all the typical clinical features, including pigmentation of the mucous membrane of the mouth and lips, while the children, in order of age, demonstrate the same phenomena in decreasing severity and distinctness. The moles, placed so prominently in the list of important clinical features by Addison, Greenhowe, and others, are specially characteristic of this family group. If less significance is attached to them, then the correctness of our diagnosis is much more open to question.

The cases were shown at a meeting of the Medico-Chirurgical Society of Edinburgh on February 1st, 1900.

Why should it not be conceivable that a tuberculous lesion so often hereditary might not exist in the same family in every child? But why is it that in so many recorded cases of Addison's disease, amounting now to several hundreds, so seldom is any reference made to plurality of cases in one family?

The future will clear up the mystery as far as this family is concerned, and the record is published in the hope that by directing attention to investigation into family history, more carefully than has been done in the past, similar instances may be met with.

PRELIMINARY NOTE ON A NEW QUANTITATIVE METHOD FOR SERUM DIAGNOSIS.

BY

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[From the Jenner Institute of Preventive Medicine.]

For the successful application of the agglutination test ("Widal" method) in the diagnosis of disease, it is universally admitted that some dilution of the blood is desirable, a dilution which should be approximately known, and which varies in practice between 1 in 10 and 1 in 100, while for the investigation and comparison of the specific agglutinating power of the blood in different cases or diseases, and under varying conditions, an accurate quantitative method for carrying out the dilutions is a necessity.

Of the various methods employed for diluting, the following may be mentioned:

1. Dilution by means of a platinum loop (McWeeney, Delépine, and others). This at the best can be but approximate.
2. Dilution by means of a capillary pipette (Wright). This is more suitable for serum than for the whole blood.
3. The dry-blood method (Johnson). This is subject to many inaccuracies: for example, variability in the amount of solids in the blood and in the dryness of the film, and the difficulty of weighing out so small a quantity as 1 milligram.
4. The hæmoglobinometric method, the blood being diluted to a given standard colorimetrically by its contained hæmoglobin, a very variable factor.
5. The capillary-tube method (McFarland). Independently of McFarland, we carried out experiments with the capillary-tube method, and early abandoned it, for we found it impossible to obtain capillary tubes of even approximately the same internal diameter, while accurate calibration by capillarity is attended with great difficulties.

We do not mean to imply that the above methods are useless, all of them give satisfactory results for rapid clinical diagnosis or in those cases where an approximation suffices, but we believe that the capillary pipette method (No. 2 above) is the only one which has any claims to accuracy. We have, therefore, devised the following quantitative method, which is suitable not only for serum but also for the whole blood, and is capable of the greatest accuracy.

Ordinary vaccine tubes are taken, the large, the very small, and any irregular ones being discarded. We prefer those varying in diameter from about 0.9-1.2 mm., they are boiled in strong nitric acid, thoroughly rinsed in tap, and then in distilled water and dried; a large number can thus be treated at one operation. The selected tubes are charged with the blood to be examined, being preferably one-half to two-thirds filled, and are carefully sealed by melting their ends in a flame in the ordinary way. In the laboratory the length of the column of blood between the bases of the two menisci is first measured by means of a scale graduated to half millimetres assisted by a lens mounted to avoid parallax. The end of the tube is then cut off by means of a fine file or writing diamond, and its internal diameter is measured. This, the most important of all the manipulations, is carried out in the following manner:—

The tube is supported in the axis of the microscope by means of two iris diaphragms carried respectively by the stage and by the substage. These close concentrically and support the tube, at the same time cutting off all light reflected from the mirror. The opaque blood in the interior of the tube in the same way cuts off all light that might pass through the lumen of the tube. The only light that thus reaches the optical system is that transmitted by the glass walls of the tube. In the field of vision the lumen of the tube now appears as a black circle surrounded by a brilliantly-illuminated annulus. Owing to the extreme sharpness of the image the measurement of the diameter of the tube can be carried out with great accuracy by means of an eye-piece micrometer arranged to give readings to the one-thousandth of a millimetre. From the formula

$$V = D^2 \times L \times 0.7854,$$

Where V = volume of the cylinder,

D = diameter of the cylinder,

And L = the length of the cylinder,

the volume of the blood is calculated. If many measurements have to be made, a Fuller's slide-rule greatly expedites the calculations. It is of the utmost importance that the diameter of the tube be accurately measured, and therefore the adjustment of the eye-piece micrometer must be carried out with great care, and the scale by which it is set should be one the accuracy of which is undoubted. For, considering the formula ($V = D^2 \times L \times 0.7854$), it will be seen that any error in the measurement of D will be increased in a geometrical ratio in the final result, hence the comparative inefficiency of a capillary-tube method in which D is measured indirectly and with less accuracy.

We have controlled the accuracy of the method by filling tubes with distilled water, ascertaining the amount of water by direct weighing, and afterwards calculating the weight of the water by the formula given above; it is easy to obtain results which are within 0.5 per cent. of the true weight. Having ascertained the volume of blood, it remains to carry out the dilutions; this is accomplished as follows:

The capillary tube is placed in a vertical thick-walled glass tube of slightly larger bore, the lower end of which is submerged in the required amount of the diluting medium (measured out by a graduated pipette) contained in a hollowed-out block of glass, such as is used in histological and microscopical work. The capillary tube with its contained blood is then crushed *en masse* by means of a soft iron plunger, and the interior of the containing tube is washed out several times by drawing up the diluting medium into its lumen, either with the mouth or by means of an india-rubber teat. The powdered glass quickly falls to the bottom, and the mixture may be employed for conducting the serum reaction either by the microscopic or by the macroscopic method.

On a future occasion we propose giving further data and details, but we may say in conclusion that this method has been tested at the Jenner Institute of Preventive Medicine, and has proved to be easily carried out, and to give satisfactory results, while the time consumed in the whole of the manipulations (5 to 10 minutes) is comparatively short.

ON THE VALUE OF THE AGGLUTINATION TEST AS A MEANS OF DIAGNOSIS OF THE B. TYPHOSUS FROM COLIFORM ORGANISMS.

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THE difficulties which surround the correct interpretation of the agglutination test are well shown by the following bacteriological examination of a drinking water derived from a polluted upland surface. The water was sent for analysis as there had been several cases of enteric fever amongst the consumers, though no real epidemic, such as might be attributed to a polluted water supply, had occurred. On arrival at the laboratory the water was found to contain a large number of micro-organisms, so the usual plan of concentrating the sample by pumping one or two litres through a Berkefeld bougie had to be abandoned. Consequently 1 c.cm. of the water was added to each of twelve broth tubes containing 0.05 per cent. of carbolic acid; all the tubes were then incubated at 37° C. At the end of twenty-four hours some of the tubes were hazy in the upper half and some in the lower half, and three tubes were turbid throughout. These three tubes were then plated out in gelatine containing 0.05 per cent. carbolic acid. After seventy-two hours' incubation at 22° C. the plates made from two of the tubes showed almost pure cultures of coliform colonies, whilst the plates made from the third tube showed numerous colonies of *B. megaterium*. Nearly all the coliform colonies were fished and their cultural characteristics studied. It was then found that the coliform organisms could be arranged in three groups.

Group I (Cultures A₂, B₂) showed the following characteristics:

Colonies on Gelatine Plates.—The surface colonies appeared as thin bluish films with an irregular margin; under a low power the centre of the colonies had a yellowish tinge and surface markings of ridges and valleys running irregularly from the centre to the periphery were seen.

Slab Gelatine.—Surface growth resembling the colonies, and a thin white growth along the stab.

Milk.—Unchanged in appearance after six days' incubation at 37° C. Neutral litmus whey tested with $\frac{N}{10}$ alkali, and compared with a control tube of the same milk kept for the same time at 37° C., showed a slight increase in acidity.

Glucose Gelatine (Shake).—Marked gas formation after twenty-four hours at 22° C.

Peptone and Salt Solution.—No indol produced after seven days' incubation at 37° C.

Potato.—Thick yellowish-white growth.

Gelatine (25 per cent., incubated at 37° C.).—Marked film on the surface after forty-eight hours' incubation.

Microscopical Characters.—A small motile bacillus.

Agglutination Test.—Typhoid serum, diluted 1 in 10, produced no traces of agglutination.

Group II (Culture A) showed the following characteristics:

Colonies.—The same as Group I.

Slab Gelatine.—The same as Group I.

Milk.—Unchanged in appearance; litmus whey showed a slight increase of acidity as compared with a control tube.

Glucose Gelatine (Shake).—No gas formation.

Peptone and Salt.—No indol produced after seven days' incubation at 37° C.

Potato.—A yellowish-coloured growth, yellow tinge not so marked as in Group I.

Gelatine (25 per cent., incubated at 37° C.).—No film on the surface; a diffused growth through the tube.

Microscopical Characters.—A small motile bacillus, about the same size and same motility as Group I.

Agglutination Test.—Typhoid serum, diluted 1 in 10, produced no traces of agglutination.

Group III (Cultures B₃, B₄, B₅, and B₆) showed the following characteristics:

Colonies on Gelatine Plates.—Identical with those of Groups I and II.

Slab Gelatine.—Surface growth the same as the colonies, which appeared to grow more slowly than B. coli and more quickly than B. typhosus planted out at the same time.

Gelatine Slope.—A thin growth which did not show the crenated margin of B. coli but was distinctly wider than B. typhosus, though not so wide as B. coli planted out at the same time. After about ten days' incubation distinct granules appeared down the centre of the streak.

Glucose Gelatine (Shake).—No gas formation.

Peptone and Salt Solution.—No indol produced after seven days' incubation at 37° C.

Milk.—Unchanged in appearance; litmus whey rendered slightly acid as compared with a control tube.

Potato.—Yellowish growth. The colour and extent of the growth varied, but when compared with B. typhosus planted out on the same potato, there was always a more marked yellowish tinge after several days' incubation.

Agar.—A white growth, not characteristic.

Gelatine (25 per cent., incubated at 37° C.).—No film appeared on the surface, but a diffused growth took place through the tube.

Microscopical Characters.—A small motile bacillus resembling B. typhosus closely.

Flagella.—Long and wavy, average number 6.

Agglutination Test.—The bacilli were completely agglutinated in thirty minutes by typhoid serum diluted 1 in 20. The cultural characteristics of the members of this group were in many ways so like the B. typhosus that cultures of them and also a pure B. typhosus were planted out at the same time on the various media, so that the growths might be compared from day to day. It was then found that the members of this group differed from B. typhosus in three ways, namely: (1) Quicker growth, (2) a tendency to produce a yellow growth on potato, and (3) the flagella averaged only six. The agglutination reaction with antityphoid serum was also further tested as follows: A twenty-four hours' agar growth of the different organisms was made into an emulsion with broth; equal quantities of the emulsion and antityphoid serum diluted with broth were then mixed so as to form dilutions varying from 1 in 10 to 1 in 2,000,000. The dilutions were examined by (a) the rapid hanging-drop method and (b) the slow method in capillary sedimentation tubes. The capillary tubes were kept at the laboratory temperature for twenty-four hours, and then examined macroscopically for sedimentation, and microscopically—namely, the tubes were opened and the contents blown out on to cover glasses and then examined in a hanging drop—for agglutination. A twenty-four hours' broth culture of the organisms was also prepared and mixed with antityphoid serum diluted with broth so as to form the same series of dilutions. A true

typhoid culture was also put up at the same time with the serum in the same dilutions. Controls of the emulsions were preserved in capillary sedimentation tubes and examined at the end of twenty-four hours for traces of agglutination. Two specimens of antityphoid serum were employed; one possessed an extremely powerful agglutinating action on the B. typhosus, but the other was weak, and when highly diluted required considerable time to bring out the specific action. The following results were obtained:

Powerful Antityphoid Serum.

Dilutions of Serum.	Culture of B. Typhosus.		Culture of Coliform Organism (Group III).	
	Hanging Drop (2 Hours).	Capillary Tube (24 hours).	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).
1-10	Complete agglutination	Complete agglutination	Complete agglutination	Complete agglutination
1-20	"	"	"	"
1-40	"	"	"	"
1-80	"	"	Incomplete (large clumps)	"
1-160	"	"	Incomplete (small clumps)	Incomplete (large clumps)
1-320	"	"	Nil	Incomplete (small clumps)
1-640	"	"	"	Nil
1-1,280	"	"	"	"
1-2,500	"	"	"	"
1-5,000	"	"	"	"
1-10,000	"	"	"	"
1-100,000	"	"	"	"
1-2,000,000	"	"	"	"

Weak Antityphoid Serum.

Dilutions of Serum.	Culture of B. Typhosus.		Culture of Coliform Organism (Group III).	
	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).
1-10	Complete agglutination	Complete agglutination	Complete agglutination	Complete agglutination
1-20	"	"	"	"
1-40	Incomplete (large clumps)	"	Incomplete (large clumps)	"
1-80	Nil	Incomplete (large clumps)	Nil	Incomplete (large clumps)
1-160	"	"	"	"
1-320	"	"	"	Incomplete (clumps small)
1-640	"	Incomplete (clumps small)	"	Nil
1-1280	"	Incomplete (clumps small and few in number)	"	"

The control hanging drops and capillary tubes showed no traces of agglutination.

An examination of the above results shows that, when working with a powerful antityphoid serum, the quick examination by the hanging drop at once brings out marked differences between the true B. typhosus and allied coliform organisms. But if a weak serum is employed, the specific action is so slow that the hanging-drop method quite fails to differentiate between the organisms, and it is necessary to prolong the examination for twenty-four hours, when distinct differences in the agglutination become apparent.

Both the serums employed were prepared by inoculating horses with cultures of B. typhosus, and it appeared possible that the agglutinative action of the serums on the coliform organisms might not be specific but simply the result of agglutinins present in the normal serum of the horse. In order to test this point the coliform organisms were examined in exactly the same manner as before; but, instead of using the antityphoid serums, normal horse serum was employed. The following results were obtained:

Normal Horse Serum.

Dilutions.	Culture of Coliform Organism (Group III).		Dilutions.	Culture of Coliform Organism (Group III).	
	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).		Hanging Drop (2 Hours).	Capillary Tube (24 Hours).
1-10	Complete agglutination	Complete agglutination	1-160	Nil.	Incomplete (large clumps).
1-20	"	"	1-320	Nil.	Incomplete (a few clumps).
1-40	Incomplete agglutination	"	1-640	Nil.	Nil.
1-80	"	"			

An examination of this table shows at once that the agglutinative actions of the antityphoid serums and the normal horse serum on the coliform organisms were practically identical.

As a final test of the nature of these organisms it was determined to immunise a rabbit with a broth culture of one (B₃) of the members of Group III. This was kindly done for me by Major Leishman, R.A.M.C. The serum so obtained was then tested, in the same manner as before, with a twenty-four hours' agar culture of *B. typhosus*, and a twenty-four hours' agar culture of the same organism which had been used to immunise the rabbit. The results shown in the table were obtained.

Anticoliciform Serum (B₃).

Dilutions.	Culture of <i>B. Typhosus</i> .		Culture of Coliform Organism (B ₃).	
	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).	Hanging Drop (2 Hours).	Capillary Tube (24 Hours).
1-10	No traces of agglutination	No agglutination.	Complete agglutination	Complete agglutination
1-20	"	"	"	"
1-40	"	"	"	"
1-80	"	"	Incomplete	"
1-160	"	"	Nil	Incomplete (large clumps)
1-320	"	"	"	"
1-640	"	"	"	"
1-1280	"	"	"	Only a few small clumps

It is, therefore, clear that the coliform organisms contained in Group III are not typhoid bacilli, though as regards their cultural reactions they differ very slightly from Eberth's organism. It is also evident that the correct interpretation of the agglutination test may be a matter of considerable difficulty. The experiments given show distinctly that there are at least two important factors which enter into the reaction, namely (1) the strength of the serum employed, and (2) the time during which the serum is allowed to act on the microbe, consequently no fixed dilution of typhoid serum can be laid down which will enable the *B. typhosus* to be at once distinguished from coliform organisms. It may be said that an organism which fails to agglutinate with typhoid serum is certainly not the *B. typhosus*; but, on the other hand, it does not necessarily follow that an organism is the *B. typhosus* because it is agglutinated by fairly dilute typhoid serum. With our present knowledge it appears essential to work with a powerful serum, also the suspected organism should be tested with the serum diluted to the highest degree which has been found capable of agglutinating a true *B. typhosus*.

INOCULATION WITH TYPHOID VACCINE AS A PREVENTIVE OF TYPHOID FEVER.

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ONE can in these days, without fear of contradiction, make the statement that typhoid fever is only too readily contracted by those who are administering to patients suffering from this disease. From my own experience of an institution where considerable numbers of these patients are treated, I can

affirm that the occurrence of typhoid fever amongst the staff is a not infrequent and a very real cause of anxiety, despite the most explicit instructions for the prevention of infection; whilst the occasional occurrence of a fatal termination casts a temporary gloom over the whole establishment. A consideration of the table given below as regards the 4½ years from 1895 to September, 1899, will only too readily give an idea of the reality of this anxiety.

In September last it seemed to me desirable to offer facilities to the staff for the injection of typhoid vaccine, and I accordingly made application to Professor A. E. Wright, of the Army Medical School, Netley. (I wish to take this opportunity of thanking Professor Wright for the extremely prompt and courteous manner in which he has responded to my requests, and I am all the more sensible of his kindness, seeing that my applications have been made at a time when the Army Medical Department has been more than usually occupied owing to the war in South Africa.)

I am well aware that the time which has elapsed since the adoption of the prophylactic measure is too short to draw any conclusive inference, yet the results so far observed are, I think, sufficiently marked to warrant this report, and even embolden one to hope that, at any rate for a limited period, either an immunity against typhoid can be thus produced, or at least one may give "an increased power of resisting infection by living typhoid bacilli."

Concerning the procedure followed in making the injection, it is sufficient to say that the instructions issued by Professor Wright along with the vaccine were carefully attended to with two exceptions. The first injection revealed the fact that the local tenderness remained after the general indisposition had disappeared, and thus caused discomfort in wearing the natural clothing, so that future injections were made over the insertion of the deltoid.

In the second place, as the nurses are usually only employed in the typhoid fever ward for a period of three months, the inoculations have only been repeated on two occasions. The inoculation has not been made compulsory upon the staff. I have simply recommended it to those attending the typhoid patients, and I have not been disappointed in the ready manner in which it has been accepted.

During the six months concerned 22 nurses have been engaged, of whom 14 received the typhoid vaccine. Of the remaining 8, 4 had previously been under my care for typhoid fever contracted during the performance of their duties, whilst the other 4 did not wish to submit to inoculation.

In general the clinical symptoms have agreed with those detailed by Professor Wright, but I should be inclined to add that headache has been consistently present in all; in several it was accompanied by general pains or aching, whilst one nurse complained of "cramps in the legs and feet." Frequently also there was nausea, and on two occasions vomiting. At the end of thirty-six hours the constitutional symptoms had disappeared, but local tenderness sometimes persisted for two or three days longer. By inoculating, therefore, in the evening the nurses were allowed to have one day in bed, and returned to duty on the following day.

Taking now the occurrence of typhoid fever amongst the staff since 1895, the following table shows the number of typhoid patients admitted in each year and the number of attacks contracted during the corresponding periods.

TABLE I.

Year.	No. of Patients Admitted.	Cases of Typhoid in Staff.
1895	229	3
1896	238	3
1897	302	4
1898	426	8
To end of September, 1899 ...	163	5
From September, 1899, to March, 1900	146	0

Concerning the 5 cases occurring in 1899, it should be men-