was there a rise of temperature or constitutional disturbance sufficient to warrant even a suspicion of a positive reaction to the tuberculin. The dosage was employed in most of the cases as above stated, and the results were eminently satisfactory.

Perhaps in none of these instances was there so striking an advantage in employing the test as in several cases of slowly-resolving pneumonia, where for a period of from five to six weeks after the acute symptoms had subsided there was evidence of consolidation of the lung, and just such a condition as might be incident to a tuberculous process. The inoculations, however, proved negative, and a favourable prognosis was in each instance given to the relatives, while the course of the disease later on to recovery amply justified the conclusions drawn. In another case of pyuria the diagnosis of tuberculosis had seemed undoubted, though careful preparations of the urinary sediment had failed to reveal the bacilli of tuberculosis. Tuberculin, when injected, gave negative results, and the subsequent operation revealed merely the presence of a renal calculus the removal of which was attended by progressive amelioration and ultimate recovery.

Of the cases of undoubted tuberculosis tested, only those were employed where there was practically an absence of fever, or fever so slight as not to interfere with the reaction. The inoculations, too, were given late at night, so that any possible rise might occur during the time of day when least of all we would anticipate a tuberculous pyrexia.

Of these, 5 were cases of undoubted pulmonary tuberculosis, 4 of tuberculous pleurisy, 1 patient with tuberculosis and pyopneumothorax, and also 1 with genito-urinary tuberculosis. In all, positive reactions were obtained. Cases with Pott's disease and morbus coxæ gave similar results, and, as a rule, marked reactions. Six were unsuccessfully inoculated in this manner, though 2 others failed to react. In each of these latter there was wasting, and a large local cold abscess associated with the primary disease. Two cases of tuberculous cervical adenitis were injected, and gave positive reactions, as did also 3 cases of tuberculous arthritis of the knee-joint.

With reference to the third point, namely, the observation of doubtful cases, such as pleurisy, where the etiology was obscure, there was some difficulty in accurately establishing the value of the test. The attempt was made to inoculate the pleural effusions into guinea-pigs, and thus to ensure a diagnosis of tuberculosis in suspected cases; but, in common with other observers, we here obtained negative results. In all cases, likewise, the suggestions of Eichhorst to inoculate the fluid in large quantities was followed, but the results, even so, proved quite unsatisfactory.

In the second place, where patients failed to react, and where it was believed that no tuberculosis was present, the cases after leaving the hospitals never reappeared for examination. Apart, however, from the 4 undoubted cases above mentioned, were 2 others in whom at first there was merely a suspicion of tuberculosis of the pleura. The diagnosis in these, established by the inoculation of the tuberculin, was later verified by development of the disease in the lungs. In one of these cases an insidious onset of the effusion and significant family history aroused the suspicion of its bacillary origin; but a guinea-pig inoculated by the fluid was unaffected; the injection, however, of $\frac{1}{2}$ mg. of tuberculin was followed by marked rigors, *malaise*, and a temperature of 104.4° . In the course of a few weeks the pleural effusion subsided, and the patient left the hospital greatly improved; two months later she returned to the outdoor department, with definite signs of pulmonary destruction in the apex of the previously affected side, obviously a tuberculous condition. The case before mentioned where a pyuria gave difficulties in diagnosis is likewise of interest in this connection, as showing the value of the reaction in clearing up obscure cases.

The examination of cases in which operations have been performed for the removal of tuberculous tissue has just been commenced, and we have injected but two patients, both of whom had undergone excision of the knee-joint. Both patients reacted mildly to the tuberculin; although there was no discoverable evidence of remaining lesions, local or elsewhere. Our investigations, however, are still in progress.

It will be seen from the cases just recorded that more than 30 patients in whom no tuberculosis could be discerned failed to respond in any degree to the inoculation of the reagent; that, on the other hand, 21 cases of undoubted tuberculosis reacted positively. Several failures remain to be recorded, however, these having occurred under small doses of the tuberculin, and before we had become aware that a dose larger than 3 milligrammes was sometimes necessary. Guttman, in his long series of cases, had observed that with a dose of 3 milligrammes he invariably obtained as marked a reaction as with any larger dose; but our own observations have led us to conclude that at times there is an individual susceptibility or idiosyncrasy which enables a very small dose to produce a marked reaction, while in other instances no result is obtained till a larger quantity is administered. It is in this way we would account for a distinct failure in a young man with incipient pulmonary tuberculosis, showing very few local signs, but in whose sputum tubercle bacilli were found. The inoculation of three successive doses of 1, 2, and 3 milligrammes was attended with negative results; and believing it wiser at the time not to administer more, the patient was allowed to leave the hospital before a larger dose could be injected. In one case of tuberculous tarsitis, and one of Pott's disease, the same negative results were obtained with similar small doses; but with these instances ends the total number of cases where the diagnostic value of the test seems doubtful.

As to the criterion of what constitutes a reaction, there has evidently been considerable variation of opinion. In our own cases, unless the rise of temperature was more than $1\frac{1}{2}^{\circ}$ to 2° F. above that previously existing, we have not considered the reaction positive; and usually the febrile condition subsided gradually in about 36 to 48 hours. In the majority of cases the constitutional symptoms were slight, including mainly headache and slight *malaise* with nausea. Distinct rigors were uncommon. The highest temperature obtained was 105° F., and that after the injection of only $\frac{1}{2}$ milligramme.

In conclusion, we would record our hearty thanks to Drs. Trudeau and Hewetson, of Saranac Lake, for their manifold kindly suggestions and generous supply of tuberculin, while we are further indebted to the clinicians in attendance at the General and Royal Victoria Hospitals for the use of material so freely granted.

A QUANTITATIVE METHOD OF SERUM DIAGNOSIS BY MEANS OF DRIED BLOOD.

BY

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[From the Laboratory of the Montreal General Hospital.]

WIDAL suggested¹ the plan of simply dissolving the dried blood drop in a fixed number of drops of water. Dacosta advocates the same plan. The objection to this is that the previous volume of the blood drop cannot be determined after it has been dried, and they have not attempted to estimate the limit of possible dilution.

The plan adopted by us is that of taking the sample by means of a loop of standard size, made of No. 20 gauge copper wire, 2 mm. inside diameter. This gives a considerable quantity of blood which, when dried and spread on a glass slide, can afterwards be dissolved by means of a definite number of loopfuls of water. The solution thus obtained is abundant enough to yield much more blood solution than is necessary for quantitative measurement of the reaction. To ensure greater accuracy a small outfit was used in which the original loop used in taking the blood is returned, and employed in making the dilutions.

The dried blood drop is dissolved in five loopfuls of water used *seriatim*, the solution from each drop being sucked up by a capillary tube, and the whole mixed. From this stock solution standard sub-solutions are made in a straight capillary tube bound by a rubber band to a thermometer scale. The advantage of this plan is that the exact quantity of blood taken is immaterial. The tube is easily washed. The

method of placing the loopfuls separately on the glass, as recommended by Delépine, was found very reliable.

In testing, a small platinum loop of the blood solutions is mixed with as many loops of water and bouillon culture as may be needed to give the required dilution, and make the volume of the diluted blood as nearly as possible equal to that of the culture. For convenience a table of the formulæ for dilutions was made, and is useful when one is ascertaining the extreme limit of dilution possible, as this often reaches into the hundreds.

For an ordinary test—to learn, for instance, if the blood reacts at 1 to 10—all that is necessary is to mix a loop of the stock 1 to 5 solution with one of the culture. By mixing it with four loops of water and five of culture, a dilution of 1 to 50 is readily obtained. The time required when this simple test only is needed does not exceed five minutes.

We have made duplicate observations to ascertain the average limit of error, and find this to be about 10 per cent. after some familiarity has been obtained with the method. It is much more accurate than a plan previously suggested by one of us of taking the standard of colour as a guide.

Comparative tests, when the duplicate samples consisted either of serum or of watery solutions of fresh blood taken by a Zeiss-Thoma barometer pipette, gave us results practically identical with those by the dry blood method, the latter being slightly lower, however, as a rule. So that if the dry blood showed a given intensity, the serum would show at least that, and perhaps 10 per cent. more.

Drying for a few days did not make any great difference in the intensity, though it slightly lessened the reaction. We defer the publication in detail of our results until our observations are more numerous.

The result of the quantitative dry method so far has convinced us that it is delicate and accurate enough to give results sufficiently close for practical purposes or for comparison with other methods of testing. It has not yet been found necessary to use the quantitative test for the ordinary routine diagnostic work; but where the results are to be used for recording doubtful or exceptional cases for scientific publication, we think quantitative data preferable.

It does not appear necessary, however, to use quantitative tests in ordinary routine diagnostic work. Our experience has been that in no case hitherto examined by us have we been able to obtain a decisive reaction by the use of quantitative methods of fluid blood or serum, when it could not also be obtained by the dried blood test without quantitative complications.

At the same time, we fully agree with the views expressed by Professor Welch on a recent occasion, that for scientific observations it was very desirable that the dried blood method should be also made a quantitative one, and our work in that direction was prompted by a friendly suggestion coming from him, for which we wish here to express our thanks.

REFERENCE.
1 Soc. de Biol., January, 1897.

THE CONDITION OF TEST CULTURES ESPECIALLY AS REGARDS TITRATION FAVOURABLE TO CLEAR SERUM REACTIONS BY THE DRIED BLOOD METHOD.

BY

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[From the Laboratory of the Board of Health of the Province of Quebec]

THIS matter was referred to in the discussion at Philadelphia on June 3rd, 1897, before the American Medical Association, by Bates Block and one of us. We found that by varying the composition of the medium and the conditions under which the culture is kept, it is possible to obtain a culture which will yield excellent results or the reverse. We can now at will obtain a bouillon culture which can be left in contact with solutions of non-typhoid blood for 24 to 48 hours without giving any reaction, and will react promptly with typhoid blood. On the other hand, we can by neglecting certain details, get a test culture which will give one of two pseudo-reactions with almost any blood solution or serum. Pseudo-reaction A is a

decided clumping of over 95 per cent. of all the bacteria, but without loss of motion. Pseudo-reaction B culture with most blood solutions will show cessation of motion, with defective clumping. The former (A) we found to occur with cultures made very active by daily transplantations, and the latter (B) to growth in rather too alkaline bouillon.

Our earliest observations happened to be made with bouillon giving satisfactory results. These became unsatisfactory for a few weeks under daily transplantations and good again on returning to monthly changes of stock cultures. After a few months a change in the laboratory personnel with different assignment of duties led to the second class of pseudo-reactions troubling us for a few weeks. Attention to the reaction of the culture medium remedied this difficulty, which seemed entirely due to a trifling increase in the alkalinity of the laboratory bouillon. At another laboratory in charge of one of us there was still a supply of bouillon remaining which gave satisfactory results. This reacted 3.5 per cent. acid to phenolphthalein and just verged on the acid point with litmus. The adoption of bouillons having a reaction requiring 3.5 per cent. of normal alkali to restore the tint of phenolphthalein led to satisfactory results once more. The reading of the end point varies somewhat with the observer and the optimum reaction varies slightly with the particular culture and the composition of the medium, but always lies between 3 and 4 per cent.

The pseudo-reactions here described occur in bouillons giving a heavy growth and some sediment at the end of twenty-four hours at 37° C. They show a tendency to the spontaneous clumping mentioned by Widal, but this is greatly exaggerated by adding blood solution. Serum does not have the same tendency to cause pseudo-reaction A. Dilution alone did not prove in our hands a satisfactory remedy.

We believe that these anomalies explain the statements of some writers that all serums tend to cause clumping sooner or later. The optimum reaction depends somewhat on the temperature of the thermostat and the age of the culture. Satisfactory cultures show gentle clouding only at twenty-four hours, and have no sediment or serum. If the culture medium is made too acid it becomes insensitive, loses motility, and does not react promptly, but gives no pseudo-reaction.

We note for the first time in the BRITISH MEDICAL JOURNAL of December 4th, 1897, that Delépine had already in the JOURNAL on April 17th, 1897, called attention to the danger of pseudo-reactions if the bouillon is alkaline. He recommends neutral bouillon, whereas we found acid bouillon preferable. The question is one of intensity of growth rather than of pure alkalinity alone, since the pseudo-reactions can also be avoided by raising the alkalinity till the growth becomes scanty. Personally, our interest lay more in deciding the most suitable reaction for dried blood work.

ON SERUM REACTION WITH BACTERIA OTHER THAN THE USUAL PATHOGENIC FORMS.

BY

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[From the Pathological Laboratory, McGill University.]

SOME observations made jointly by us have shown the occurrence of a serum reaction when cultures of the specific organism of the Picton cattle disease are brought in contact with the serum of cattle attacked by this disease or with that of other animals (goat, rabbit, and guinea-pig) infected with it. The blood of healthy animals did not give the reaction. Its diagnostic value has not yet been thoroughly tested clinically. The reaction was obtainable in dilutions of 1 in 25, and was evidenced by the formation of distinct clumps, the control specimens remaining free from them after many hours.

Injection of cultures sterilised by heat produced a moderate blood reaction and some degree of immunity. The results will be more fully reported elsewhere by E. W. Hammond. The organism in this disease is a small non-motile diplobacillus, the constant occurrence and specific relation of which to this disease was established some years ago—in 1893—by Dr. Adami.