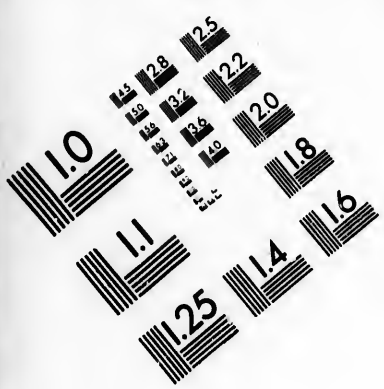

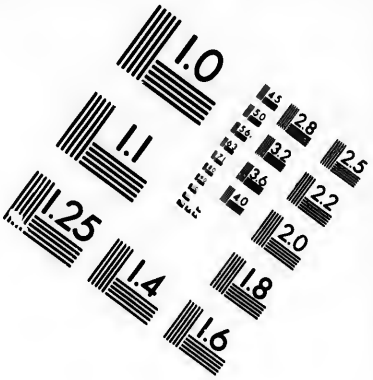

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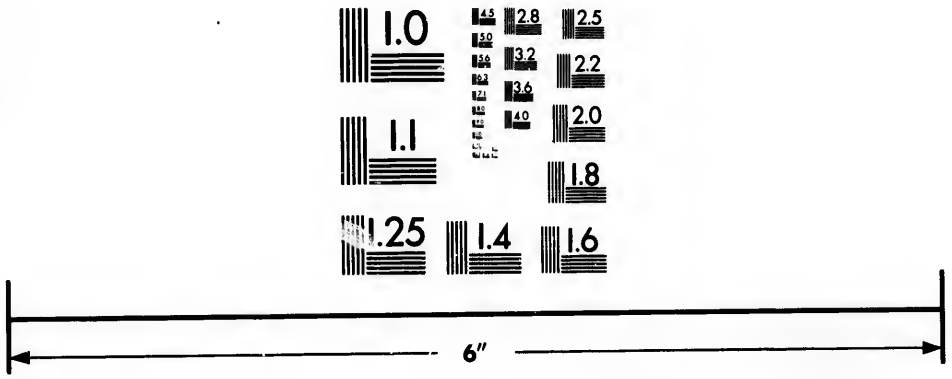

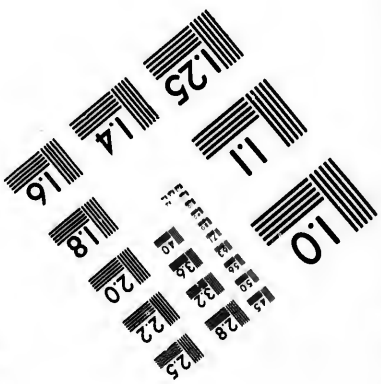

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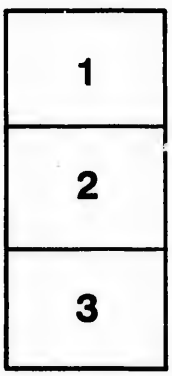

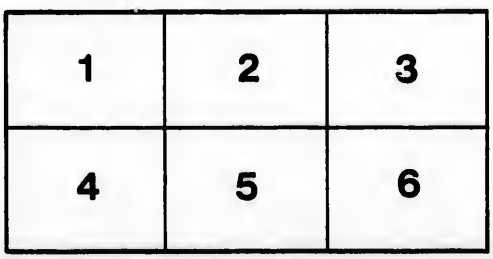

ON THE DIFFERENCE BETWEEN SERUM AND BLOOD SOLUTIONS, THE CONDITION OF THE TEST CULTURE AND THE SIGNIFICANCE OF BACTERIUM COLI INFECTION IN RELATION TO TYPHOID DIAGNOSIS.

BY

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(From the Laboratories of the Board of Health of the Province of Quebec and the Montreal General Hospital.

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We wish to report some details concerning technique which we find necessary in order to insure successful results in Serum Diagnosis by the dried blood method, with which we have now tested over 500 bloods.

We mention the facts only in so far as they have a direct practical bearing on diagnostic work.

Our results already published were as follows :

1. Out of 129 cases, which we had good reason to regard as true typhoid, if we exclude a few cases where the first samples were taken at a very early stage and no re-examinations could be obtained, and also a few cases first examined late in convalescence, we have met with but one apparently genuine case of severe typhoid, which, when re-examined under satisfactory conditions, did not give a decisive reaction by the dry blood method and this one also gave no reaction by the serum method. Occasionally the first appearance of the reaction is delayed beyond the end af the first week.

2. We have never met with a well marked reaction under conditions where there were not strong reasons for believing it to be due to typhoid.

3. In a few cases where the result of the blood examination remained in doubt the mild type of the fever made an accurate clinical diagnosis impossible. In such cases, we believe bacteriological examination to be the most exact method of procedure.

4. We have not yet met with a case of typhoid where a decisive reaction was obtainable by the serum method and not by the dried blood method.

5. We found that pseudo-reactions may be avoided by attention to the character of the culture media. We have found that by using an attenuated or quiescent stock culture grown at room tempera-

ture, and transplanted at intervals of about one month, a suitable degree of sensitiveness was obtained. From such stock cultures a 24 hour bouillon at 37°C., with a moderately diluted blood solution, or serum would give prompt and decisive reaction within a few minutes in the case of typhoid patients, while concentrated solutions of non-typhoid blood or serum were found to give no reaction, even at the end of 24 or 48 hours, hence estimation of the amount of dilution is not necessary for ordinary diagnostic work. (See circular Board of Health, Prov. of Quebec, Jan. 7th, 1897.)

The reaction, although specific in degree, is now generally considered to be quantitative, and small amounts of the agglutinative substances are admitted to be present in varying amounts in non-typhoid bloods. The specific substances are, however, a hundredfold more abundant in typhoid blood.

With virulent cultures the presence of agglutinative substances in non-typhoid bloods may lead to pseudo reactions occurring which can usually be excluded by estimating quantitatively the intensity of the reaction. These pseudo reactions we have found to be characterized by a rapid clumping, without the corresponding loss of motion so characteristic of the true reaction. If watched for some hours these clumps tend to break up.

Quantitative estimation is now generally done by diluting the typhoid serum, but may also be doue by modifying the virulence of the culture.

The degree of dilution which can be employed with a given blood solution or serum, while still producing a decided reaction, will depend entirely on the activity (virulence) of the culture employed. This factor has been too much left out of the reckoning in much of the work already published, and it probably affords a natural explanation of the widely different results obtained by competent observers.

Cultures which are made active and virulent by frequent (daily) transplantation and growth at body temperature, are much more sensative to the agglutinative substance than cultures which have become quiescent and attenuated by infrequent (monthly) transplantation and growth at room temperature.

This is apparently at variance with Pfeiffer's statement (*Cent f. Bakt* XIX, p. 594), that highly virulent cultures are less influenced by typhoid and cholera sera than less virulent ones. No details are given by Pfeiffer as to the conditions under which his non-virulent cultures were used. Pfeiffer's statements refer to serum and not to blood solution, he pays little attention to the agglutinative and much to the paralytic phenomena of the reaction, and attaches most

importance to certain disintegrative changes produced by his special method of testing *in vivo*. We have stated elsewhere that highly active cultures, if left for a few hours longer than usual between the times of transplantation, rapidly undergo involution changes, and while in this condition are far more liable to show agglutination than was the case with the same cultures tested a few hours earlier. We have found that for class purposes involution forms in cholera are as abundant and striking in a virulent culture left unchanged for three or four days as would be the case with a non-virulent culture, grown at room temperature, if left without transplanting for as many weeks or months. Bouillon cultures, which have stood long without transplanting, show a tendency to spontaneous partial clumping, which is quite absent during the first 24 hours. For this reason we prefer to use 24 hour bouillons, which are free from sediment, for the test.

The peculiar disintegration obtained by Pfeiffer in typhoid cultures placed directly in the peritoneum of a specially immunized animal, do not tend to occur where the serum is tested in vitro by the hanging-drop method. With blood solution, however, this peculiar phenomenon is frequently witnessed. The clumped bacteria, if watched, for an hour or so, may be seen to break up in granules, which gradually become indistinct and vanish whilst under observation until practically no trace remains of the clumps which shortly before studded the entire field of the microscope. The change is more liable to occur in cultures some days old than in young cultures and more, perhaps, with attenuated than virulent cultures. It does not occur with all samples of typhoid blood, and is not well marked in very dilute blood solutions.

This greater tendency to bacteriolytic action in blood solutions often makes the reactions obtained with them look at first sight less striking and intense than that obtained with serum where the clumps usually remain intact Apparently, however, the difference indicates that a large amount of the bactericidal substances originally found in the plasma do not permanently remain as constituents of the serum. This not only has an obvious bearing on serum therapeutics, but explains how the action of serum may be modified by mechanical mixture with the fibrin elements of the blood.

Quantitative estimation of the degree of dilution in the case of blood solutions is possible by hæmometry as well as by making direct measurement. With samples of freshly dried blood, sufficiently accurate observations can be made to express the degree of dilution in multiples of 10—($\frac{1}{10}$ $\frac{1}{20}$ $\frac{1}{50}$, etc.)

We have employed a cell having a depth of 0·85 mm. and giving

with a Fleischl's hæmometer a tint reading 100 p.c., with $\frac{1}{10}$ dilution of normal blood. In anæmic cases the dilution will vary with the degree of anæmia, which can readily be determined. Blood dried for some time gradually yields less and less hæmoglobin, owing to the change of this substance into the hæmatin compounds. This change goes on rapidly in air where gas is being burned and slowly in pure air. In any case, the error is in the direction of a less dilution than that shown by the hæmometer. As a matter of experience, we find exact estimation of the dilution, while interesting for scientific purposes is not necessary for the practical purposes of the test if attenuated cultures are used and the establishment of fixed arbitrarytime limits, as recommended by Grünbaum seem only of use in avoiding pseudo results, due to the use of highly virulent cultures.

Grünbaum, being enthusiastic for exact estimation of dilution in all cases, claims (*Lancet*, Sept. 19, 1896), that though most sera will in time produce clumping, that typhoid serum can still be specifically identified by its being the only serum, which, with free dilution in a ratio of 16 to 1, will produce a complete clumping and arrest motion in 30 min. A fixed dilution ratio, with an arbitrary time limit, appears to us quite uncalled for as a routine diagnostic practice, and has no standard value unless a culture of fixed virulence is used.

Since writing the above we find that Grünbaum has now stated on theoretical grounds "that possibly the use of attenuated cultures would enable us to dispense with the dilution" (*Lancet*, Dec. 19, 1896.)

We had anticipated *a priori* that the solution obtained from the dried blood would be less sensitive as a reagent than the fresh liquid serum. We find the blood solution on the contrary to be apparently more potent than the serum, in causing the agglutination though not as to the paralytic effect, and perhaps to give the reaction at a somewhat earlier stage of the disease. This view agrees with the researches of Widal, who found that the agglutinating substance was contained in the globulins and fibrinogen, and that the serum albumin and corpuscles contained none. Thus the blood serum contains only a part of the agglutinative substance. Dr. A. H. Appel of the U. S. Army has also recently made studies and observations showing the greater agglutinative properties of solutions of the whole blood as compared with that of the serum. A decided agglutination can be obtained from weak solutions of the entire blood when none is produced by stronger solutions of the serum. While Widal places the limits of dilution with serum below 1 to 200, R. Stern who employed solutions

of the entire blood in bouillon reports reactions with dilutions of 1 to 2000.[1]

Owing to the greater sensitiveness of blood solutions as compared with typhoid serum, there is a greater tendency to pseudo-reactions if active virulent cultures are used, than is the case in working with serum. This difficulty is, however, completely obviated by employing attenuated cultures for testing. Cultures which exhibit darting movements in hanging drops are too sensitive for the dry blood test. Those cultures having a quiet but rapid gliding motion in hanging drops have given us uniformly good results. If the movements of the culture become sluggish, one or two daily transplantations at body temperature will make it more active and sensitive. One or two cc., of the living bouillon cultures injected into the peritoneum of a guinea pig produce immunity and a marked blood reaction without injuriously affecting its health.

Clean preparations containing very little fibrin can readily be obtained if care is taken not to stir up the film of blood clot and to use plenty of water for dissolving.

We find that the blood dries in a few minutes sufficiently to be enclosed in an ordinary letter.

Our routine method of testing is to place a large drop of water from capilliary pipette, on the film of dried blood and let it stand for a minute or two. A loop full of the solution so obtained is taken *from the top of the drop* and mixed with a loop full of the bouillon culture, or may, if desired, be diluted further.

For the re-examination of cases giving a negative reaction, a somewhat more virulent culture can be used or a quantitative estimation also made by the serum method. We have not succeeded however in obtaining a decided reaction by the serum when the result with the dried blood was inconclusive and now attach equal importance to a negative result by the dried blood test.

Our published observations (*N. Y. Med. Journal*, Oct. 31, 1896, *British Medical Journal*, Dec. 5, 1896), on the dry blood method

[1] We observe that Widal, who was the first to show that dried blood could produce the reactions, and already, in June, 1896, obtained reactions from serum after four months drying, has recently (*Semaine Med.* Jan. 13, 1897), reported that he has been able to obtain successful results by the dried blood method in the earliest stages of the disease and that the blood after six months drying retained the power of producing the reaction. The dried blood also gave him positive reactions late in convalescence in cases where agglutination had become very feeble. We are glad to find our published results on these points agree with those of so high an authority. We have found that with those who have had difficulties with the dried method, these have been due to their having acted upon the erroneous idea that the blood solution was much weaker than the serum whereas, even with attenuated cultures, we have got a reaction readily with it in dilutions as high as 1 to 125.

were made with attenuated cultures, and pseudo reactions were practically never encountered.

Later on, for a few weeks we tried active virulent culture transplanted daily at 37°c., but these gave us with the dried blood solution numerous and very peculiar pseudo-reactions, i.e., reactions not due to existing typhoid. For instance, the blood of one of us (W. J.) when dissolved gave prompt and abundant agglutination with a virulent culture, while we habitually use it as a suitable negative control blood with attenuated cultures. A solution of the blood of the other (D. D. McT.) gave no reaction. (W. J. had typhoid fever 16 years ago; D. D. McT. has never had it). W. J's blood serum gave no pseudo-reaction with the virulent culture.

On resuming the use of the attenuated cultures described above, the pseudo-reactions disappeared. On re-examining, the blood drops which had given them with the virulent cultures, no longer did so when tested with attenuated cultures, although dry blood from genuine cases taken at the same time still reacted typically.

For practical diagnostic work it may be stated that when a blood does not show a decisive reaction in a serious case of fever which has lasted over a week, the fever is almost certainly not typhoid. In very mild febricular cases the result may remain doubtful, unless investigated by an early bacteriological examination of the spleen pulp or stools.

In this connection we may state that we find that Elsner medium containing 25 per cent. gelatine instead of 10 per cent. will remain solid at a temperature about 30 C., and give visible typhoid colonies within 24 hours.

Reaction with the Colon Bacillus.

Very little attention has as yet been paid to the clinical significance of serum reactions with colon bacillus. Courmont and Rodet have stated that typhoid blood serum reacts with colon cultures, while Achard and Chantemesse state that it does not. Widal states that he has studied quantitatively the intensity of reaction of typhoid sera with Coli, but has been unable to draw any important diagnostic conclusions from the results.

Various observers have reported colon reaction as being present occasionally in different chronic and acute diseases. This can readily be understood in the light of our present knowledge of terminal infections. One case which at first strongly resembled typhoid but gave no serum reaction, has been recorded by Vedel who found a marked colon reaction and looked upon it as only colon infection, this opinion being confirmed by the subsequent events. Personally we have found

reactions with the colon bacillus to be rare with typhoid blood or serum (even in cases when perforative peritonitis had occurred) provided the typhoid reaction was well marked. On the other hand we have been struck by the large proportion of positive colon reactions obtained in cases having step-ladder temperature and other symptoms strongly resembling typhoid but without the typhoid serum reaction. We think that under these circumstances the colon reaction may have a real diagnostic importance, and indicates that the colon infection whether occurring alone or as a secondary complication of typhoid may be playing an important part in the production of the patient's condition. The whole question of associated colon infection deserves further study.

The reaction can be tested with ease by placing a duplicate drop of blood solution or serum on the cover slip with the drop to be tested by typhoid culture and mixing it with a drop of colon bacillus culture. Pseudo-reactions can be avoided by using stock cultures kept at room temperature, and transplanted infrequently. Test cultures grown in bouillon from the stock at room temperature for 24 hours are free from scum or sediment, and give reliable results. The conflicting results just mentioned may have been due to pseudo-reactions having been taken seriously.

In our case of apparently genuine typhoid without serum reaction, (on which, by the way the test was first applied during the third week) the blood reacted very decidedly to B. Coli, producing typical clumping. The same held good of four other blood samples referred to us for examination as having a clinical course like typhoid, but with negative serum reaction. A complete colon reaction we have found to be exceptional in ordinary typhoid and its presence would indicate a condition of Coli intoxication sufficient to explain the existence of many symptoms giving to typhoid its ordinary clinical features. Whether this excludes typhoid, is another question. W. H. Park has observed a case of fever with no typhoid serum reaction, where he was able to cultivate the typhoid bacillus by spleen puncture. Later on in the case however a relapse occurred and the reaction appeared. The possibility of a latent typhoid infection overshadowed by toxic phenomena, due to concurrent action of the colon bacillus is quite consistent with the generally accepted opinion that many of the symptoms in typhoid and especially the intestinal ones are due to secondary infection by B. Coli. It follows that in severe cases of typhoid type, with no typhoid reaction, the blood should be tested with a culture of B. Coli and a bacteriological study made by examination of the stools or by spleen puncture.

In a few cases we have met with a partial typhoid reaction only, in mild cases clinically fibricular, where the fever subsided by lysis in within two weeks of the onset. Here, the possible presence of typhoid appeared to indicate the prudence of keeping the patients in bed and avoiding articles of diet which are contra-indicated in typhoid. Our experience has been that fibriculæ, with completely negative blood reaction, get suddenly well after a few days of fever. Here, also, spleen puncture, as in Dr. W. H. Park's case, might enable a decided diagnosis to be made earlier than by the blood test alone. Wesbrook recommends spleen puncture under the circumstances. The possibility of infection by organisms resembling the typhoid bacilli must naturally be borne in mind.

Diabetic blood has been found by Block and by W. H. Park, to give a decided agglutination. We have examined two cases of diabetes which both gave perfectly negative results.

Conclusions.

The difference in reaction observed between typhoid blood solution and blood serum is not simply due to varying intensity, but to an alteration in the relative prominence of the agglutinative, paralytic and disintegrative phenomena which constitute the reaction. The extent of this difference also varies with the virulence of the culture, but the difference probably depends also on the presence of part of the specific substances elsewhere than in the blood serum.

Blood solution has a greater capacity than blood serum for producing the disintegrative (bacteriolytic) changes described by Pfeiffer Descriptions of this phenomena are conspicuously absent from the many recent accounts of the reactions with typhoid serum as observed in hanging drops.

The paralytic effect is relatively more marked with serum than with blood solutions.

Agglutination without stoppage of motion is more readily occasioned in virulent cultures by blood solution than by serum, and does not indicate existing typhoid.

It appears preferable that for the dry blood method only attenuated cultures should be used. These have the advantage of being more easily kept in readiness than virulent cultures, and are less sensitive to changes of temperature. With the serum method virulent cultures give prompt results. Dried blood serum can be readily obtained and transmitted to the laboratory by pushing aside the edge of a blood drop which has clotted for a few minutes but has not dried

and collecting the serum beneath it on the tip of an ivory vaccine point. etc. This does not, however, give a quantitative result.

For ordinary diagnostic purposes, the simplicity of the method as originally described does not require modification, provided attenuated cultures are used.

A drop of the solution obtained from a dried typhoid blood drop, mixed with a drop of the culture, will give the reaction promptly, without any special attention to the degree of dilution. In order, however, to obtain the best results, it is well to dilute freely and especially to avoid having a sticky solution of syrup-like consistency.

In cases where the clinical type strongly resembles typhoid and where the serum does not give the typhoid reaction, a decided reaction with cultures of the colon bacillus may explain the symptoms.

Our results with the dried blood test have been very satisfactory, giving uniformly positive results with genuine and well marked typhoid cases, and not reacting with non-typhoid bloods when attenuated cultures were employed.

Although the use of serum undoubtedly enables the results to be recorded and compared with greater scientific precision, we find that dried blood answers just as well for routine diagnostic work.

The alterations in reaction, induced by very slight modifications of the manner of testing, help to explain differences in the results reported by experienced and careful observers. With the same blood and culture, the amount of dilution possible largely depends on whether plain bouillon, bouillon culture or water is used for diluting. Opinions also vary as to what should be regarded as constituting a reaction. Personally, we do not think that anything less than complete clumping and total arrest of motion obtainable by the dry as well as the moist test in a young attenuated culture, should be regarded as typical.