923–1926, provided additional evidence in support of this indisputable fact.

Finally, it may be stated here that it was the opinion of older clinicians (Curschmann, 1904) that mixed infections of typhus and typhoid fever do not occur at all. Zlocisti (1918) on the strength of his own observations and those published by other workers (Munk, 1916) concluded that the evidence accumulated during the war was in support of this older view. In any case, it would appear, from the data published by all workers who had the widest experience with typhus fever in various countries, that such mixed infections must be considered as entirely exceptional.

(*d*) *The nature of "synchronous and sympathetic" stimulation of inoculation agglutinins in the course of enteric infections.*

So long as preventive inoculation was performed with the monovalent T. vaccine only, the study of the interaction of inoculation and infection agglutinins was less complicated and could afford unmistakable results. At that time several careful investigations on this matter were published, yielding completely uniform results.

Grattan and Harvey (1911) were the first to demonstrate the danger of errors in diagnosis arising from increase in inoculation agglutinins. They showed (in India before the war) that in undoubted cases of paratyphoid A. fever in T.-inoculated individuals (proved by successful blood culture) a marked increase in the agglutinins for *B. typhosus* was noted. These results were confirmed in later communications by Grattan and Wood (1911) and Safford (1913) from India.

Martin and Upjohn (1916), again in the study of paratyphoid (A. and B.) infections in T.-inoculated patients, came to the following conclusions: "The development of the well-marked agglutinins to typhoid bacilli weeks before the specific agglutinins arrived, suggests another interpretation [*i.e.* other than co-agglutinins], namely, that in an inoculated person, the mechanism for the

manufacture of typhoid agglutinins being already laid down, the introduction of paratyphoid organisms stimulates this mechanism to further activity."

Hall (1916), studying the same problem in the same type of patients (viz. paratyphoid A. and B. infections in T.-inoculated patients) by means of absorption methods, concluded: "...that the rise is not due to any group, or co-agglutination effects, but is specific for each type of agglutinin." In a second communication Hall, Hiles and Nicholls (1917) summarised their results as follows: "These findings in bulk confirm for man the observations which Dreyer and Ainley Walker record in their experiments upon rabbits, namely, that after immunisation with dead *B. coli*, the subsequent injection of killed emulsions of *Staphylococcus aureus*, the bacillus of Friedländer, or a Streptococcus increases the production of the specific primary agglutinins, so long as the animal retains any measurable degree of acquired immunity. Perhaps the figures may be interpreted so as to carry the matter a little further and to suggest that inoculations with allied, or group, organisms, point to the probability that typhoid and paratyphoid bacilli differ so much in their essential metabolism as to classify them individually as heterologous organisms. The results of our previous absorption experiments and their inferences as to the absence of co-agglutinins in sera containing mixed antibodies lend support to the surmise."

The important difference in opinion between these last-mentioned workers and Dreyer and his co-workers, may best be illustrated by the following quotation from a paper of Ainley Walker (1916) (p. 897): "These subsidiary rises in agglutinin titre are almost certainly to be explained as due to a re-stimulation by infection with a closely allied organism of the mechanisms already trained by inoculation to produce particular series of agglutinins."

In the light of the present knowledge of the double type of antigen and antibodies (Weil and Felix, 1920) the labilotropic H agglutinins are strictly specific in the enteric group of organisms and they are the only ones concerned in all the investigations quoted above. The synchronous changes of their respective indices in the course of typhoid and paratyphoid A. and B. infections must, accordingly, be considered as due to a non-specific, *i.e.* heterologous stimulus. In spite of their being closely related members of one group of organisms, manifesting this relationship through common components of their stable O antigen, the labile H components of their respective body substance "differ so much in their essential metabolism" (in the expression of Hall, Hiles and Nicholls, 1917) as to classify them as strictly heterologous antigenic substances. The re-stimulation of these heterologous H agglutinins in the course of an enteric infection, can, therefore, only be of the same non-specific character as in the case of any other febrile condition.

In concluding this discussion concerned with establishing that the re-stimulation of inoculation agglutinins is of the same non-specific character in the course of an enteric infection, or in typhus fever, or in any other febrile

condition—it may be of interest to compare some of the figures given by various workers as regards the incidence of the phenomenon.

The figures given by Weil and Felix (1916) for typhus fever are: (*a*) in 15 out of 28 cases fluctuation in still persistent T.-inoculation agglutinins was observed (*i.e.* 53 per cent.); (*b*) in 18 out of 55 cases re-appearance and fluctuation of T.-inoculation agglutinins was observed, which before the beginning of the disease had already fallen below the limit of normal agglutination (*i.e.* 33 per cent.).

If the figures for both groups, as being alike in character, are combined, then the result is that re-stimulation of T.-inoculation agglutinins occurred in 33 out of 83 cases in typhus fever (*i.e.* in 40 per cent.).

Martin and Upjohn (1916) state that the inoculation with paratyphoid A. and B. bacilli was followed by the considerable development of typhoid agglutinins in about half of those persons who had previously received T. vaccine. They do not give figures concerning the occurrence of re-stimulation amongst their paratyphoid A. and B. patients.

Hall, Hiles and Nicholls (1916) make the following statement (p. 284): "In the presence of infective paratyphoid agglutinins, the inoculation titre remains steady in 53 per cent. of cases, but in the remainder it varies and often rises considerably. This rise is higher with paratyphoid B. than with paratyphoid A. infections."

The numerous publications by Dreyer and his co-workers as well as the paper by Perry (1918) do not contain any figures on the incidence of the synchronous fluctuations in inoculation agglutinins in the course of active enteric infections, although the occurrence of the phenomenon is always mentioned. The same unfortunately is to a certain extent the case with the most comprehensive account of the results obtained by Dreyer's method in the British armies in France which was published by Leishman (1924). He gives a detailed analysis of the agglutination results obtained in 1110 cases of enteric infections, of which 516 had been non-inoculated or inoculated with the monovalent T. vaccine and 594 with the polyvalent T.A.B. vaccine; in both these groups were included 20 and 280 cases, respectively, classed finally as "enteric group." This detailed analysis clearly discloses two facts: (*a*) that re-stimulation of inoculation agglutinins occurs in enteric fevers very often, and (*b*) that the occurrence of this phenomenon caused great trouble in the laboratory diagnosis of enteric infections in the inoculated. It should be noted, however, that Leishman's general conclusion from analysis of the whole data available was expressed in the following terms: "...it will probably be admitted that there is no justification for the pessimistic view that triple inoculation has rendered the differential diagnosis of the enteric fevers by agglutination tests impossible" (p. 245).

5. Review of Observations on the non-specific Stimulation of the Weil-Felix Reaction.

As soon as the important rôle was recognised, which non-specific stimulation of inoculation agglutinins played in the use of the Widal test, the suspicion arose that the phenomenon would in an analogous way interfere with the application of the Weil-Felix reaction. Soon after its introduction in the routine diagnosis of typhus fever, it was observed that agglutinins for *B. proteus* X 19 may persist in the serum for many months or years after the convalescence; it was also established that the duration of this persistence depends upon the height of titre, which had reached its maximum at the time of defervescence (Felix, 1917, 1; Zlocisti, 1917). A re-stimulation of these persistent agglutinins for *B. proteus* X 19, similar to that known from the typhoid inoculation agglutinins, was expected to occur in the course of various febrile diseases, and to create an additional danger of errors in diagnosis.

The behaviour of persistent X 19 agglutinins, due to a previous typhus infection, was, accordingly, thoroughly investigated but no change due to re-stimulation could be noted (Felix, 1917, 1). Zlocisti (1917 and 1918), who paid particular attention to this question, was unable to establish, in typhus convalescents, any fluctuation in the X 19 agglutination titre as the result of various intercurrent febrile diseases, or as the result of subsequent inoculation with T. vaccine. Mühlens and Stojanoff (1917) and Seyfarth (1918) who, in Bulgaria, observed typhus fever in various combinations with relapsing fever and with benign and malign malaria, held the view that the non-specific stimulation of X 19 agglutinins is very likely to occur, but were unable to establish it in any single case.

It is surprising to find, upon carefully reviewing the very numerous papers on the X 19 agglutination, only a very few observations contrary to those quoted above. Arnstein (1917) observed in a case of pyrexia of doubtful origin a rise in X 19 agglutination titre from 1 : 25 to 1 : 100. The arguments offered by the author which are claimed to prove that a non-specific re-stimulation was set up in this case, can certainly not bear criticism.

The second observation, published by Starkenstein and Zitterbart (1918) is no more convincing. Shortly after inoculations against typhoid and cholera an X 19 titre of 1 : 200 was observed in a typhus convalescent whose serum had reacted ten months previously to a titre of 1 in 50 only. The same authors were unable to reproduce the phenomenon by injections of milk and of deuteroalbumose (according to Fleckseder, 1916) and in two cases of dysentery infection during late convalescence after typhus they recorded re-stimulation of T.-inoculation agglutinins only, whereas the X 19 agglutination titre remained completely unaltered.

Anders (1919) and Kraus and Barrera (1922) published observations on one patient and on two patients respectively, which they considered to be instances of non-specific re-stimulation of X 19 agglutinins. But in both these

papers the diagnosis of the cases has been assumed on entirely insufficient grounds.

The remarkable difference in the response to a non-specific stimulus, as observed in the Widal test and in the Weil-Felix test, was clearly formulated by Zlocisti (1918) and attested by Wolff (1922) and by Weigl (1924). Later, the same phenomenon was established in the experimental infection of the rabbit with typhus virus (Weil and Felix, 1921).

The striking difference in the behaviour of the curve of agglutinins for *B. typhosus* and *B. proteus* X 19 was welcomed by some workers as additional evidence in support of the view almost universally held, of the non-specific nature of the Weil-Felix reaction. Wolff (1922), for example, in a review on this reaction, used this argument in support of the much-favoured theory that the agglutination with *B. proteus* X 19 is not due to true immune bodies, and is only the expression of physico-chemical changes in the blood serum of typhus patients. As these physico-chemical changes are peculiar to typhus fever and cannot be reproduced by other conditions, the agglutination reaction with *B. proteus* X 19 cannot be re-stimulated by other febrile diseases.

The following investigation was undertaken with the view of testing this hypothesis.

6. A COMPARATIVE STUDY OF THE BEHAVIOUR OF AGGLUTININS FOR *B. TYPHOSUS* AND *B. PROTEUS* X 19 IN RESPONSE TO A NON-SPECIFIC STIMULUS.

The following tables are published to afford additional evidence of the reliability of the qualitative serum analysis in enteric fevers. It is not proposed to furnish any further data of the kind already discussed in the previous sections of this paper, which were directed to prove that the usual quantitative method of the agglutination test is not capable of differentiating inoculation and infection agglutinins. Such additional data appear to be superfluous.

It is also not proposed to give statistical figures on the application of the qualitative method. Since the publication of the writer's previous observations (1924, 1 and 2) this method has been used exclusively in the routine of the Widal test in the laboratory of the Hadassah Medical Organisation in Jerusalem. Table A shows the total number of examinations performed, together with a specification of cases of enteric infections which are included in the total number of examinations.

It will be sufficient to state that the conclusions arrived at in the previous papers (Felix, 1924, 1 and 2) were fully confirmed.

It is true that the number of cases of enteric infection in the inoculated recorded in Table A is relatively small, owing to the fact that in Jerusalem the majority of enteric infections occurs in children, which are not included amongst those persons compulsorily inoculated as immigrants. But the number of examinations of the serum of inoculated adults suffering from various non-enteric diseases was sufficiently great to convince the writer again that it is only thanks to the existence of the qualitative method that the Widal

reaction could usefully be applied as a laboratory test in inoculated persons. The clear negative diagnosis: "Widal due to inoculation" was not less helpful to the clinician than the answer: "Enteric group, type serologically unable to be differentiated owing to previous inoculation." In many instances the diagnosis "Widal due to inoculation" had to be given in the case of patients, whose blood samples were sent in with the explicit remark "non-inoculated." The presence in the serum of labilotropic H agglutinins for T. A. and B. simultaneously made this diagnosis, nevertheless, quite certain, and subsequent enquiry of the patient or checking of the inoculation records of the Department of Health, proved invariably the correctness of the laboratory diagnosis.

Table A. *Total Widal tests performed by means of the qualitative method at the Hadassah Laboratory in Jerusalem during* 1923–1926.

Hadassah Laboratory Jerusalem	Widal reactions performed: Total number of sera examined	Widal reactions performed: Number of sera yielding positive agglutination with T. A. or B.	Number of cases of one of the enteric fevers diagnosed by agglutination: Typhoid	Para. A.	Para. B.	E.G.*	Total	Number of cases diagnosed by successful blood culture: Typhoid	Para. A.	Para. B
1923	243	116	43	6	9	4	62	30	1	.
1924	394	120	41	1	17	16	75	18	.	1
1925	690	271	153	2	21	40	216	99	2	11
1926	894	440	150	2	40	74	266	67	.	6
1923–1926	2221	947	387	11	87	134	619	214	3	18

* E.G. = enteric group, type unable to be differentiated serologically.

On the other hand, it was an extremely rare occurrence that clinical and laboratory diagnosis were finally discrepant. The number of cases of pyrexias in inoculated individuals constantly negative to cultivation and undiagnosible as enteric group by agglutination, owing to the absence of O agglutinins, but sufficiently typical from the clinical side to be considered as enteric infections—was extremely small. The absence of O agglutinins in such cases is, of course, of the same significance only as is the constantly negative Widal test in a non-inoculated patient, viz. it is not decisive.

It must be stated that in a considerable proportion of the cases recorded in Table A the difficulties in the interpretation of agglutination results seemed at the beginning almost insurmountable. The adult patients admitted to the Jewish Hospitals with which the laboratory was connected, had in many cases the most complicated history as regards inoculation and previous enteric and typhus infections. Those who were immigrants came in the majority from Russia and Poland, where both these diseases are not only endemic since pre-war times, but have raged in epidemics of enormous extent during and after the war. Many of these adults had, as soldiers or civilians, received during the war repeated inoculations, mostly of the monovalent T. vaccine, and on entry into Palestine, all these immigrants were, in addition, inoculated with

T.A.B. vaccine. Here they were again exposed to both these infections, as enteric fever is prevalent throughout the country and typhus fever is endemic in many places. The smaller part of the adult patients were natives of Palestine or had been living there for many years and had not been recently inoculated with T. or T.A.B. vaccine; but many of them had a history, dating from the war, of enteric or typhus infection or of both these diseases.

The sera of these patients were the proper material for a comparative study on the behaviour of agglutinins for *B. typhosus* and *B. proteus* X 19 in response to a non-specific stimulus. This study, it was hoped, would give an answer to the following two questions:

(1) As regards *B. typhosus* and *B. paratyphosus* A. and B.—is the qualitative serum analysis, being based solely upon stabilotropic O agglutination, capable of distinguishing definitely agglutinins due to the specific stimulation in active enteric infection from those re-stimulated unspecifically by various febrile diseases?

(2) As regards *B. proteus* X 19—is there existing any difference between stabilotropic O agglutinins for *B. proteus* X 19 and those for *B. typhosus* and *B. paratyphosus* A. and B. as far as non-specific re-stimulation is concerned?

Table I. *Persistence, reappearance and non-specific fluctuation of labilotropic (H) agglutinins due to previous enteric infection.*

Non-inoculated patients.

No.	Name	Age	Time elapsing since previous typhoid infection	At present suffering from	Day of illness	Blood culture	Titre of agglutination with strains: Ty 901 l.	G 2 s.	A 1 l.	B 2 l.	X 19 s.
1	D. R.	35	20 years	Papatacci fever	6	Sterile	200	0	0	0	0
2	H.	29	21 years	Tuberculosis	7	Sterile	500	0	0	0	0
3	E. K.	29	26 years	Malaria	9	Sterile	200	0	0	0	0
4	F. T.	29	5 years	Pleurisy	6	Sterile	500	0	0	0	0
					8	Sterile	500	0	0	0	0
					12	Sterile	500	0	0	0	0
5	K. M.	24	10 years	Malaria	7	Sterile	500	0	0	0	0
					15	Sterile	500	0	0	0	0
					30	Sterile	500	0	0	0	0
6	M. A.	29	19 years	Typhus (1923)	9	Sterile	500	0	0	0	100
					13	Sterile	2000	0	0	0	1000
			20 years	Papatacci fever (1924)	5	Sterile	200	0	0	0	50
7	H. G.	45	5 years ago typhoid	Typhus	10	Sterile	200	0	0	0	500
			9 years ago paratyphoid A.		13	Sterile	500	0	200	0	2000
8	S. B.	30	5 years	Subphrenic abscess	30	Sterile	0	0	0	0	0
					39	Sterile	100	0	0	0	0
					46	Sterile	200	0	0	0	0
					51	Sterile	200	0	0	0	0
9	E. C.	19	1. vi. 1924	Typhoid	10	*B. typhosus*	2000	100	0	0	0
			14. xi. 1924	Typhus	9	Sterile	100	0	0	0	50
			16. xi. 1924		11	Sterile	200	0	0	0	200
			18. xi. 1924		13	Sterile	1000	0	0	0	2000

Technique: see Felix (1924, 2). Ty 901 = *B. typhosus* No. 901. G 2 = *B. enteritidis* Gärtner No. 2. A 1 = *B. paratyphosus* A. No. 1. B 2 = *B. paratyphosus* B. No. 2. l. = labilotropic (= large flakes). s. = stabilotropic (= small flakes). Titre 0 = a negative result in dilution 1 : 25 with X 19 or a negative result in dilution 1 : 100 with all the other organisms.

The observations demonstrated in the following tables give the answer to these two questions. The cases included in these tables are typical examples selected out of the great number recorded in Table A[1].

The first two tables illustrate the fact that the behaviour of labilotropic H agglutinins is exactly the same in the case of agglutinins due to a previous enteric infection (Table I) as it is in those due to previous prophylactic inoculation (Table II).

Table II. *Persistence, reappearance and non-specific fluctuation of labilotropic (H) agglutinins due to previous inoculation.*

			Inoculated						Titre of agglutination with strains				
No.	Name	Age	In	Time elapsing since inoculation	Vaccine	At present suffering from	Day of illness	Blood culture	Ty 901 l.	G 2 s.	A 1 l.	B 2 l.	X 19 s.
1	H. D.	18	Palestine	5 years	T.A.B.	Malaria	9	Sterile	200	0	0	0	0
2	G.	26	Palestine	6 years	T.A.B.	Typhus	16	Sterile	500	0	100	100	10,000
3	A. B.	26	Palestine	8 years	T.A.B.	Papatacci fever	6	Sterile	200	0	200	500	0
4	R. N.	27	Russia	9 years	*B. typhosus*	Typhus	7	Sterile	200	0	0	0	500
5	S. Y.	48	Palestine (1914)	12 years	*B. typhosus*	Malaria	12	Sterile	200	0	0	0	0
6	A. S.	26	Russia	7 years	*B. typhosus*	Papatacci fever	5	Sterile	100	0	0	0	0
				8 years		Influenza	4	Sterile	100	0	0	0	0
7	O. S.	30	Palestine	3½ years	T.A.B.	Tuberculosis	16	Sterile	0	0	100	0	0
							22	Sterile	0	0	200	100	0
							30	Sterile	0	0	500	0	0
8	E. A.	23	Palestine	2½ years	T.A.B.	Typhus	5	Sterile	100	0	0	0	0
							8	Sterile	100	0	0	0	25
							13	Sterile	500	0	100	0	1000
9	G. B.	26	Palestine	2½ years	T.A.B.	Typhus	9	Sterile	0	0	0	0	500
							15	Sterile	100	0	0	100	5000
10	N. G.	24	Palestine	1 year	T.A.B.	Malaria	15	Negative	500	0	200	0	0
							25	Negative	200	0	200	0	0
11	A. A.	14	Palestine	6 months	T.A.B.	Typhus	6	Sterile	0	0	0	0	25
							8	Sterile	100	0	0	0	500
							12	Sterile	200	0	0	0	1000
12	Z. S.	25	Palestine	3 months	T.A.B.	Pleurisy	13	Sterile	0	0	0	0	0
							15	Sterile	200	0	0	0	0

Table I clearly demonstrates the fact that, as a rule, agglutinins persisting for a long time in individuals who have suffered from an enteric infection, are of the labilotropic H type. The consistently negative results recorded under the heading for *B. enteritidis* Gärtner indicate the absence of stabilotropic O agglutinins for *B. typhosus*. With the single exception of case No. 7 in Table I concerning a patient who had suffered previously from both a typhoid and a paratyphoid A. infection, all these sera contained labilotropic H agglutinins for *B. typhosus* only. The same is seen in Table II in cases 4, 5 and 6 who had been inoculated with monovalent T. vaccine only. On the other hand, in those cases of Table II who had received injections of the polyvalent T.A.B. vaccine, the persisting or reappearing and fluctuating H agglutinins are some-

[1] For the friendly co-operation from the clinical side during the course of this work I am indebted to Dr Salkind (Hadassah Medical Organisation, Rothschild Hospital), Dr Wallach (Shaare Zedek Hospital), Dr Neuman (Bikur Cholim Hospital) and Dr Rokach, all from hospitals in Jerusalem.

https://doi.org/10.1017/S0022172400009712 Published online by Cambridge University Press

times noted for all three organisms concerned and sometimes for two or one only.

The same extremely wide range of variation in strength and persistence of inoculation agglutinins, well known from the numerous investigations in healthy individuals, is again met with in connection with non-specific re-stimulation. It appears quite conceivable that this range of variation should even be wider in re-stimulation, as here in addition to the factor of individuality a second variable factor is operating: viz. the degree of stimulation which may vary not only in different diseases, but even in one particular disease, according to the severity of the case.

It is well known that the sensibilisation of the human organism caused by an attack of typhoid is considered to be of life-long duration. Positive Widal tests persisting for several decades have long ago been recorded in such cases. As regards inoculation agglutinins, it was assumed, at first, that they would signify a transitory disturbance only. But it is now established beyond any doubt that this interference is a lasting calamity. Dreyer, Walker and Gibson (1915) had already observed T.-inoculation agglutinins in an individual inoculated 7 years previously. In a previous paper (Felix, 1924, 2) I had expressed the belief that as far as agglutination is concerned the sensibilisation of the organism by inoculation may be of as long a duration as that caused by an attack of enteric fever. Table II gives further evidence in support of this view.

Owing to this behaviour of the labilotropic H agglutinins, identical in nature in the case of inoculation agglutinins and in those due to a previous enteric infection, it appears entirely hopeless to develop an agglutination technique based on this type of agglutinins, for which diagnostic significance in individuals inoculated with T.A.B. could be claimed. Therefore, the proposed qualitative method of the Widal test entirely eliminates H agglutinins from any consideration in the diagnosis of active enteric infection in triply inoculated individuals and solely relies on the reaction of stabilotropic O agglutinins. Especial attention must therefore be paid to the smooth-rough variation of the cultures used (Arkwright, 1921) since it is the stabilotropic O agglutination which is affected by this variation (White, 1925). A suspension is essential which is purely smooth and contains no rough element. This is important for two reasons: (1) the spontaneous agglutinability of rough bacteria in salt solution, (2) the tendency of rough bacteria to agglutinate with non-specific sera (Schütze, 1922 and White, 1927).

In the following tables O agglutinins for *B. proteus* X 19 and for *B. typhosus* and *B. paratyphosus* A. and B. are shown in their behaviour to a non-specific stimulus.

Whereas in the case of H agglutinins, those due to inoculation and to a previous enteric infection could be compared as to their response to a non-specific stimulus, an analogous comparison could not be made in the case of stabilotropic O agglutinins. This type does not persist in human serum in

any appreciable amount as the result of prophylactic anti-enteric inoculation (Felix, 1924, 1 and 2), but it is known, especially from the work of Schiff (1922 and 1924) that the normal agglutinins in human beings as well as in some species of animals are exclusively of the stabilotropic O type. These normal O agglutinins for *B. typhosus*, *B. paratyphosus* A. and B. and *B. proteus* X 19 were, therefore, compared with the respective immune O agglutinins persisting in the serum of individuals who had passed through an attack of typhoid, paratyphoid A. or B. or typhus fever respectively.

Some remarks on normal agglutinins may be included here. It is known that considerable discrepancies exist in the opinions held concerning the relationship between normal and immune agglutinins. Schiff (1924) rightly emphasised that some of the points at issue might be elucidated by comparing, by means of the qualitative method, normal agglutinins with immune agglutinins of the same type, *i.e.* with stabilotropic O agglutinins.

In connection with the following tables, one point only needs especial consideration, it is the limit of agglutination titre for normal agglutinins. It is well known that as regards the amount of agglutinins contained in normal serum, very distinct differences exist not only in various species of animals, but also in individuals of the same species. On the other hand, it is equally well known that the agglutinability by normal serum varies most widely not only in different bacterial species, but also in individual strains of the same species.

As regards the first factor, the individual variation in the blood serum of members of the same species, it must be admitted that it is very difficult to fix a certain amount of serum, *i.e.* a certain serum dilution, as an absolutely valid limit. With increase of experience the critical limit has continually risen. It would appear that nature does not afford the sharp distinctions that are so desirable for our purposes.

In regard, however, to the second variable factor, namely, the different agglutinability by normal serum of individual strains of the same bacterial species, the qualitative receptor analysis has already afforded some light. Weil and Felix (1920) found in *B. typhosus* important differences in the sensibility of the stable O antigen in various strains; between the types of extreme sensitiveness and extreme unsensitiveness the difference in agglutinability by immune O agglutinins may be expressed by multiples of 10, 50 and even more. The degree of sensitiveness of the stable O antigen is strictly constant in every individual strain; in *B. typhosus* 901—a strain of the type of *B. typhosus* Rawlings, which is much used in this country—a perfectly constant maintenance of its remarkable sensitiveness has now been observed for as long as ten years. Similar conditions could, in the course of this time, be noted in *B. paratyphosus* A., whereas in *B. paratyphosus* B. this difference in individual strains seems to be far less conspicuous. [It may be added that entirely analogous differences in sensitiveness to bactericidal serum action are due to the same factor, as the immune body concerned in this reaction is identical with the stabilotropic O agglutinin (Felix and Olitzki, 1926).]

Since the stabilotropic O character of agglutination in the normal human serum has been established for the typhoid-paratyphoid group of organisms (Rotky, 1921), (Schiff, 1922), it became obvious that the limit of normal agglutination is dependent upon the sensitiveness of the O antigen of the strains used. For the strain *B. typhosus* 901, constantly used in our laboratory in Jerusalem in the routine of the Widal test, it had already been insisted upon (Felix, 1924, 1 and 2) that only a strongly positive agglutination in the dilution 1 : 100 should be regarded as a positive reaction. On the strength of additional experience I should rather prefer the dilution 1 : 200 to be taken as this limit, in spite of a certain loss in the important advantage in the earlier diagnosis of cases, which is the consequence of this precautionary measure.

Table III. *Normal (O) agglutinins for* B. proteus *X* 19 *in the course of a non-typhus disease.*

Non-inoculated patients.

No.	Name	Age (years)	Diagnosis	Day of ill-ness	Blood culture	Titre of agglutination with strains: Ty 901 l.	Ty 901 s.	G 2 s.	A 1 s.	B 2 s.	X 19 s.
1	H. S.	1½	Typhoid	12	*B. typhosus*	0	500	100	100	0	100
				21	Negative	0	2000	200	100	0	100
2	E. G.	21	Typhoid	8	*B. typhosus*	0	200	0	0	0	50
				18	Sterile	0	2000	100	200	0	50
3	E. A.	12	Typhoid	8	*B. typhosus*	2000	200	100	0	0	50
				75	Sterile	2000	0	0	0	100	50

Table IV. *Normal (O) agglutinins for* B. typhosus *and* B. paratyphosus *A. and B. in the course of various non-enteric diseases.*

No.	Name	Age	Diagnosis	Day of ill-ness	Blood culture	Titre of agglutination with strains: Ty 901 s.	G 2 s.	A 1 s.	B 2 s.	X 19 s.	Remarks
1	O. A.	28	Papatacci fever	5	Sterile	200	0	0	0	0	Inoculated
				11	Sterile	200	0	0	0	0	2 years ago (T.A.B.)
2	H. J.	28	Influenza	3	Sterile	200	0	0	0	0	Non-inoculated
				5	Sterile	200	0	0	0	0	
3	S. A.	19	Malaria quartana	7	Sterile	100	0	0	0	0	Non-inoculated
				10	Sterile	100	0	0	0	0	
				12	Sterile	100	0	0	0	0	
4	B. Z.	15	Tuberculosis	7	Sterile	200	100	100	100	50	Non-inoculated
				23	Sterile	200	100	100	100	50	
				48	Sterile	200	100	100	100	50	
				60	Sterile	200	100	100	100	50	
				94	Sterile	200	100	100	100	50	
				138	Sterile	200	100	100	100	50	

In the case of *B. proteus* X strains, it was stated by Weil and Felix (1916, 2) that agglutination in a dilution 1 : 25 of normal serum occurs in some 10–12 per cent. with the strain X 2 and in some 7 per cent. with the strain X 19 (Felix, 1916). Other workers prefer to fix the limit for normal agglutination with X 19 much higher, mostly in the dilution 1 : 100, some of them even at 1 : 200. In countries with endemic typhus, where the possibility of persistence in the serum of X 19 agglutinins due to a previous infection must be taken

into account, absolute diagnostic significance cannot be claimed for any of these limits.

In Tables III and IV is shown the behaviour of normal O agglutinins for X 19 and for the typhoid-paratyphoid group of organisms respectively. Cases were selected with the view of demonstrating normal agglutinins with the highest observable titre exposed to the non-specific stimulus of various febrile diseases. Every possible care was taken to obtain a reliable history of each patient in order to exclude a previous infection with the organism concerned.

It will be noted that no fluctuations whatever could be observed in the titre of these normal O agglutinins. For the sake of brevity, the results had to be tabulated in this somewhat condensed form, otherwise it could have been clearly brought out that the intensity of agglutination in the respective dilutions invariably remained unchanged. The precaution already mentioned in Section 4 was also taken in the course of this investigation; in many instances the sera taken on successive occasions from the same patient were, after having been tested, stored in the ice box and then simultaneously re-examined.

Table III demonstrates the steadiness of a relatively high titre of normal O agglutinins for X 19 in the course of typhoid infection. Similar observations had already been made by Weil and Felix in the early days of the use of their reaction, and were, in the course of the following years, repeated on a very great number of patients. The normal O agglutinins for *B. typhosus* and *B. paratyphosus* A. and B., demonstrated in Table IV, show exactly the same behaviour.

It may at first appear to be superfluous to bring forward observations of this kind in regard to normal agglutinins, as the fact described is known from the older work of the founders of immunology and is generally accepted as one of the fundamental principles of serological specificity. Nevertheless, in the course of the discussion of the theory of the Weil-Felix reaction, this fact has often been questioned. Doerr and Pick (1919), for instance, in a study on the typhus infection in the rabbit, were led, by the observation of an increase in normal agglutinins for *B. typhosus*, to the erroneous conclusion, that the simultaneously observed rise in X 19 agglutinins was equally an "entirely non-specific" phenomenon. This untenable argument has not yet been eliminated from the discussion on the X 19 agglutination.

The correctness of those observations which stated an increase in normal immune bodies, as a result of various non-specific stimuli is, of course, not called in question. In these cases, the rise in the titre of the blood serum becomes manifest immediately after the inoculation (in the course of a few hours) and is, therefore, considered to be caused by a rapid mobilisation of pre-formed antibodies from the internal organs. The present study is not concerned with this phenomenon which, if properly taken into account, will hardly ever interfere with serum diagnosis.

In Table V persisting immune O agglutinins for *B. proteus* X 19, due to

a previous typhus infection, are demonstrated under the stimulus of various febrile diseases. The majority of cases described in this table are typhoid infections, as the opportunity of getting an adequate number of blood samples at the appropriate intervals presented itself more often in this type of patient. Cases of typhoid relapse were examined with particular care. Table V shows

Table V. *Immune (O) agglutinins for* B. proteus *X* 19, *due to a previous typhus infection, in the course of various non-typhus diseases.*

No.	Name	Age	Time elapsing since previous typhus infection	At present suffering from	Day of ill-ness	Blood culture	Titre of agglutination with strains: Ty 901 l.	Ty 901 s.	G 2 s.	A 1 s.	B 2 s.	X 19 s.	Remarks
1	T. C.	21	5 years	Typhoid	14	Sterile	0	2000	200	100	100	100	
					20	Sterile	0	2000	200	100	100	100	
					61	*B. typhosus*	0	100	0	0	0	100	Typhoid relapse
					71	Negative	0	500	100	0	0	100	
2	W. S.	22	6 years	Typhoid	6	*B. typhosus*	0	200	0	0	0	100	
					9	*B. typhosus*	0	500	100	100	0	100	
					19	Sterile	0	500	100	100	0	100	
					39	*B. typhosus*	10,000	200	100	0	100	100	Typhoid relapse
3	C. F.	25	6 years	Typhoid	7	Negative .	0	500	100	100	100	100	
					14	*B. typhosus*	0	500	100	100	100	100	
					18	*B. typhosus*	0	1000	200	200	200	100	
					21	Negative	200	1000	200	200	100	100	
4	S. D.	19	3 years	Typhoid	8	Sterile	0	500	100	200	200	100	
					72	*B. typhosus*	2000	500	100	0	100	100	Typhoid relapse
5	B. E.	24	5 years	Influenza	9	Sterile	0	0	0	0	0	200	Inoculated
					12	Sterile	0	0	0	0	0	200	4 years ago
					15	Sterile	0	0	0	0	0	200	(T.A.B.)
6	E. G.	23	6 years	Angina	5	Sterile	0	0	0	0	0	200	
					9	Sterile	0	0	0	0	0	200	
7	M. Y.	42	5 years	Pneumonia	6	Sterile	0	0	0	0	0	100	
					11	Sterile	0	0	0	0	0	100	

that no fluctuation whatsoever was noted in the titre of the persisting immune O agglutinins for X 19, in spite of the well-marked changes in the titre of the specific agglutination (H and O) for *B. typhosus* and of the group agglutination (O) for *B. paratyphosus* A. and B. The steadiness of the stabilotropic X 19 titre also in the cases 5–7 showed that there was no suspicion of an active typhus infection being involved.

Table VI shows that analogous conditions are obtaining in the stabilo-

Table VI. *Immune (O) agglutinins for* B. typhosus *and* B. paratyphosus *A. and B., due to a previous enteric infection, in the course of various non-typhoid diseases.*

No.	Name	Age	Time elapsing since previous typhoid infection	At present suffering from	Day of ill-ness	Blood culture	Titre of agglutination with strains: Ty 901 s.	G 2 s.	A 1 s.	B 2 s.	X 19 s.	Remarks
1	Z. F.	18	2½ years	Papatacci fever	8	Sterile	0	0	500	100	0	Non-inoculated
					12	Sterile	0	0	500	100	0	
2	T. L.	25	2 years	Typhus	11	Sterile	500	100	0	200	25	Non-inoculated
					15	Sterile	500	100	0	200	200	
					17	Sterile	500	100	0	200	500	
3	S. B.	23	6 months	Undulant fever	6	Sterile	100	0	0	0	0	Non-inoculated
					45	Sterile	100	0	0	0	0	Titre of agglutination with
					71	Sterile	100	0	0	0	0	Micrococ. melitensis
					102	Sterile	100	0	0	0	0	1 : 2000
					173	Sterile	100	0	0	0	0	1 : 2000

https://doi.org/10.1017/S0022172400009712 Published online by Cambridge University Press

tropic O agglutination for *B. typhosus* and *B. paratyphosus* A. and B. Independently of the formation of the specific agglutinins (for X 19 in Case 2 and for Micrococcus melitensis in Case 3) the persisting stabilotropic agglutinins for the typhoid-paratyphoid group of organisms invariably remained unaltered in titre. This steadiness of their stabilotropic O titre forms a striking contrast to the fluctuations seen in their labilotropic H titre.

The criticism might be raised that the cases brought forward in Tables V and VI cannot serve as valid evidence to prove the existence of this difference in the behaviour of O and H agglutinins, although the various diseases, whose effect on persistent O agglutinins is investigated in these tables, are all known as being capable of re-stimulating persistent H agglutinins. As this latter phenomenon does not occur constantly but in a certain proportion of cases only, it remains unproved, that the non-specific stimulus actually acting in the particular cases described in Tables V and VI was strong enough to produce the phenomenon even in H agglutination. Some examples are, therefore, given in Table VII, demonstrating various O and H agglutinins simultaneously persisting in a patient's serum and exposed to the non-specific stimulus of an intercurrent disease.

Table VII. *O and H agglutinins, simultaneously present in a patient's serum, under the non-specific stimulus of an intercurrent disease.*

							Titre of agglutination with strains							
							Ty 901		G 2	A 1		B 2		X 19
No.	Name	Age	*History*	At present suffering *from*	Day of ill-ness	Blood culture	l.	s.	s.	l.	s.	l.	s.	s.
1	U. A.	29	4 years ago T.A.B. inoculation	Pleurisy	9	Sterile	0	100	0	100	0	0	0	100
					12	Sterile	0	100	0	500	0	0	0	100
					15	Sterile	0	100	0	1000	0	0	0	100
2	K. T.	25	4 years ago T.A.B. inoculation	Papatacci fever	6	Sterile	100	0	0	0	0	0	0	100
			15 years ago typhus		9	Sterile	100	0	0	200	0	0	0	100
3	B. M.	28	14 months ago T.A.B. inoculation	Typhus	10	Sterile	100	500	0	100	0	0	0	25
			2 years ago typhoid		13	Sterile	100	500	0	100	0	0	0	100
					15	Sterile	100	500	0	100	0	100	0	200
					17	Sterile	100	500	0	200	0	100	0	500
					21	Sterile	100	500	0	1000	0	200	0	200

It is clearly shown in Table VII that the non-specific stimulus exerted in these particular cases of pleurisy, papatacci fever and typhus fever was sufficiently strong to bring about a well-marked fluctuation in the titre of H agglutinins, but was unable to cause any fluctuation in the respective titres of simultaneously present O agglutinins. In Cases 2 and 3, labilotropic agglutinins for A. and B. respectively were re-stimulated, which, before the beginning of the disease had already fallen below the limit of normal agglutination, whereas the stabilotropic agglutinins for *B. typhosus*, persisting in Case 3 in a remarkably high concentration (1 : 500), remained completely unaltered. Cases 2 and 3 also show again that, out of several different H agglutinins due to a previous sensibilisation, on occasion, one or other only may undergo re-stimulation when exposed to the same non-specific stimulus; and that agglu-

tinin, which persists in a higher concentration in the serum, is not necessarily the more liable to show the phenomenon.

The following directions were drawn up for the interpretation of agglutination tests in patients with this complicated history of previous typhus or enteric infections, and T.A.B. vaccinations, on the basis of the work discussed above:

(*a*) The appearance of or the fluctuation in titre of stabilotropic O agglutinins for *B. proteus* X 19 and for the enteric group of organisms, was considered to be significant for an active typhus or enteric infection respectively.

(*b*) A different significance, however, was attached in the two groups of infections to the absence of O agglutinins or to the steadiness of their titre, when observed throughout the whole course of the disease:

(i) as regards enteric infection this negative result was not considered to be decisive (in analogy to the negative Widal in non-inoculated patients);

(ii) as regards typhus infection this negative result was, as a rule, considered to be decisive, as the complete absence of X 19 agglutinins throughout the whole course of the disease, is entirely exceptional.

If these conclusions will be adopted and tested by further clinical observations on a large scale, it will be found that an advance has been made in the agglutination diagnosis of typhus and of enteric fevers.

Thus, the answer to each of the two questions put forward at the beginning of this section is in the affirmative:

(1) The proposed qualitative method seems to be definitely capable of distinguishing agglutinins due to the specific stimulation in active enteric infection from those re-stimulated unspecifically by various febrile diseases.

(2) As far as non-specific re-stimulation is concerned, no difference whatsoever seems to exist between stabilotropic O agglutinins for *B. proteus* X 19 and those for *B. typhosus* and *B. paratyphosus* A. and B. The striking difference known to obtain in this respect between the Widal test and the Weil-Felix test disappears entirely, when the labilotropic component of the Widal test is eliminated and the purely stabilotropic Weil-Felix reaction is compared with the stabilotropic component of the Widal reaction only.

The results obtained in the course of this investigation were always uniform; the difference in the behaviour of labilotropic and stabilotropic agglutinins in response to a non-specific stimulus was invariably constant in the serum of patients suffering from various febrile diseases. This fact would seem to suggest that the phenomenon is an additional observation in the series of qualitative differences already established in regard to certain physical, chemical, and biological properties of the two different kinds of antigen and antibody. The existing evidence, however, from animal experiments, seems to indicate that this difference is more likely one of degree than one in nature of

response. This problem has not yet been investigated adequately by means of the qualitative method, and some preliminary experiments on rabbits only gave results from which no conclusion could be drawn. But the existing literature, especially the more recent papers by Tsukahara (1921) and Jaggi (1923), contains many indications that in animal experiments non-specific re-stimulation of agglutinins has been demonstrated in bacterial species which possess both the H and O antigen as well as in those possessing O antigen only. This question needs still further investigation.

SUMMARY OF SECTIONS 1–4 (pp. 419–433).

(1) The review of the published data furnishes additional evidence in support of the view that no technique whatsoever, Dreyer's technique included, based on the quantitative method of the agglutination reaction hitherto used, is capable of affording a differentiation between inoculation and infection agglutinins.

(2) These techniques are concerned always exclusively in the demonstration of the labilotropic H agglutinins of *B. typhosus* and *B. paratyphosus* A. and B. and it is the behaviour of these agglutinins that is the responsible factor in producing the phenomena.

(3) In various febrile conditions in inoculated individuals these H agglutinins undergo a re-stimulation resulting in a curve of agglutination which is indistinguishable from that due to specific stimulation. The re-stimulation of the labilotropic inoculation agglutinins is of the same non-specific character (*i.e.* heterologous) in the course of enteric infections as in the course of other febrile diseases.

(4) The observation of this non-specific re-stimulation is independent of the technique used; living bacilli and suspensions preserved with phenol or formalin (Dreyer's technique included) do not in this respect behave differently.

(5) The proposed qualitative method for the Widal test depends, in inoculated individuals, exclusively upon the behaviour of the stabilotropic O agglutinins. In their presence it is capable of affording the certain diagnosis of an enteric infection; in their absence the negative result of the test is not conclusive; if T.A.B. vaccine has been used it is only possible to diagnose enteric group without being able to differentiate typhoid from paratyphoid A. or B.; if T. vaccine has been used then A. or B. infection can be differentiated but not T.

SUMMARY OF SECTIONS 5–6 (pp. 434–445).

(1) The conclusions previously arrived at by means of the qualitative method of the Widal test were fully confirmed. By eliminating the labilotropic H agglutinins from any consideration—in the case of previously sensibilised individuals—agglutination due to the specific stimulation in active enteric infection can be distinguished definitely from that due to the non-specific re-stimulation by various febrile diseases.

(2) Normal and immune O agglutinins for *B. typhosus* and *B. paratyphosus* A. and B., as well as those for *B. proteus* X 19, are not liable to non-specific stimulation in the course of various febrile diseases.

(3) One more of the supposed differences in nature between the Widal test and the Weil-Felix test is thereby eliminated.

(4) The difference in the response to non-specific stimulation shown to exist in stabilotropic and labilotropic agglutination seems more likely to be one of degree than one in nature and needs further investigation.

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(*MS. received for publication* 14. XI. 1928.—Ed.)