

Johnston (W.)

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ON THE APPLICATION OF THE
SERUM DIAGNOSIS OF TYPHOID FEVER
TO THE REQUIREMENTS OF
PUBLIC HEALTH LABORATORIES.*

BY WYATT JOHNSTON, M. D.,

MONTREAL,

BACTERIOLOGIST TO THE BOARD OF HEALTH FOR THE PROVINCE OF QUEBEC;
PATHOLOGIST TO THE MONTREAL GENERAL HOSPITAL.

GREAT interest attaches to Widal's important communication to the effect that the serum of persons suffering from typhoid fever, even in the early stages, is capable, when mixed with a pure culture of the typhoid bacillus in bouillon, of arresting the active movement so characteristic of this organism and causing the bacilli to agglutinate into clumps resembling zoogloea. The serum of typhoid convalescents and immunized animals had been shown by Pfeiffer, Durham, and Gruber to possess this property. But Widal has certainly been the one to demonstrate its great clinical value. With the serum of an undoubted case of typhoid fever we are able to apply what appears to be the most conclusive

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of the tests at our disposal in deciding whether a given organism is really the typhoid bacillus or not. On the other hand, with a culture of the genuine typhoid bacillus, we are able to decide whether a doubtful case is or is not typhoid fever.

Although the test is so recent in origin, those who have tried it appear practically unanimous as to its being of great delicacy, and, in particular, the negative results which it furnishes are of nearly as much practical value, something which can scarcely be said of the routine bacterial tests for tuberculosis.

Widal's original method was to obtain the serum from the vein of a patient's arm by means of a sterilized syringe, decanting the serum after it had separated and adding it to bouillon culture of typhoid bacilli. This was then placed in the incubator, and showed, after several hours, a flocculent precipitate composed of the immobilized and agglutinated bacilli and a clearing of the upper part of the fluid. This was found by Widal to be characteristic of typhoid blood. The blood in other febrile disorders, such as malaria, typhus, tuberculosis, pyæmia, etc., as well as the serum of healthy persons, were found to have no power of producing this phenomena when mixed with typhoid cultures. Those who have repeated Widal's experiments have also been able to confirm his statements that the colon bacillus does not give this reaction with typhoid blood.

Widal was fortunately led to simplify the method materially by taking a few drops of blood from the finger tip, and as soon as the serum was separated from the edge of this mixing it with a drop of actively mobile typhoid culture, whereupon the reaction could be satisfactorily observed under the microscope and was usually

complete in a few minutes.* Dieulafoy testifies to the remarkable accuracy of the test and its value in diagnosing obscure cases.

My attention was first directed to the test through having been consulted by physicians as to the nature of suspected cases of typhoid, and my experience has been thoroughly in accord with that of Widal and others as to its great value as an aid to clinical diagnosis.

As the reaction appeared to depend probably upon the presence of some substance analogous to the ordinary toxines, and as many of these preserve their characteristics in a dry state, it naturally occurred to me that this might be true of the substance producing the serum reaction. The advantage of being able to operate with a dried substance was obvious, especially with reference to the possible application of the method to the rapid bacteriological diagnosis of typhoid fever in municipal laboratories, just as is now done in the case of diphtheria, and my observations have been made with this end in view.

Instead of taking the serum as soon as it exuded, I allowed the drop to dry, and found that upon moistening it subsequently the solution obtained was just as efficacious as the pure serum for the diagnostic purposes of the test.†

This power appears to remain practically unimpaired even after the blood has been allowed to dry for many

* This plan of observing the reaction directly under the microscope had been published by Gruber and Durham some months previously.

† Since writing the foregoing I have been able to obtain fuller accounts of Widal's work than were at first available, and find it stated by him (*Bulletin médical*, 12 août, 1896, p. 267) that dried serum, and to a lesser extent dried blood, are capable of furnishing the reaction. This circumstance does not appear to have been hitherto utilized practically.

days. My experiments upon how long the blood will continue to react when in this dry state are not yet finished, but blood drops dried for from two to four weeks still give the reaction.

In this manner I have tested the blood of ten patients suffering from undoubted and typical attacks of typhoid. The reaction was obtained conclusively in every instance. In eight cases the loss of mobility and the agglutination was complete in from two to fifteen minutes. Of the two others, one, in a very early stage of the disease, required thirty minutes for the completion of the reaction, while the other in a very late stage, following a relapse, required one hour.

Control Cases.—The blood of ten other hospital patients, as well as a number of healthy individuals, was next tested, and in no single instance was the reaction obtained. Occasionally a pseudo-reaction with some agglutination was observed within a few minutes of the mixture of blood solution and culture, but some movements of translation (wandering through the field) always persisted on the part of isolated bacilli, and these gradually increased in number and activity till, in an hour or two, lively motion was resumed, and was found to be still present on the following day and, in some instances, where it was followed up, at the end of a week. With the typhoid bloods nothing but the oscillating or "Brownian" movements were seen, as a rule, though where the proportion of serum added was very small peculiar revolving and tugging movements, apparently due to the action of the flagella, could be made out, movements from one part to another of the microscopic field being, however, completely abolished.

In two doubtful cases examined for diagnosis the

results were negative. In one of these the malaria plasmodium was subsequently detected. The other left the hospital before the diagnosis was cleared up, but her temperature had remained normal for two weeks, and her only symptoms were persistent headache and giddiness. One of the control cases, examined with negative results, had a history of typhoid two years previously.

[In making a communication upon this subject before the American Public Health Association at Buffalo, N. Y., on September 17, 1896, I subjected the method to what I considered to be a fair practical test as to its applicability to public health purposes. I left instructions for Dr. D. D. McTaggart, resident pathologist, to forward by post to my destination, after I had left Montreal, a letter containing dried blood drops from several cases of undoubted typhoid fever and also dried blood drops for control from other hospital cases, preferably patients suffering from febrile conditions, but making sure that they had not had typhoid recently. All these blood drops were to be numbered and a key giving the clinical diagnosis in each case placed within a separate sealed envelope.

I left Montreal September 13th. Samples of blood from six patients were collected, and forwarded as directed on September 14th. On September 16th the letter was delivered unopened at Buffalo, N. Y., to Dr. Bissell, the City Bacteriologist for Buffalo, who kindly took charge of the key. At the end of an hour spent in examining the specimens, I wrote my diagnosis upon the outside of the sealed envelope. It will be seen from the subjoined signed statement, which Dr. Bissell kindly made at my request, that the results were perfectly in

accord with the clinical diagnosis in each case, while the specimens, which were then examined by a number of competent bacteriologists, showed that good objective grounds existed for arriving at the conclusions given.

Statement by Dr. McTaggart, Resident Pathologist, Montreal General Hospital.—The samples of blood were mailed to Dr. Johnston one day after he had left Montreal. Dr. Johnston had no knowledge of the contents of the "key," and no private means of knowing which of the numbers referred to typhoid and which to non-typhoid blood. (Signed.) D. D. McTAGGART.

Statement by Dr. Bissell, City Bacteriologist, Buffalo, N. Y.—Buffalo, September 16, 1896: Received to-day from Dr. Wyatt Johnston a sealed letter, mailed in Canada, with postmark, "Montreal, September 14, 1896." This was opened by me and found to contain (a) six glass cover slips, numbered from 1 to 6, with a drop of dried blood on each, and (b) also a sealed envelope marked "key." Received from Dr. Johnston, after examining the blood by the (Widal) serum diagnostic test, the following report: No. 1, typhoid; No. 2, typhoid; No. 3, typhoid; No. 4, not typhoid; No. 5, not typhoid; No. 6, doubtful, probably not typhoid. The key was then opened by me and the clinical diagnosis from all cases found as follows; No. 1, typhoid; No. 2, typhoid; No. 3, typhoid; No. 4, malaria; No. 5, enlarged glands of neck; No. 6, heart disease.

(Signed.) WILLIAM E. BISSELL.

It will be noticed that a qualified though correct opinion was given at the time of my making the report in one of the negative cases (No. 6). This doubt was owing to the fact that it was the last specimen examined, and that a partial agglutination appeared to take

place at first, though motion was not abolished. Subsequent examination some hours later showed such lively motion that I should have had no hesitation in declaring it not to be typhoid had the circumstances permitted that much delay before an opinion was given.]

A ready means of diagnosis in typhoid fever is something which has long been desired by sanitary officials. The medical profession is proverbially lax with regard to the notification of typhoid cases, and we may assume that this neglect is in part due to the want of any adequate *quid pro quo* in return for such notification. Probably the assistance derived from a prompt bacteriological diagnosis or even corroboration of diagnosis in the early stages of typhoid will lead to the more uniform reporting of cases. Besides distinguishing typhoid from such well-characterized diseases as tuberculosis and malarial disease, this test may also be expected to clear up the mystery which surrounds those doubtful cases of so-called bilious fever, remittent fever, gastric fever, typhomalarial fever, etc., which are so common in times and places where typhoid is prevalent, and rare in the absence of typhoid, at least in temperate regions which are free from malaria.

Those who are called upon to investigate epidemics of typhoid are much perplexed by the large number of cases of ill-defined and transitory fever occurring among those personally exposed to the infection, and the impossibility of coming to anything like a definite conclusion upon the evidence hitherto obtainable as to whether these are to be regarded as cases of abortive typhoid or not. In my own experience, such cases have usually equaled or outnumbered the cases where the symptoms justified a definite diagnosis.

I may add a few words with regard to technic. I use a dry lens of about one-fourth-inch focal distance. The dry blood drop is partly dissolved with germ-free water and a drop of the solution obtained is placed upon a cover glass which has just been passed through a flame and mixed with a drop of a typhoid bouillon (a watery suspension of an agar culture also answers very well). This is placed over a hollow cell sealed by vaseline. I control the examination by comparing it with a blood drop from an undoubtedly typhoid case and also with normal blood. It is also advantageous to place a minute drop of the blood solution upon the cover slip alongside the mixture of culture and serum, so as to satisfy one's self in negative cases that the blood contains no motile bacteria. Uniformity of temperature is the chief detail to be attended to, as the agglutination does not take place so well if the movements are sluggish. A hot-water dish filled with warm water forms a cheap and convenient substitute for an incubator, and a simple warm stage made of a sheet of copper is also useful. In a well-warmed laboratory, however, the use of these adjuncts is unnecessary. Hollow cells are convenient, but not indispensable. For collecting the blood drop any smooth surface suffices; cover glasses or slides have the advantage of being clean and sterile, but I have found ordinary writing paper or smooth cardboard most convenient, as it could be more easily labeled or forwarded. The swabs used for diphtheria outfits will answer, but the presence of extraneous substances, such as fibres, was found annoying. The presence of blood pigment is rather an advantage, as it enables the drop to be more easily focused. The small fibrin particles of clot sometimes bear a superficial resemblance to the

islets of agglutinated typhoid bacilli, but are readily distinguished from them by the presence of leucocytes in their meshes.

One advantage of having the blood dried is that it insures it against contaminating growth occurring during shipment. In case any doubt as to the reaction exists at first, it will usually be dispelled by watching the preparations for some hours, or, if necessary, for a day or two. This permits a decided and progressive increase of motion in non-typhoid cases and allows the more perfect agglutination in the genuine ones.

The one indispensable factor is perfect purity of the culture. The one which I use was kindly forwarded me by Mr. J. J. Mackenzie, Bacteriologist to the Ontario Provincial Board of Health, and was stated to have come originally from the Berlin Hygienic Institute. It grows typically on gelatin, potato, bouillon, agar, and milk; reacts typically with litmus agar, produces no indol or gas, and shows the motility and staining reactions characteristic of the Eberth bacillus.

I have made this communication because the method here suggested seems better adapted than those hitherto employed for bringing this test within the range of ordinary public-health laboratory work and enabling it to be dealt with, if I may so express it, in a wholesale manner.*

* Drying the blood as a preliminary step has enabled the Board of Health of the Province of Quebec to offer to the medical profession here a free public service of typhoid diagnosis by the serum method similar to that which is followed in diphtheria. Outfits consisting of a folded and sterilized piece of paper, in which the blood drop is sent inclosed in a suitable envelope, are placed in convenient depots. In case of negative results an additional sample taken by collecting a few drops of blood in a small glass tube is examined, but this extra precaution is sel-

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Herbert Durham (abstract). *Proceedings of the Royal Society*, vol. lix, No. 355, January 23, 1896, p. 224; abstract also in *Epitome, British Medical Journal*, March 14, 1896.

Grunbaum. *Lancet*, September 19, 1896, p. 806.

Gruber and Durham have announced a paper to appear shortly in the *Archiv f. Hygiene*.

French work (I have to thank Dr. E. P. Benoit for calling my attention to the very full and satisfactory report of Widal's work in the *Bulletin médical*):

F. Widal. Soc. des hôpitaux, June 26, 1896; *Bulletin médical*, 1896, p. 618.

F. Widal. Congrès français de médecine, August 6, 1896; *Bulletin médical*, 1896, p. 766.

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Widal and Sicard. Acad. de méd., September 29, 1896; *Bulletin médical*, 1896, p. 934.

Achard. Soc. des hôpitaux, July 31, 1896; *Semaine médicale*, 1896, p. 295.

dom necessary. As to the degree of accuracy which this application of the test may afford, it is too early to speak positively. From my experience hitherto I am inclined to believe that it will compare not unfavorably with those obtained in the cases of diphtheria and tuberculosis. In one case the reaction was present on the fourth day.

Achard and Bensaude. Acad. des sciences; *Bulletin médical*, 1896, p. 933.

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Haushalter. Troisième Congrès français de méd. à Nancy (39 cases); *Bulletin médical*, 1896, p. 769.

See also observations by Nicolle and Hallipré and by Lemoine, cited by Widal, *Bulletin médical*, 1896, pp. 736 and 934.

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