SEROLOGICAL DIAGNOSIS OF THE ENTERICA.

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(With 1 Figure.)

Introduction.

This paper is written from the practical point of view of a laboratory worker who is daily in direct contact with the cases submitted to him for diagnosis. Its purpose is to prove the inefficiency of the Widal reaction as a diagnostic test, and to urge its replacement by a more satisfactory method. Such a method is to be found in the application of the discoveries and theories on agglutination of Weil and Felix (1920), Felix (1924 and 1929), and Felix and Olitzki (1928 and 1929).

By the Widal reaction is meant the agglutination of a chemically preserved suspension of dead typhoid bacilli by patients' serum. Its unsatisfactory nature was particularly brought home to me through my observations in South Africa, extending over the last 9 years and comprising well over a thousand cases of enterica. The exceptionally close contact between the laboratory, the clinician, the hospital ward, and the public health authorities which is a feature of my work, impressed upon me the need for a reliable diagnostic method for the enterica, such as few laboratories seem to have felt. The difficulties I experienced with the Widal were therefore not of my own making, but resulted from the situation. A laboratory which is kept in touch with all its diagnostic failures cannot afford to employ the Widal reaction as a diagnostic method. I do not refer here to the difficulties which have arisen for the Widal reaction through prophylactic inoculation. Put briefly, I found that the Widal reaction only gave from 50 to 60 per cent. positives, and this naturally condemned the reaction as a diagnostic test.

The Widal reaction in South Africa.

On a former occasion (1923) I showed that out of 61 culturally-positive cases of typhoid fever only 28 gave a positive Widal reaction, and that of 59 clinically well-established cases only 29 did so. The tests were done with all due precautions and many refinements. The first blood sample only from each patient was considered, but with three exceptions they were all taken after the first 10 days of illness.

In 1928 the estimated population of the Union of South Africa was 1·7 million Europeans and 6 million non-Europeans (chiefly Bantu). During that year 2557 European cases of enterica were notified and 3230 non-European.

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The incongruity of these figures is partly due to defective notification, but also to a large extent to inefficient serological diagnosis, or in other words, missed diagnosis. The relatively high incidence of enterica in South Africa is certainly due to "missed" cases and "missed" carriers, and it can safely be said that the incidence figures will have to become a good deal higher (through improved serological methods), before they can be expected to come down (through appropriate measures).

Most general practitioners and health authorities have had sufficient experience of the Widal reaction to have lost all faith in it and this has very unfavourably influenced their opinion of laboratory diagnoses in general. The inadequacy of the Widal reaction used to be freely admitted in the *Annual Reports of the South African Institute for Medical Research* (from 1920 onwards).

Recent proof of the failure of the ordinary Widal reaction as a diagnostic method will be found in the tables of this paper.

THE WIDAL REACTION ELSEWHERE.

In other countries this inherent diagnostic weakness of the Widal reaction has apparently not become so evident. This is partly due to the lower incidence of enterica, and partly to the greater feasibility of cultural methods. Also, attention has been diverted from the real issue by the difficulties arising out of prophylactic inoculation agglutinins. Lastly, tradition and sentiment create a reluctance to condemn ancient methods.

Few publications thus deal directly with this problem, but the papers of Schmitz (1916), Kalthoff (1917), Koritschoner (1917), Bitter (1917 and 1920), Kleinsorgen (1921), Hadjopoulos (1922), Löhr (1924), Oster (1925), Bohnenkamp and Kliewe (1927) and Herderschêe (1929) disclose that only between 40 and 60 per cent. of typhoid cases could be diagnosed by means of the Widal reaction, at a reasonably early date.

THE WIDAL REACTION IN GENERAL.

It is clear that if there is a difference between the inefficiency of the Widal reaction in South Africa and elsewhere, the difference is only one of degree, and slight. South African strains of typhoid bacilli, in my experience, are lacking in O-sensitiveness, and this may have some bearing on the findings. The distribution of blood groups in South Africa corresponds to that in middle Europe, and therefore the observations of Groetschel (1927) and Hilgers, Wohlfeil and Knötzge (1928) on the correlation of blood groups and agglutinin formation give no guidance. According to rabbit experiments of Pijper and Pullinger (1926), the high degree of insolation in South Africa may adversely affect the production of agglutinins, but only to a slight extent. The question of the preponderance in South African serums, of O-agglutinins over H-agglutinins as compared with other countries, will be discussed later. The results of the Widal reaction in South Africa do not differ much from those elsewhere, but under South African conditions its weakness revealed itself

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more readily. The traditional excuse for its failure, that a positive result cannot be expected early in the disease, is refuted by the observation that few serums here reach the laboratory in the first week of illness. Local conditions preclude the use of subsidiary methods such as blood culture. The need for a better serological method is quite imperative.

Complement-fixation as a diagnostic method.

From 1922 till 1928 I used complement fixation for the diagnosis of typhoid fever. I could show (1923) that this gave 96 per cent. positive results, and Pijper and Pullinger (1928) showed that it was a very suitable method for the detection of carriers. These and other advantages of the complement-fixation reaction over the Widal only came to be understood through the work of Weil and Felix, Felix, and Felix and Olitzki, mentioned above.

Weil and Felix's qualitative receptor analysis.

There is no need to describe here in detail the history, the principles, and the technique of this new diagnostic method. I shall assume all that as known. For simple clinical purposes enough information is found in the publication of Stuart and Krikorian (1928), who have applied the method in a large number of cases and completely confirmed the claims made for it.

The introduction of the strain *B. typhosus* O 901 (Felix and Olitzki) has done away with the difficulties in reading results that used to be brought up against the method. It is much more useful in this respect than the routine use of Gaertner strains or alcoholic suspensions for this purpose, because it is much more sensitive. Still, I would advise anyone taking up qualitative receptor analysis to prepare and work with artificially prepared rabbit serums made with several kinds of bacilli, both 60° and 100° serums, as I have done. In this way one can confirm certain principles underlying the method for oneself and at the same time get the necessary practice in reading H- and O-agglutination.

During the past twelve months I have done a large number of agglutination reactions with the blood serum of both typhoid and paratyphoid patients, using certain strains kindly given to me by Dr Felix of the Lister Institute, certain suspensions obtained through the kindness of Dr Gardner of the Standards Laboratory of Oxford, and certain strains and suspensions of local origin. The strains from Dr Felix were: *B. typhosus* H 1 (Weil and Felix), *B. typhosus* H 901 (Weil and Felix), *B. typhosus* O 901 (Felix and Olitzki), *B. gaertner* 2. From Oxford I used both the standard saline and alcoholic typhoid bacilli suspensions. The results were recorded in the usual way, both for H- and O-agglutination, and are chiefly found in Tables I and II. They refer to the first blood sample taken from any patient, so as to give a true picture of what happens under ordinary routine conditions of laboratory work. The serums were used unheated, and the reactions were done at 37° for 2 hours and finally read after a further 22 hours on the bench, except the tests

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with Oxford suspensions which were done at 50° and also read after 24 hours. In most cases only one serum dilution was used, 1 : 100. A lens was always used for reading results.

For various reasons not all the patients could be tested with all the different strains and suspensions. Tables I and II are best read as follows: the first

Table I. *Showing agglutination results of unheated patients' serums with various suspensions. Diagnosis established by blood culture* (B. typhosus). *Dilution of serum* 1 : 100. *Readings after* 24 *hours.*

Type of agglutination	*B. typhosus* H 901 (alive)	*B. typhosus* "Pretoria" (alive)	*B. typhosus* 1 (alive)	*B. Gaertner* 2 (alive)	*B. typhosus* H 901 killed and phenol	Alcoholic suspension Oxford Standard Laboratory
+ + + H and O	10	0	2	0	2	0
+ + + O	10	0	0	1	0	18
+ + H and O	5	3	2	0	1	0
+ + O	9	4	4	7	3	0
+ H and O	0	1	1	0	0	0
+ O	4	9	13	7	2	1
Doubtful	1	3	4	5	1	4
Negative	1	13	6	12	2	3
Total positives	38 out of 40	17 out of 33	22 out of 32	15 out of 32	8 out of 11	19 out of 26

Table II. *Showing agglutination results of patients' serums with various suspensions. Diagnosis established by clinical signs and course. Cases chiefly typhoid, but some paratyphoid A. Dilution of serum* 1 : 100. *Readings after* 24 *hours. Serums not heated.*

Type of agglutination	*B. typhosus* H 901 (alive)	*B. typhosus* "Pretoria" (alive)	*B. typhosus* 1 (alive)	*B. Gaertner* 2 (alive)	*B. typhosus* H 901 killed and phenol	*B. typhosus* "Pretoria" killed and phenol	Alcoholic suspension Oxford Standard Laboratory
+ + + H and O	42	2	12	0	3	2	0
+ + + O	29	1	4	6	4	1	50
+ + H and O	19	11	15	0	3	3	0
+ + H	0	1	1	0	0	0	0
+ + O	33	9	16	28	2	3	3
+ H and O	0	9	9	0	0	1	0
+ H	1	1	2	0	0	0	0
+ O	18	20	16	31	6	2	3
Doubtful	4	12	10	9	4	1	1
Negative	2	39	41	52	12	10	18
Total positives	142 out of 148	54 out of 105	75 out of 126	65 out of 126	18 out of 34	12 out of 23	56 out of 75

column shows that out of 40 and out of 148 first blood samples of patients, a positive result was recorded respectively 38 and 142 times, when a live suspension of the strain *B. typhosus* H 901 was used, and in Table I there are ten such positive results which showed + + + H- and O-agglutination. It would have taken too much space to quote the complete range of the reactions of every individual.

These tables call for the following comments:

1. In Table II the column headed "*B. typhosus* 'Pretoria,' killed and phenol," represents the ordinary Widal. It confirms that with the ordinary

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Widal only about 50 per cent. positives are obtained. I have in 24 additional cases of typhoid substituted formol for phenol and got exactly 12 positives. Other local strains have also been tried under similar conditions in a smaller number of cases, and gave similar results. I also tried Standard Agglutinable Culture *B. typhosus* from the Oxford Standard Laboratory in 20 cases, again with similar results, *i.e.* barely 50 per cent. positives.

2. Felix and Olitzki (1928) have shown that low concentrations of formol or phenol inhibit O-agglutination, and have no such effect on H-agglutination. This phenomenon helps to explain negative Widals, but it cannot be the whole explanation, as this inhibition is not absolute, and many of my cases still give O-agglutination with suspensions containing these chemical preservatives. This inhibition, and also its partial nature, is well illustrated in Table III.

Table III. *Showing the difference in agglutinability of* B. typhosus *H* 901, *alive, and after heating at* 60° *and adding formol to a concentration of* 0·1 *per cent. in the agglutination tube. Readings after* 24 *hours.*

Patient	*B. typhosus* H 901 (alive)	*B. typhosus* H 901 heated and formol added
1	+ + +H and O	+ +H and O
2	+ + +H and O	+ + +H and O
3	+O	—
4	+ + +O	+O
5	+ + +H and O	+ + +H and O
6	+O	—
7	+O	—
8	+ +H and O	+H and O
9	+ + +H and O	+ + H and O
10	+ + +O	—
11	+ + +H and O	+ +H and O
12	+ + +H and O	+ + +H and O
13	+ + +O	—
14	+ +O	—
15	+ +O	+ +O
16	+ +O	+ +O

That it is not only the addition of preservative chemicals which results in too small a number of positive reactions, is shown by another column in Tables I and II, from which it appears that *B. typhosus* "Pretoria," used alive, did not work satisfactorily.

3. It is quite clear from Tables I and II that the best results are obtained with live suspensions or alcoholic suspensions, and that the strain *B. typhosus* H 901 (Weil and Felix) is by far the most sensitive. It works much better than *B. typhosus* 1 or the strain "Pretoria," which is an old laboratory strain, isolated many years ago from the blood of a Pretoria patient. Quite good results are obtained with the alcoholic suspension of the Oxford Standard Laboratory, but not so good as with *B. typhosus* 901.

The secret of the problem of a sufficient number of positive agglutination results obviously is bound up with the factor "sensitiveness."

4. Felix's demand that live suspensions shall be used for agglutination reactions (with the exception perhaps of alcoholic suspensions for O-agglu-

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tination) is quite justified by Tables I and II. The sensitiveness of the very sensitive strain *B. typhosus* 901 is clearly spoilt by heating and the addition of chemicals. I have also made a series of tests using this strain as an alcoholic suspension, prepared according to the usual method, but this treatment also damaged its sensitiveness to a marked extent.

5. It has been suggested by Gardner (1929) that in South Africa patients form relatively more O-agglutinins than in Europe. Tables I and II seem to support this view. The quantity of O-agglutinin formed may be a variable factor, depending on season, country, or infective agent. Felix thinks that O-agglutinins are more abundant in the first and last stages of the disease, and as far as I can gather from my own figures, this is the case. Table II cannot be used in this discussion as it stands, because it contains a certain number of cases of paratyphoid A. But Table I also shows a relatively large number of cases that produce O-agglutinin only. In my opinion the possibility exists that there is no difference in this respect between European and South African cases, but that just through the use of a highly sensitive reagent in the shape of live suspensions of *B. typhosus* H 901, such cases are diagnosed as typhoid, which might not have been the case otherwise. I believe that many such cases escape recognition in South Africa, and perhaps elsewhere. Clinicians are inclined to attach too much importance to the traditional text-book picture of enteric. I therefore think it worth while to illustrate this point by some temperature charts of enterica cases observed by me. In Fig. 1 six cases are shown. The first one was diagnosed serologically as enteric quite early in the disease (at "Aggl."). As the temperature chart was so very irregular, much more like a septicaemia than an enteric, blood culture was attempted (at "C") and resulted in a pure growth of *B. paratyphosus* A, and another positive agglutination reaction with *B. typhosus* 901. The second case was ill for a few days, and was diagnosed as enteric (at "Aggl.") by a positive agglutination with *B. typhosus* 901. The patient, another native boy from a mine hospital, then recovered and wanted to go back to his work. He returned to hospital a few days later, and ran a temperature for another week. During that week a pure growth of typhoid bacilli was obtained from his blood (at "C"). Such cases are by no means exceptional. The third case illustrated had fever for 12 days only, which came down by crisis, and would probably have been diagnosed as pneumonia, if (at "C") the blood culture and the agglutination reaction with *B. typhosus* 901 had not been positive. The last three cases of Fig. 1 are all native mine labourers. The first two are brought up to show that many patients are able to walk about whilst having typhoid fever and only go to hospital when a perforation of an enteric ulcer, such as was found at the *post-mortem* in these two cases, compels them to do so. The last case was first regarded as an appendicitis and duly operated on. The appendix was found normal, but typhoid ulcers were seen, and thus fully confirmed the correctness of the positive agglutination reaction found in the blood the next day. Here again there was nothing in the clinical signs to suggest typhoid.

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6. There is no doubt that O-agglutination is the most important factor in the serological diagnosis of the enterica. Apart from what appears in Tables I and II, I met with many cases of typhoid fever which would have escaped

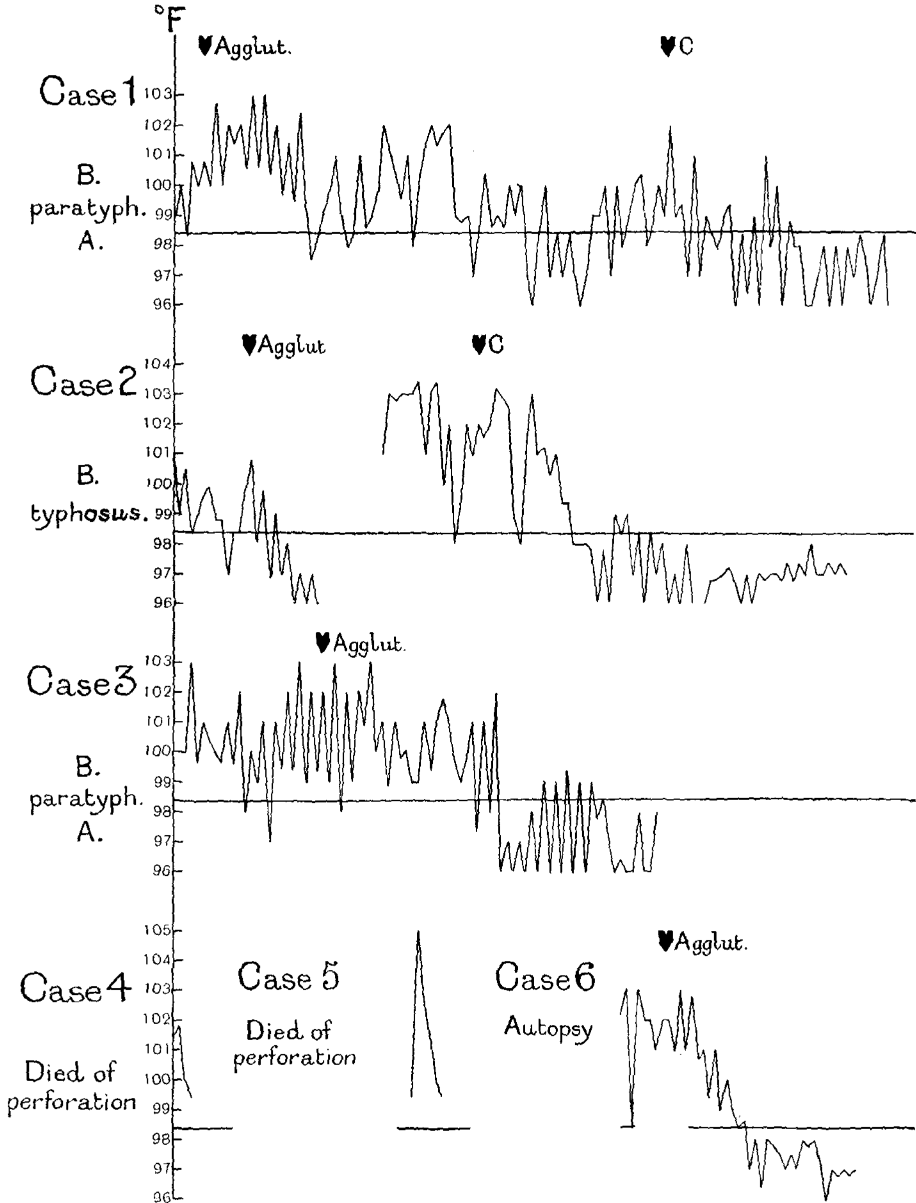

Fig. 1.

recognition if it had not been for a slight degree of agglutination of the O-type with live suspension of *B. typhosus* 901. Felix (1929) has stated that O-agglutinins are absent very rarely. The diagnosis of the enterica, in my experience,

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hinges on the demonstration of O-agglutination. One should not lose sight of the H-agglutination as naturally O-agglutination only allows of a diagnosis of enteric being made, the H-agglutination solely being able to indicate which specific group of typhoid or paratyphoid bacilli is concerned. For ordinary clinical purposes one has often to be content with a diagnosis of "enteric" without further specification, when insufficient H-agglutinins are formed to allow of a specific diagnosis.

7. The cases tabulated in Table II are of two kinds. Some are Pretoria and Transvaal cases, others are native mine labourers from the Witwatersrand. Many of these do not belong to the Union of South Africa, and there is not much contact between these natives, living in compounds, and the surrounding population. In and around Pretoria, and probably in the whole Transvaal, paratyphoid fever is non-existent. Amongst hundreds of strains of typhoid bacilli isolated during the last 9 years, I had never met with a paratyphoid strain. I had therefore at first not felt the necessity of putting up a para-

Table IV. *Serum dilution* 1 : 100. *Readings after* 24 *hours. Typhoid and paratyphoid A patients. Serums not heated.*

Origin of patient	*B. typhosus* H 901 (alive)	Alcoholic suspension Oxford Standard Laboratory	*B. paratyphosus* A (alive)	Blood culture
Native, Mine compound	+ + O	+ + + O	+ + + H and O	*Paratyphoid* A
Native, Mine compound	+ + + O	+ + + O	+ + + H and O	*Paratyphoid* A
Indian, Pretoria	+ + + H and O	+ + + O	+ + + O	*B. typhosus*
Native, Mine compound	+ + + H and O	+ + + O	+ O	*B. typhosus*
Native, Pretoria	+ + + H and O	+ + + O	+ + O	*B. typhosus*
Native, Pretoria	+ + H and O	+ + + O	+ + + O	*B. typhosus*
Native, Mine compound	+ O	+ + + O	+ + H and O	Not done
Native, Mine compound	+ + + H and O	+ + + O	+ + O	Not done
Native, Pretoria	+ H and O	+ + + O	+ O	Not done

typhoid agglutination test along with the typhoid. After a while it began to strike me that the blood specimens from these native miners gave H-agglutination much more rarely than the other cases, whilst their O-agglutination often was particularly strong. A special visit to the mine for the purpose of making blood cultures was rewarded by four positive blood cultures, two of which were pure typhoid, and two paratyphoid A. One of these paratyphoid A strains was henceforward used for routine agglutinations, and some of the results are shown in Table IV. Here the principle of qualitative receptor analysis as introduced by Weil and Felix certainly, and rather unexpectedly, proved its worth.

QUALITATIVE RECEPTOR ANALYSIS AND PROPHYLACTIC INOCULATION.

As prophylactic inoculation against the enterica has not been largely practised in the Union of South Africa, and is now even being superseded by the Besredka method of oral vaccination, I have not had much opportunity of studying this side of the agglutination problem. In one such case I could

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deny the likelihood of the clinical diagnosis of typhoid being correct on account of failing O-agglutination (and complement-fixation) in the presence of H-agglutination, and was completely justified by the further course of the disease. In two other cases of this nature I could affirm a diagnosis of typhoid, O-agglutinins being found, and again the diagnosis was justified, this time by blood cultures.

In two urinary carriers of typhoid bacilli, who only showed O-agglutination, the appearance of H-agglutinins was observed shortly after a course of subcutaneous injections of a typhoid vaccine was started.

Carriers and qualitative receptor analysis.

My observations on carriers of typhoid bacilli, as tabulated in Table V, indicate that they showed O-agglutination exclusively. This may be a coincidence, but again the superior sensitiveness of the strain *B. typhosus* 901 was very evident.

Table V. *Agglutination reactions of typhoid carriers with various suspensions. Serum dilution* 1 : 100. *Readings after* 24 *hours. Serums not heated.*

No.	*B. typhosus* found in	*B. typhosus* H 901 (alive)	*B. typhosus* 1 (alive)	*B. typhosus* "Pretoria" (alive)	*B. typhosus* H 901 killed and phenol	*B. typhosus* "Pretoria" killed and phenol	Alcoholic suspension Oxford Standard Laboratory	Complemen fixation
1	Urine	+ + + O	+ + + O	+ + + O	+ + + O	+ + + O	—	+ + + +
2	Urine	+ + O	+ + O	+ O	+ + O	Neg.	+ + +	—
3	Urine	+ + O	+ O	—	—	—	—	+ + + +
4	Urine	+ + O	+ O	Neg.	Neg.	—	Neg.	—
5	Gall-bladder	+ + + O	+ + O	—	—	+ O	—	+ + + +
6	Stool	+ + O	—	Neg.	—	—	+ + +	—
7	Urine	+ + + O	+ + O	+ + O	—	Neg.	+ + +	+ + + +

Sensitiveness of strains of typhoid bacilli.

Weil and Felix (1920) have divided typhoid strains into three groups according to the readiness with which they give O-agglutination. Their strain *B. typhosus* H 901 was the only representative of the most readily agglutinating variety, and their strain *B. typhosus* 1 the only representative of the next best group. The authors specially point out that poor O-agglutination in typhoid strains is not due to absence of O-receptors, but is due to certain physical factors. Heating makes such strains more agglutinable, and so does addition of phenol to the culture medium.

I have examined 26 locally isolated strains in their first culture from the blood, and found that although all of these gave good H-agglutination, their O-agglutination as observed in a 100° rabbit serum was poor. No O-agglutination at all was given by 12 strains, some O-agglutination was given by 5 strains in a serum-dilution of 1 : 100, and by 4 strains in a serum dilution of 1 : 400. A serum dilution of 1 : 1600 still had some effect on 2 strains, and a serum dilution of 1 : 3200 affected only 3 strains very incompletely.

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The poor agglutinability of local fresh strains improved on further cultivation. That this poor agglutinability is not due to absence of O-receptors was pointed out by Weil and Felix (1920). I have confirmed this by the following observations.

When these strains were heated to 100° for two hours, O-agglutination improved very much, without however reaching the high level of *B. typhosus* 901. Also, when rabbit immune serum was left in contact with such strains in the usual way of absorption experiments, the supernatant fluid after centrifuging could be shown to have no agglutinins left for *B. typhosus* 901, which is the most sensitive reagent. This experiment only succeeded when the serum was used in a dilution of at least 1 : 100. Lastly, an experiment was performed following the usual cross-absorption technique to see whether the old laboratory strain *B. typhosus* "Pretoria" absorbed as many O-agglutinins from a rabbit serum as the strain *B. typhosus* 1, which usually agglutinated at a much higher dilution. There was no difference in this respect between the two strains.

There is therefore no difference between different strains of typhoid bacilli as regards the presence or avidity of their O-receptors, but the strain *B. typhosus* H 901 (Weil and Felix) is in a unique position in that it will show visible agglutination much more readily than any other strain.

THE O-AGGLUTININS IN PATIENTS' SERUMS.

I have encountered a rather large number of typhoid patients who possessed O-agglutinins only. Cultures from their blood at the same time in some instances showed a complete lack of O-agglutinability. Such patients then did not agglutinate their own strains at all, and gave good agglutination with *B. typhosus* 901, sometimes complete up to 1 : 1000. The cultures gave good H-agglutination. Felix (1924) has also described such occurrences. With the ordinary Widal many such cases will give a negative result.

I have further examined the serums of twenty such cases which gave positive O-agglutination with a live suspension of *B. typhosus* 901, ranging from + to + + +, and no agglutination at all with other suspensions (*B. typhosus* 1, *Gaertner* 2, *B. typhosus* "Pretoria," alive and dead). The serums were mixed with these suspensions which they could not agglutinate, and centrifuged after 24 hours. On retesting the supernatant fluid with *B. typhosus* 901, alive, it was seen that hardly any of these serums gave positive agglutination. A "negative Widal" therefore does not imply that the patient does not possess O-agglutinins. It may only mean that the reagent used to demonstrate their presence was not sufficiently sensitive.

The following experiment may throw some light on the question whether local strains provoke more or less O-agglutinin than, for instance, the strain *B. typhosus* H 901. Two groups of six natives in whose blood no agglutinins were present at the start, were prophylactically injected with a vaccine of typhoid bacilli, heated to 100° for 2 hours. The first group received two doses

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of *B. typhosus* 901, was then tested for the presence of O-agglutinins, and then injected twice with local strains, and retested. The second group first received the local strains, was tested, and then received the *B. typhosus* H 901 vaccine, and retested. There was no appreciable difference in the results of the agglutination reactions of the two groups.

O-AGGLUTININS AND COMPLEMENT FIXATORS.

Weil and Felix (1920) noticed that O-agglutinins and complement-fixing power of artificial immune serums often went parallel. Weil (1921) could not definitely claim the identity of O-agglutinins and complement-fixators, but concluded that complement-fixators are primarily provoked by the O-receptors. Marked H-agglutination could take place without affecting complement. He also noticed, always working with artificial serums, that O-agglutination might not become visible in a given case, whilst at the same time complement-fixation took place, and concluded that a change might occur in the bacilli which did not show itself as agglutination, but was really identical with O-agglutination. He did not find all strains equally suitable for complement-fixation. Typhoid bacilli were on the whole quite suitable for this purpose, more so than paratyphoid-bacilli, and a paratyphoid serum would fix complement with typhoid bacilli. Sometimes complement-fixation was found by Weil to be more sensitive than O-agglutination. Felix and Robertson (1928) have also produced evidence for the identity of complement-fixators and O-agglutinins in working with artificial serums against spore-bearing anaerobes.

My former experience with the complement-fixation reaction as a routine diagnostic method, referred to above as completely successful, can be regarded as another argument for the close relationship of O-agglutinins and complement-fixators, especially as I could show that prophylactic inoculation as a rule did not give rise to the appearance of complement-fixators (1923).

I have now further demonstrated the close relationship of the two kinds of antibodies by performing complement-fixation tests and O-agglutination reactions with the serums of 42 typhoid patients. In most cases a close correspondence in results was observed, except in two cases, where a positive O-agglutination was registered with *B. typhosus* 901, and a negative complement-fixation.

Absorption experiments with rabbits' immune serums gave similar results. Such serums, both 100° and 60° were mixed, undiluted, with large quantities of a suspension of *B. typhosus* H 901, which had been heated to 100°. Examination of the serums after this absorption had taken place showed that by one such treatment the H-agglutinins were practically unimpaired and that the O-agglutinins had diminished in quantity. Repeated absorption never made all the O-agglutinins disappear, but such a treated serum was no longer capable of giving positive complement-fixation. Also, if such a partially absorbed serum was mixed with the usual antigen for complement-fixation

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reactions, and live suspension of *B. typhosus* 901 was added after an hour's incubation, no agglutination took place. Unabsorbed serum could be mixed with the complement-fixing antigen without losing appreciably in agglutinative power. The complement-fixing antigen consisted of a saline suspension of typhoid bacilli, filtered through a Berkefeld filter. Details of its preparation were given by Pijper and Pullinger (1928).

These experiments support the view that O-agglutinins and complement-fixators are closely associated, but not identical.

It was also found that *B. typhosus* H 901 was less suitable for complement-fixation reactions than the strain *B. typhosus* "Pretoria." A strain of paratyphoid A bacilli fixed complement particularly badly with typhoid serums.

TITRE-LIMIT OF NORMAL O-AGGLUTININS.

Normal agglutinins are exclusively of the O-variety. Felix (1929) has pointed out that of course the limit of normal agglutination is dependent on the sensitiveness of the O-receptor used. For the strain *B. typhosus* 901 he wishes that only a strongly positive agglutination in the dilution 1 : 100 should be regarded as positive, and he would rather prefer the dilution 1 : 200 to be taken as this limit.

Bound up with this question of titre limit of normal agglutinins at least for practical diagnostic purposes, is the question of immune agglutinins persisting after prophylactic inoculation or previous infection. I have not sufficient material at the moment to express an opinion on this last matter, but must refer to the recent publications of Felix (1929) and Gardner (1929).

My experience with normal agglutinins so far has been that they very rarely occur in such quantity as to give even just visible agglutination in a dilution of 1 : 100 with live suspension *B. typhosus* 901. I have only met with two cases, amongst several hundreds of negative controls, that did this and thus might be regarded as "false positives." But even in these two cases typhoid fever could not be excluded with certainty.

I must add that lately I have examined a certain number of blood samples from a native compound, where typhoid and paratyphoid A fever was endemic, which samples sometimes gave a positive reaction in a dilution of 1 : 100 without these persons showing other signs of illness. I have failed to find such reactions in natives who did not live in a compound. Now the conditions in native compounds lead to that high degree of "faecal communion" which is essential for the persistence of paratyphoid A infection in a community (Snijders, 1927). Paratyphoid A has not been able to get a footing in the Transvaal outside native compounds. Therefore the possibility that here one dealt with mild infections, sub-infections, and carriers, must be left open. A similar position (in a mental hospital) was discussed by Gardner (1929).

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Conclusions.

Under South African conditions the shortcomings of the Widal reaction have become more manifest than in other countries.

The faulty Widal reaction is responsible for the high incidence of enterica in South Africa, because it leaves many cases and many carriers undiagnosed.

My observations confirm the claims made by Felix for the diagnostic method called qualitative receptor analysis, as far as inoculated persons and cases of paratyphoid A are concerned, but I have met with a rather large number of cases of enterica where a sufficient quantity of H-agglutinins had not, or not yet, been formed to allow of more than a group diagnosis of enterica, the specific type of infection remaining undecided.

Paratyphoid fever being rare in South Africa, the chief importance of Felix's work for South Africa lies in the elucidation of the characteristics and importance of O-agglutination, and the methods indicated by him to bring to light O-agglutinins in the blood.

The characteristics of O-agglutination explain the shortcomings of the Widal reaction to a large extent.

The close association of O-agglutinins and complement-fixators explains the good results I formerly achieved in the diagnosis of enterica in South Africa by means of the complement-fixation method.

Practically all cases of enterica in South Africa were found to possess O-agglutinins, but in order to demonstrate their presence a very sensitive reagent is necessary. South African strains proved unsuitable. The strain *B. typhosus* 901 was found to be the most suitable, if used as a live suspension. The sensitiveness of various strains and suspensions was carefully compared.

For routine work I have found one serum-dilution adequate, and I chose the dilution of 1 : 100. Below this dilution inhibition may occur (Burnet, 1924). Higher dilutions do not give more information for routine purposes than the reading of the strength of the reaction in the dilution 1 : 100 can give.

I would advise the performance of agglutination tests with the following live suspensions, prepared as demanded by Felix (1924). The most important one is *B. typhosus* H 901 (Weil and Felix), and if one can add *B. typhosus* O 901 (Felix and Olitzki), this will be very helpful for the differentiation of H- and O-agglutination. Unless the possibility of paratyphoid and prophylactic inoculation can be definitely excluded, one must add a strain of paratyphoid A and B bacilli. I found it particularly useful to add one further suspension, the alcohol preparation of typhoid bacilli as supplied by the Standards Laboratory of Oxford. This suspension is not so sensitive as *B. typhosus* 901, used alive, but it is always ready for use, gives very clear-cut results, is not subject to the mishaps that occur with live suspensions, and is more sensitive than other preparations I have tried. Also, I have witnessed a few instances where this alcoholic suspension gave a positive result and live suspensions a negative. For this I cannot give an explanation.

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