

of six months gradual diminution, and finally entire disappearance of the abscess, followed—a result which was claimed as a supreme triumph for domestic surgery. It may be pardonable to remind you that the spontaneous disappearance of abscesses is brought about by the gradual absorption of the fluid contents leading to inspissation; the treatment by aspiration imitates this sometimes natural process and is therefore based upon perfectly rational grounds.

You have without doubt noticed that I have spoken only of simple aspiration without the injection of iodoform or any other germicide. In the commonly used text-books and in other places will be found recommendations, sometimes very strong, for the use of iodoform injections, mainly in the form of emulsion, in all tuberculous abscesses. A fair trial of the drug in this relation led me some time since to the conclusion that no obvious advantage arises from its use. Indeed, I am one of those who rarely use iodoform for any purpose. In operative work I never use it—a fact which is familiar to those who have seen anything of my practice. It may, indeed, be known to some of you that my first admonitions to a house surgeon coming freshly to my work are that he shall be careful about absolute cleanliness and that the use of iodoform shall not be a routine treatment with him. For my own part, I feel bound to say that although I have seen harm come from the use of the drug I have never seen it effect any advantage worthy of mention in operations in any part of the body. In certain stinking or phagedænic sores of a venereal sort it may at times be used with benefit, but in operative work properly conducted it is, in my belief, valueless. On the other hand, I have seen undoubted instances of poisoning from it, and I have seen cases in which, to the best of my judgment, grave complications have arisen solely in consequence of the employment of iodoform. The indiscriminate use of the drug has without doubt vastly diminished of late, but even now by some surgeons, operations, however clean, are rarely completed without its application. For example, the wound in the abdomen after the removal of an appendix during the quiet stage is by some filled with iodoform; the wound resulting from the removal of a breast is frequently freely dusted with it; and I have actually seen the drug freely sprinkled over the parts after an operation for hare-lip. Personally I find it difficult to understand why a drug the germicidal qualities of which cannot be trusted until it has been sterilised in order to kill its own peculiar micro-organisms should be accorded a respect which with some of us exalts it almost to the position of a fetish. Of the evil effects of the drug I could give you sufficient examples were not the matter beyond the scope of the present lecture.

Returning to the main subject which is before us, let me repeat that I wish it to be distinctly understood that I have no desire to advocate the indiscriminate use of aspiration in chronic abscess to the exclusion of the more scientific treatment by free incision and scraping. All that I am anxious to do is to point out that aspiration, in the manner which I have indicated, offers a means of dealing with certain chronic abscesses, especially of the "residual" kind, which can be with safety carried out when the treatment by incision and scraping from the circumstances of the patient, and possibly of the practitioner, is extremely undesirable, if not actually dangerous; and further, that in many cases the aspiration treatment can be adopted without interfering with the patient's ordinary vocation for a single day—an advantage the importance of which in some cases can hardly be over-rated. It is, moreover, a method which offers but trifling difficulty in the matter of sterilisation and needs no complicated dressings or other troublesome details—facts which to those who have to practise, as some of you may have to do, in circumstances of difficulty in the matter of antiseptics and of hygiene, must be of great moment. In this respect it may not be amiss to remind you that it is impossible for all of us, much as we may desire to do so, to practise amongst the wealthy and in the advantageous conditions which can be afforded by riches, or to have at hand the means which are available in a great general hospital like this. To those whose practice lies in the less advantageous circumstances I can with confidence recommend the method as one which, although it may be "old-fashioned," may be used with safety when more radical means are so difficult as to be almost impossible with due regard to the patient's welfare; and further, I am sure, no matter what the facilities of the practitioner may be, that there are many cases of the kind of which I have been speaking in which the use of the aspirator with discrimination may prove of incalculable advantage.

ON A METHOD OF MEASURING THE BACTERICIDAL POWER OF THE BLOOD FOR CLINICAL AND EXPERIMENTAL USES.

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WHATEVER may be the exact rôle played by the bactericidal substances of the blood and lymph in connexion with the destruction of bacteria in the animal organism there can be no doubt as to the important influence which these substances must exert. The quantitative study of the variations in the bactericidal power may therefore serve quite well to elucidate many pressing questions. In the first place, it may furnish valuable data upon which to base a diagnosis and prognosis in cases of specific fever. Again, the quantitative study of the alterations in the bactericidal power of blood which are produced by the addition to that blood (*in vitro* or *in vivo*) of a particular "therapeutic serum" may quite well yield important information with regard to the utility or otherwise of the particular "remedial agent" in question. Furthermore, in the case of animals inoculated with particular bacterial vaccines, a comparison of the bactericidal power of their blood subsequent to inoculation with the bactericidal power of the blood anterior to inoculation may quite well afford valuable indications as to the presence or otherwise in the bacterial vaccines in question of the immunising substances which are desired. Again, quantitative estimations of the bactericidal powers of the blood of normal persons and of persons who have been inoculated with bacterial vaccines may quite well furnish important indications, in the first case as to the expediency of inoculation, and in the latter case as to the successful issue, or otherwise, of the inoculation. Lastly, by means of quantitative estimations of bactericidal power many theoretically important questions may be resolved. Among these may be mentioned the question as to the co-existence and interrelation between the agglutinating and bactericidal powers of the blood. Without in any way undervaluing the important results already obtained by the initiating work of Buchner, it will be manifest that the investigation of most of these questions has hardly as yet been entered upon. It has been retarded by the fact that the technical methods which have been hitherto employed have necessitated the employment of relatively considerable quantities of blood, and have, in the case of agglutinating bloods, been associated with a serious source of fallacy, arising from the fact that the bacteria, which would normally be separately disposed, are under the influence of such bloods agglutinated together, with the result that the individual colonies which develop on the nutrient medium may be the outcome, not of a single surviving bacillus, but of a whole group of surviving bacilli. By means of the technique described below the sources of difficulty and error, which have been referred to above, can be effectually and, it seems to me, easily disposed of. The method is also a comparatively rapid one, it being possible, after practice has been acquired, to carry out about four quantitative determinations in the hour.

The principle of the method.—With a view of determining in a quantitative manner the bactericidal power of the blood measured volumes of serum and of graduated dilutions of serum are introduced into a series of "capillary cultivation tubes," along with a series of equal volumes of a gelatin culture containing an appropriate number of bacteria. Mixture of the contents of the serum and culture is then effected in the capillary tube. It will be noticed that by this arrangement the serum comes in contact with the bacteria only after these have been suspended in a fluid which is sufficiently viscid to make it impossible for them to come together into groups. After the gelatin has solidified the tubes are incubated for a period of two or more days. The number of colonies of the specific organism which develop is then elicited by counting under a microscope, the results of the enumeration being in each case compared with the results of the enumeration of the colonies in a series of control tubes filled with an equal volume of the gelatin culture duly diluted with an indifferent diluting fluid.

It will be convenient to begin the description of

procedure by considering how the desired dilutions of the blood are made and the desired quantities of bacterial culture and serum are measured out. For these purposes it is convenient to make, as can readily be done by the following method, an accurately graduated capillary pipette.

Method adopted for dividing an ordinary capillary glass tube so as to make a graduated capillary pipette.—A piece of ordinary glass tubing of about a quarter of an inch (roughly 0.6 centimetre) in diameter is drawn out in the middle into a capillary tube of an approximate diameter of from $\frac{1}{8}$ th to $\frac{1}{10}$ th of an inch (from 0.4 to 0.25 millimetre). Before the glass has quite cooled the capillary end of the tube is bent round so as to make a right angle with the wider portion of the tube. This done a stout glass capillary pipette, such as the five cubic millimetre pipette, which is supplied with Gowers's hæmocyto-meter, is taken and filled in up to the calibration mark with a solution of eosin or other coloured fluid. The extremity of the ungraduated thin-walled capillary tube which is to be calibrated is now broken off and the tip of the stout glass capillary pipette is brought into apposition with the broken end, this last being during the operation held vertically upwards. By gentle blowing the measured volume of coloured fluid is now transferred to the ungraduated thin-walled capillary tube. The transference of the coloured fluid in an unbroken column can be effected without difficulty provided that the end of the thin-walled capillary tube has been broken off in an accurately transverse direction, so as to make it impossible for bubbles of air to be sucked into the orifice.

When the measured volume has been transferred without loss and in unbroken column into the capillary tube, the point of this latter is held downwards in such a manner as to allow the fluid to flow back to the point. A mark with a coloured wax pencil is then placed on the capillary stem to indicate the upper limit now attained by the coloured fluid. After the first division has been thus placed upon the tube the further calibration is undertaken in the following way. The point of the tube is again raised in such a manner as to allow the column of fluid to run back from the point until the distal end of the column of fluid corresponds with the division mark which has been placed on the tube. The point where the second division is to be placed on the tube is now manifestly indicated by the proximal end of the column of coloured fluid. Two divisions having been placed on the tube with a view to obviate, so far as possible, a progressive loss of fluid by adhesion to the walls of the tube, sufficient additional coloured fluid is introduced to fill the tube up to the second division. This done, the point of the tube is again raised and the fluid is allowed to flow back from the point until its distal limit is in apposition with the first division. The proximal end of the column of fluid will now indicate the place where the third division ought to fall. Proceeding in the same way—the next time filling in the tube up to the third division with coloured fluid—the fourth division mark can be placed on the stem of the capillary tube. A fifth and subsequent divisions if desired can be obtained in a similar manner. For the purposes of the method which is to be described below it will, when operating with an original volume of five cubic millimetres, be convenient to place only three marks on the tube in the manner indicated, and then by a subsequent operation to subdivide each of these divisions into two equal subdivisions.

The process of subdivision in question is very simply achieved by the following system of trial and error. The first step is to guess the point on the tube where the first subdivision mark ought to be placed. This point may conveniently be indicated by a light pencil mark. This done, the coloured fluid is filled in up to this tentative subdivision mark. By tilting the tube the column of fluid is now allowed to run down from the point until its proximal end just touches the first of the original (five cubic millimetre) division marks. If it is now found that the distal end of the column corresponds accurately with the tentative subdivision mark, this mark will manifestly have been placed exactly at the proper point. If, on the other hand, the distal end of the column of coloured fluid now overlaps or falls short of the tentative subdivision mark, a second light mark is to be placed on the tube to indicate the point upon which that distal end falls. The true point of hemisection will now fall on a point of the stem midway between these two light marks. Having by the above procedure

found the point corresponding to half the volume originally taken—in other words, to 2.5 cubic millimetres—the points corresponding with one and a half and two and a half volumes respectively are easily found by a procedure exactly identical with that described in placing the original, five cubic millimetre, division marks upon the capillary stem.

It is to be noted that it is not essential that the tubes should be graduated in terms of 2.5 cubic millimetres, or indeed of any definitely known measure; it will suffice for almost all purposes if the capillary stem is divided into equal arbitrary divisions by the method described in the paragraph last but one above. At the same time the calibration in terms of 2.5 cubic millimetres is convenient as giving definite indications of the actual quantities of bacterial culture and serum actually employed.

Method for obtaining the fresh serum required for the estimation.—The next point to consider is the method of obtaining blood serum for examination. Blood may be conveniently obtained from a prick in the finger and may be filled into blood capsules of the pattern elsewhere figured by me.¹ The blood capsule is, in accordance with a suggestion received from Major W. B. Leishman, R.A.M.C., introduced into a small hand centrifugal machine as soon as the blood has clotted. A clear serum is thus readily obtained within a few minutes after drawing off the blood.

Preparation of the bacterial culture employed in measuring the bactericidal power of the blood.—It will suffice under this heading to give indications as to how the bacterial culture is prepared in the case of the bacillus typhosus. The simplest method of operating is here to make a superficial stab into a gelatin tube (inoculating for this purpose from a broth cultivation) and to incubate this for from 12 to 18 hours at a temperature of about 22° C. If after this time the very first indications of growth can, on careful examination, be made out the culture may, after duly melting and mixing, be employed for the estimation of bactericidal power.

Failing a young gelatin culture such as is just described a suitable culture can be obtained by placing 2.5 cubic millimetres of a tenfold diluted young broth cultivation of the typhoid bacillus into a tube of melted gelatin. Similarly, if only an older stab cultivation of the typhoid bacillus is available, this may be diluted 10 or more times with gelatin. In this last case the amount of dilution necessary to give the required number of colonies in the culture tube will readily after a little practice be learned. Until this experience shall have been gained it will be safer to employ only quite young gelatin cultures. It is convenient to employ for the estimation of bactericidal power a culture containing not less than 50, and not more than 200, colonies in 2.5 cubic millimetres of gelatin.

Method of making the blood dilutions.—The indications given under this heading will again relate only to the case where the bactericidal effect upon typhoid bacillus is to be elicited. In connexion with such determinations it will be convenient to employ, in addition to undiluted blood, dilutions of five-, ten-, twenty-, and forty-fold. Dilutions of this order when mixed with equal volumes of gelatin may be expected to show bactericidal effects. Higher dilutions will occasionally be required. The dilutions are made by means of the capillary pipette, the motive force required being obtained by the compression or relaxation of a rubber teat fitted on to the broad end of the pipette. The means for making the five-fold dilutions are provided by the division marks which have been placed on the stem of the pipette. The higher dilutions can be made from these primary ones. The procedure for measuring out equal volumes of typhoid gelatin and serum or serum dilution, and for introducing these into the capillary cultivation tubes, and for affecting the necessary mixture must next engage our attention.

Description of capillary cultivation tubes.—The capillary tubes which are illustrated in Figs. 1 and 2 are easily made in pairs by taking a piece of glass tubing of about three-sixteenths of an inch in diameter and drawing this out in a flame into a capillary stem of from one-sixtieth to one-hundredth of an inch in diameter. Before the glass cools the wider ends of the original piece of tubing are bent round upon the capillary stem in such a manner as to form a right angle with it. Finally, the capillary stem is burnt through in the middle, a slight pull being, during this process, exerted upon the ends of the tube in order to obtain

the very fine terminal hollow filaments which are represented in Fig. 1. The reason for these procedures will presently appear.

Provision for warming the capillary cultivation tubes and for keeping the gelatin culture liquid.—For this purpose a beaker of warm water may be used; it is, however, more convenient to employ a hot-water box, fitted with a warm shelf for the reception of capillary cultivation tubes and sterile watch-glasses, and with provision, in the form of a series of metal tubes let into the face of the box, for the reception of the tubes of the bacterial culture and diluting fluid. A tube of boiling sterile water is to be kept ready at hand for the purpose of warming and washing out the capillary pipette between the separate operations.

Procedure adopted for filling in the tubes.—The simplest procedure to adopt would at first sight appear to be to measure out with the capillary pipette into a sterile watch-glass the required quantities of the bacterial culture and serum and to draw these up, after mixture has been effected, into an ordinary straight capillary tube. In actual practice,

the tube as indicated in Fig. 1. The difficulty can be further circumvented by reserving, after the expulsion of the required quantum, a certain residuum of fluid in the capillary stem. In actual practice this resolves itself into reserving, as indicated in Fig. 1, at least one measured volume of fluid in the stem. In the procedure described below, in which both these safeguards against frothing are employed in conjunction, the measured quantum of serum and bacterial gelatin culture can in each case be filled into the cultivation tube without either wastage or breaking of the column. The rationale of the procedure adopted in filling in the tubes having been explained I may now proceed to the description of the operation.

The capillary pipette is filled up to the second division with the particular serum or serum dilution which is to be tested. The point of the capillary pipette is then introduced into the liquefied gelatin culture² and one volume of this culture is drawn up into the pipette. When this has been done the proximal end of the column of fluid will stand opposite to the third division of the stem, the second and

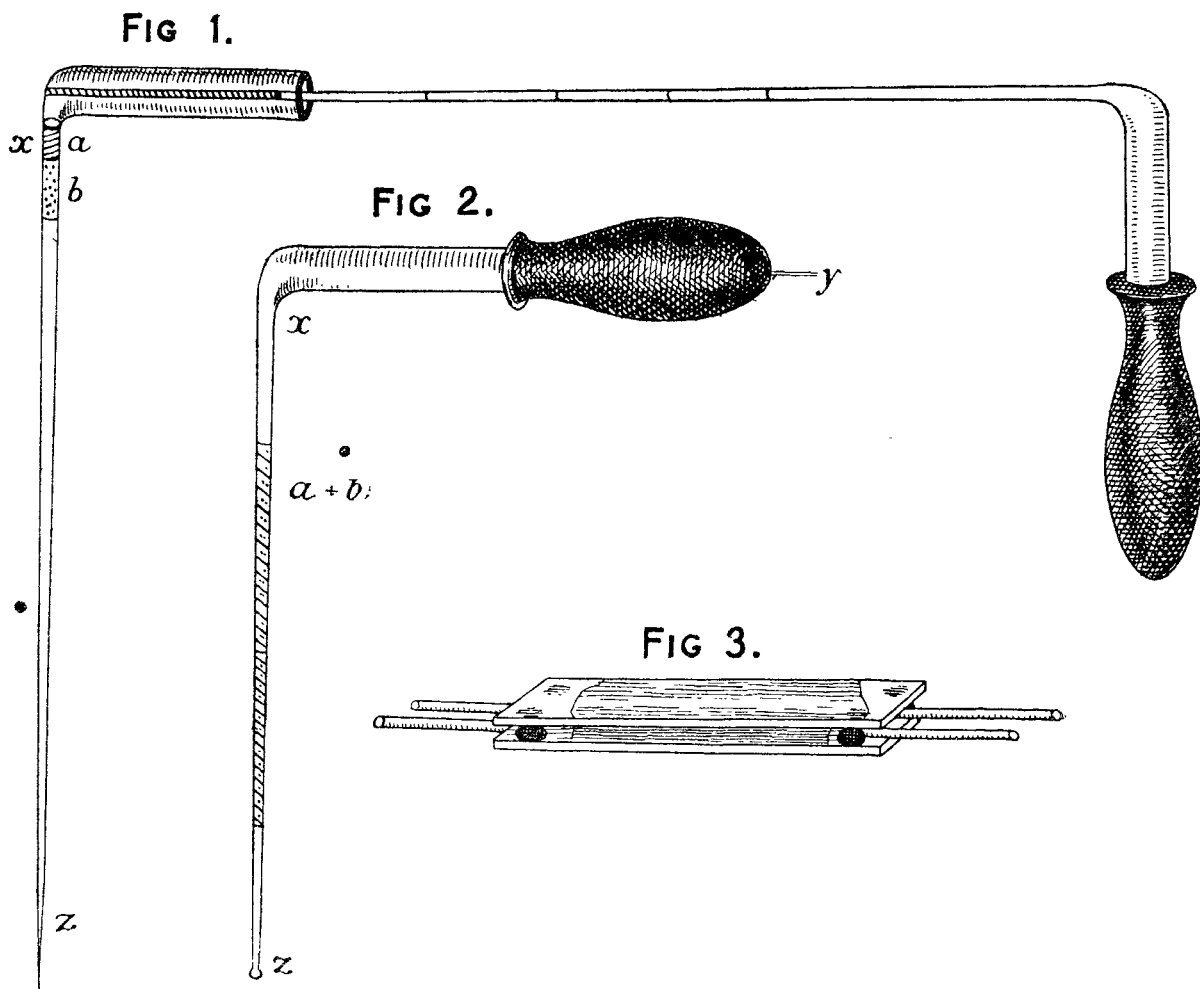


Fig. 1. Capillary cultivation tube on the left and capillary pipette on the right, the point of the pipette being shown in position against the back wall of the cultivation tube. The gelatin culture (*b*) and the serum (*a*) have lodged in the neck of the tube. One volume of serum is shown as retained in the stem of the capillary pipette.

Fig. 2 shows the column of mixed serum and gelatin culture (*a + b*) which has, after mixing, been driven down into the capillary stem of the cultivation tube. The end of the tube (*z*) has been sealed in the flame, and the rubber teat is shown transfixed by the hollow glass filament *y*. The point at which the tube is afterwards burned off is indicated by *x*.

Fig. 3 shows the arrangement for mounting the tubes for microscopic examination.

however, this method proves unsatisfactory, as attempts to mix together the serum and the gelatin lead to the formation of a tenacious froth. As a result, not only is the greater part of the material lost, but it becomes practically impossible to draw up the mixture into the capillary tube in an unbroken column. To avoid these difficulties the procedure which is described below is adopted. Before describing it it will be convenient to consider the following. The cause of the frothing is, as reflection will show, to be found in the fact that the confined air on escaping from the capillary pipette becomes engaged in viscous fluid enveloping the mouth of the tube. In conformity with this the difficulty in question may be overcome by arranging that the viscid fluid shall on emerging from the mouth of the capillary pipette impinge on a perpendicular surface in such a manner as immediately to flow away, leaving the orifice of the tube unengaged. This indication may be complied with in practice by employing a cultivation tube such as is shown in Figs. 1 and 2, and by blowing out the contents of the capillary pipette directly on to the posterior wall of

third divisions being occupied by serum and the first by the gelatin culture. The end of the capillary pipette is then introduced into the mouth of one of the capillary cultivation tubes which has just been taken down from the warm shelf. The tip of the tube having been placed in position against the posterior wall the column of fluid is blown out, on to this perpendicular surface, until the proximal end of the column in the capillary pipette stands level with the first division mark on the stem (as shown in Fig. 1). Of the two volumes of fluid which will thus have been expelled from a capillary pipette the first is, as will be remembered, the gelatin culture, the second is the serum. These flow down in succession and lodge in superposed strata a little below the neck of the tube at the point indicated by the letter *x*. This done the tube is replaced on the warm

² A minute bubble of air may, if desired, be allowed to intervene between the two volumes of serum and the one volume of gelatin. After a little practice this bubble of air is best dispensed with.

shelf. A whole series of tubes are filled in in this manner, each tube corresponding to a particular dilution of the blood which is to be tested. A series of control tubes, in which the serum is replaced by an indifferent diluting fluid, is then filled in. The next step is to effect in each case a mixture of the contents.

Method adopted for mixing the culture with the serum in the capillary cultivation tube.—It will be remembered that the serum and the melted gelatin are forming two separate layers in the cultivation tube. The procedure adopted for mixing these will best be understood if the principles of that procedure are first set forth. In the case of viscid fluids, such as liquefied gelatin and serum, an attempt to produce mixture by allowing air to bubble through the fluids would, owing to the frothing which would be produced, be inadmissible. Effectual mixture can, however, be obtained by causing the contents to rise and fall in the funnel-shaped upper part of the cultivation tube by an alternate rarefaction and condensation of the air in a rubber teat affixed to the upper end of the tube. Certain difficulties are, however, incident to the procedure. If the teat is placed upon the upper end of the tube without special precautions it will be found in nine cases out of ten that the air in the interior of the tube will either have been rarefied or condensed. In the case where a positive pressure has been

at any stage of the procedure. If the tip of the tube has been broken off it will, of course, be necessary, before making the correction, temporarily to seal up the open tip again in the flame.

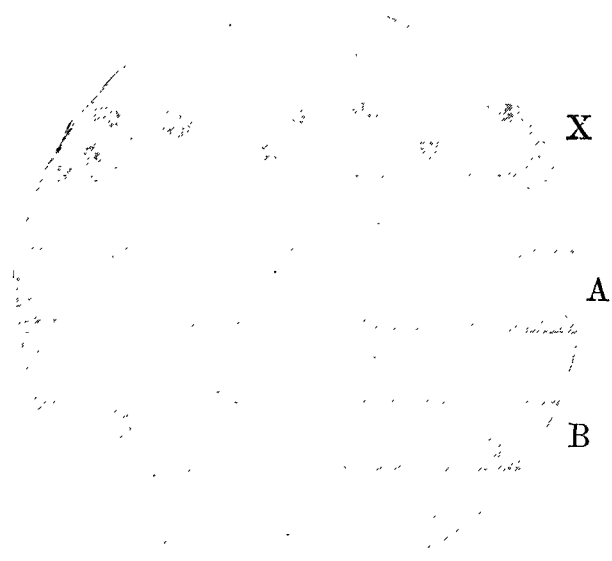
A further point is to be noted. Where the calibre of the stem of the capillary cultivation tube is disproportionately large in proportion to that of the capillary tube which serves as a vent the movement of the fluid in the tube will not be sufficiently under control, with the result that the column of fluid may be subjected to sudden jerks, which may lead to the dislocation of parts of the column or to the bubbling up of air through the fluid. With a view to avoiding these accidents it is well in every case to draw out the lower end of the capillary cultivation tube into a very fine filament, such as is represented in Fig. 1, z. If this has not been done in the making, the end of the tube may be drawn out in a peep-light immediately before the teat is placed upon the tube.

Recapitulation and amplification of the account given of the procedure so far as it relates to the filling and mixing of the tubes.—To recapitulate: after the required dilutions of serum have been made in sterile watch-glasses and the gelatin culture has been prepared the capillary pipette is first washed out in boiling sterile water. Two volumes of serum or, as the case may be, of serum dilution are taken up into the pipette and are followed by one volume of the gelatin culture. The capillary cultivation tube is then taken down from the warm shelf and two out of the three volumes of fluid which have been filled into the pipette are expelled on to the posterior perpendicular surface of the cultivation tube. The cultivation tube is then laid down again on the warm shelf. After a series of tubes corresponding to each of the serum dilutions have been filled in in this way the mixing process is undertaken. For this purpose a teat provided with a capillary vent is fitted on to the cultivation tube, the fine capillary filament at the end of the tube is broken off, and the culture is thoroughly mixed with the serum or serum dilution by causing it to rise and fall in an alternating manner in the neck of the tube. This done the fluid is blown down again into the stem and the tube is sealed. The dilution is then inscribed with a wax pencil on the broad end of the cultivation tube and the series of tubes is placed in a test tube labelled with the date and the name of the patient. When this process has been completed the tubes are placed in an incubator and are incubated for two or three days at a temperature of 22° C. They are then mounted for microscopical examination.

Method of mounting and examining the capillary cultivation tubes.—The capillary stem of each tube is first melted off in a small peep-light at the point indicated by the letter x in Fig. 2. Provision for mounting these has previously been made in the following way. An ordinary microscopic slide is taken and a little pillar of sealing-wax is placed at each corner. Canada balsam or cedar oil is then poured upon the slide. This is then covered up by a second microscopic slide, the ends of which have been heated in a flame so as to cause them to adhere to the top of the sealing-wax pillars. In pressing down the covering slide upon the sealing-wax pillars care is taken to allow sufficient interspace between the slides for insertion of the capillary tubes. These last are introduced from the side. They then appear as seen in Fig. 3. The slide thus prepared is transferred to the stage of the microscope and the enumeration of the colonies is undertaken with an objective of comparatively low magnifying power—a two-thirds of an inch objective is convenient. The microscopical appearances seen in the control tubes and in the serum tubes respectively are represented at x and A and B respectively in Fig. 4. By enumerating the number of colonies in the various dilutions in this manner it is ascertained (a) in what dilution the blood kills all the bacteria which have been introduced; (b) in what dilutions the bacterial colonies are appreciably diminished as compared with those in the control tubes; and (c) in what dilution the serum admits of the unrestrained growth of the bacteria.

Conditions which are essential to accuracy.—After a little experience with this method it will be found that extremely accurate results can be obtained, the differences in the number of colonies which develop in a series of control tubes being then almost negligible. The chief points to be attended to to secure accuracy in the results are the following. *First*, the bacteria must be evenly distributed throughout the gelatin culture which is employed for testing the bactericidal power. This object is in practice readily attained by pouring the liquefied gelatin 25 times from one

FIG. 4.



Shows the appearance of the cultivation tubes as seen under the microscope. X represents a control tube containing numerous typhoid colonies. A represents a serum tube filled in with equal volumes of normal blood and serum. B represents a serum tube filled in with a higher dilution of serum which has a marked but not complete bactericidal power.

produced the breaking off of the end of the capillary tube will now be followed by the forcible expulsion and loss of the contents of the tube. Where, on the other hand, a negative pressure has been produced by affixing the teat in an unexpanded condition, the contents will run up into the teat as soon as the tip of the capillary stem is broken off, and, as before, be lost. It will be necessary, therefore, before breaking off the tip to take steps to equalise the pressure inside and outside the teat. This may be effected in a very simple manner by employing a rubber teat the wall of which has been pierced by an extremely fine hollow glass filament. Manifold advantages result from this arrangement. If, for instance, the teat happens to have been placed upon the tube in a fully expanded condition, and it is desired that the first movement of the contents shall be in the upward direction, the negative pressure which is required may be obtained without removing the teat by pressing out through the capillary vent provided any desired quantum of air and then relaxing the pressure of the finger and thumb. If, on the other hand, the teat has been placed upon the tube in a quite unexpanded condition, and it is desired to impart to the fluid in the neck of the tube first a downward movement, the air may be simply allowed to run into the teat through the vent until a sufficient cushion of air has been obtained. Corrections of this kind can, if required, be made

TABLE SETTING FORTH THE BACTERICIDAL EFFECT OF NORMAL HUMAN BLOOD AS DETERMINED BY THE METHOD DESCRIBED.

Initials or other indication of the source of the blood.*	Date of examination.	Number of typhoid colonies in the serum tubes.							Number of typhoid colonies in the control tubes.		
		Serum dilutions.†									
		2	10	20	40	50	80	100	Tube 1.	Tube 2.	Tube 3.
Serum of 10 normal men (pooled)‡	1900. Oct. 11th.	0	0	0	0	—	—	10	}	5	5
N. S. W.	„ 13th.	0	0	—	36	—	51	73			
G. H.	„ 13th.	0	3	—	20	—	43	—			
R. F. M. F.	„ 13th.	0	1	—	17	—	69	—			
S. B. S.	„ 18th.	0	0	—	Over 200	—	Over 200	—	Over 200	Over 200	Over 200
Captain C. W.	„ 23rd.	0	0	—	—	Over 100	—	100	}	Over 200	Over 200
W. R.	„ „	0	0	0	—	4	—	24			
R. N. H.	„ „	0	0	0	—	49	—	8			
G. H.	„ 26th	0	0	1	7	—	20	—			
G. H.	„ 29th.	0	0	0	—	Circ. 100	—	—	Circ. 200	Circ. 200	Circ. 200
J. G. G. S.	„ 29th.	0	3	4	—	Circ. 100	—	100	150-200	150-200	150-200
W. A.	„ 30th.	0	0	Over 200	Over 200	—	Over 200	—	Over 200	Over 200	Over 200
A. P.	Nov. 1st.	0	12	105	Over 200	—	—	—	„	„	„
S. A. R.	„ 5th.	0	0	0	0	—	100	100	}	„	„
C. W. M.	„ 5th.	0	0	0	0	—	18	—			
E. P.	„ 6th.	0	0	0	—	40	—	55			
J. L. J.	„ 6th.	0	0	0	—	20	—	72	}	150-200	150-200
W. M. B. McC.	„ 8th.	0	0	0	—	2	—	39			
W. H. L.	„ 8th.	0	0	0	Over 200	—	Over 200	—			
W. D. P.	„ 8th.	0	0	0	100	—	100	—	}	200-300	200-300
A. W. G.	„ 12th.	0	0	0	0	—	0	13			
A. P.	„ 12th.	0	0	10	9	—	54	—			
G. G. S.	„ 13th.	0	0	8	86	—	Over 200	—	}	Over 200	Over 200
T. F.	„ 13th.	0	0	0	5	—	22	—			
S. B. S.	„ 15th.	0	0	0	13	—	55	—			
A. W. C. Y.	„ 15th.	0	0	5	44	—	57	—	}	Over 300	Over 300
H. R. B.	„ 15th.	0	1	0	30	—	70	—			
R. B. B. F.	„ 15th.	0	12	9	28	—	52	—			
H. G. P.	„ 15th.	0	0	3	31	—	46	—			

In every case young sub-cultures of a culture of a bacillus typhosus which had been recently isolated from a fatal case of typhoid fever were employed.
* The bloods employed were drawn off from healthy young men. In all but two instances these were Surgeons on Probation at Netley whose ages ranged between 21 and 28 years.
† The dilutions indicated by the figures represent the proportion borne by the whole contents of the cultivation tube to the contained serum.
‡ The serum in question was obtained by mixing together equal volumes of the serum of 10 Surgeons on Probation.

test-tube into another. Secondly, the capillary pipette must be carefully cleaned and warmed by washing it out each time in boiling sterile water. Thirdly, the gelatin culture must be kept well above its melting-point from start to finish. The cultivation tubes also should be kept on a warm shelf, both before they are filled in, and afterwards, during the interval between filling in and mixture. Fourthly, the mixture of the serum, or serum dilution, with the gelatin culture must be efficiently carried out. If this has not been done isolated colonies may, even in the case of tubes containing a highly bactericidal serum, make their appearance at one portion of the tube while the remainder of the tube is found quite sterile. Another point in connexion with the operation of mixing which has to be borne in mind relates to the shape of the cultivation tubes. In making these care must be taken to allow the glass to cool sufficiently to prevent it kinking at the elbow when it is bent round. Kinking of the tube at this point frequently entails the breaking of the column during the operation of mixing. Lastly, attention may be drawn to the fallacies which may be introduced into the observations by the use of an inappropriate diluting fluid. In the case of the bacillus typhosus normal salt solution is a very unsuitable diluting fluid. Where it is employed the number of colonies in the higher dilutions of the serum will uniformly be found less than in some of the lower dilutions. In other words, the suspension of the bactericidal powers of the serum obtained by dilution is, where normal salt solution is used as a diluting fluid, over-compensated for by the bactericidal powers of this last fluid. On the other hand, ordinary broth or, even better, saltless peptone broth, constitutes a very satisfactory diluting fluid, the number of colonies being in such a case

always more numerous in the higher than in the lower dilutions of serum.
Preliminary account of some of the results obtained.— Having described the most important points of the procedure adopted, it may be well, in conclusion, briefly to glance at some of the results which have been obtained by the application of the above method. It need hardly be said that these are here dealt with only in a preliminary way, for it will be manifest that the problems which are opened up would require the unremitting labour of months, if not of years, for their effective solution. It will be convenient to begin with the bactericidal effects exerted by normal human blood upon the bacillus typhosus. The results of close upon 50 examinations of normal bloods (all instituted with young subcultures of a typhoid bacillus recently isolated from a fatal case of typhoid fever) show somewhat remarkable results. The bactericidal effect was in all cases found complete in a two-fold dilution. In 22 out of the 28 blood examinations separately tabulated above a complete bactericidal effect was obtained also in a 10-fold dilution. In 50 per cent. of the bloods examined the complete effect was obtained also in a dilution of 20-fold. In 15 per cent. it was again complete in a 40-fold dilution. Only in one case was the bactericidal effect complete in a dilution higher than 40. A conspicuous feature in the results is the very considerable individual variation in bactericidal power. It must be carefully understood with regard to these results that they apply only to the particular culture which was used. In a subsequent paper the question will be considered as to whether a greater bactericidal effect is exerted on cultures which have been attenuated by cultivation in artificial media than on cultures the virulence of which has been exalted by a series of

passages through animals. Further, it is to be noted that the data which are given above apply in each case to the effect of sera which have been brought into application within two or three hours after the blood has been drawn off from the body. When sera are kept for a day, and even more so when they are kept for 48 hours, a very considerable decline in bactericidal power makes itself manifest. The data concerning this will also be given in a subsequent paper. Suffice it for the present that the results which have been obtained confirm in a general way those of Buchner. The same holds true with regard to the effect which is exerted on the bactericidal power by temperatures of from 56° to 60° C.

The modifications in the bactericidal power of human blood produced by additions to that blood *in vitro* of specific anti-typhoid sera may next be considered. Up to the present two different anti-typhoid sera have been studied from this point of view. Both of these were highly agglutinating sera placed on the market for therapeutic use. The effect produced by both these anti-typhoid sera were to a large extent similar. In every case even quite small additions of these sera to normal human blood brought about a complete, or all but complete, abolition of the normal bactericidal power of the blood, colonies of the typhoid bacillus making their appearance in large numbers even in mixtures containing one part in four of normal blood. Up to the present no increase of bactericidal power has been ascertained to result from the addition to a normal blood of the "therapeutic sera" in question. The importance of the issues thus opened up is immediately evident, for it is not clear that benefit would be derived from the therapeutic administration of a dose of serum which produces effects like these. It is conceivable that experiments of the kind now in question might throw an important light on the utility or otherwise of various other therapeutic sera, in particular of certain anti-streptococcus and anti-plague sera.

The question of the alterations in the bactericidal power of the blood which are associated on the one hand with attacks of typhoid fever and with convalescence from this disease, and on the other hand with inoculation with dead cultures of the bacillus typhosus, must be postponed for discussion in a future communication. With regard to the first of these questions it will here suffice to say that it seems possible, from the data which are already to hand, that indications which may be of use in prognosis may be deducible from observations on the variations in bactericidal power during the course of a typhoid fever. In connexion with the second question it will suffice to put on record that after an interval (during which it is possible that the bactericidal power is reduced) a definite increase in the bactericidal power of the blood comes under observation in guinea-pigs which have been inoculated with sterilised cultures of the bacillus typhosus. The data, which have up to the present accumulated in connexion with the effect of inoculation on the bactericidal powers of the blood in man, are not as yet sufficiently numerous to allow of a definite statement being formulated. There appear, however, to be indications that the course of events in the case of man will show a general correspondence with that which comes under observation in animals. In conclusion, it may be recorded that a large series of comparative quantitative estimations of the agglutinating and the bactericidal powers of the blood have shown (as was surmised by many observers, of whom the present writer was not one) that there is no *direct* association between these properties of the blood. I give a table which sets forth certain of the data obtained in connexion with the bactericidal power of normal human blood.

Netley.

FUNGUS DISEASE OF THE EAR.

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IN order to show the frequency with which fungus disease of the ear is met with in Bombay during the rainy season I (W. K. H.) have collected all the cases treated at the Jamsetjee Jeejeebhoy Hospital during the month of October, 1899. I have verified diagnosis by microscopical examination and in

several instances Dr. Row has kindly made a culture on agar-agar. Medical practitioners in Bombay often speak of the liability to disease of the external ear in this climate and they generally diagnose the conditions as furunculosis. I am convinced, however, that in most cases the disease is really aspergillosis and the small pustules seen in the canal are merely the result of a growth of a fungus. Von Roosa in his able work has tabulated several varieties and he states that in his opinion the fungus is the cause of the eczematous condition of the canal and not secondary to it. I quite agree with this opinion; moreover, it will be seen from the tabulated cases that in only one was there any pre-existing disease of the ear; this patient had a perforation and discharge some months before which had been treated and stopped by means of nitrate of silver. The ear remained well until the appearance of a fungus; there was therefore no discharge seen before the symptoms were experienced. Formerly there had been several recurrences of discharge with inflammatory symptoms from the affected ear and on none of these occasions was any fungus found, so that I may say that the fungus in all the cases was really primary. There appears to be a considerable difference in the symptoms due to fungus, varying from slight to considerable deafness and attended by pain which is occasionally severe. There is also a good deal of discomfort, generally described, in fact I may say almost universally, by native patients as "heaviness" and sometimes also "stiffness," but this symptom varies, I think, according as to whether the canal is blocked up by epithelium and fungus or whether the growth is merely a coating to the canal of slight thickness. In most cases the membrana tympani is obscured from view by the growth, or red patches may be seen on it here and there. In a very few instances I have observed small patches of growth on the tympanum only, without any signs of inflammation. Roughly speaking, cases may be divided clinically into dry and moist; in the latter class the symptoms of eczema are present to a greater or less extent and there is therefore a watery or slightly purulent discharge from the ear, and slight pain and deafness with a feeling of heaviness are usually complained of. In the majority of cases the aspergillus niger is found. There is a quantity of moist-looking epithelium on which black particles are plainly visible, having an appearance of grains of gunpowder. If the particles are plentiful there is more black than white visible, but if there are only a few it may not be easy to distinguish them readily. After syringing and the removal of the mass the walls of the canal are seen to be red and denuded of epithelium and often irregular, with small furuncles and swellings, and the membrana tympani may be bright red in colour or dull and sodden in appearance. Often the aspergillus flavus can be seen growing on the surface of small superficial pustules and if in any quantity the small balls of sporangia are plainly visible. The growth of penicillium glaucum gives a fluffy appearance to the surface.

In the "dry" variety the symptoms of pain, uneasiness, and deafness are also complained of, but there is no discharge, and the canal on examination may be found either to be stuffed full of epithelial debris with yellow, black, or brownish-looking particles sprinkled on the surface, or the walls of the canal are coated with a crust, usually of a darkish colour, on which the fungus is seen growing. The appearance is not unlike that of rhinitis when dry crusts coat the surface of the mucous membrane; the tympanum is therefore visible, but the surface is generally partially coated with a similar fungus to that on the canal. Sometimes white patches on the tympanum also are met with and they are difficult to remove. After syringing the walls of the canal appear red but dry and the membrana tympani is not so often inflamed as in the moist variety. Diagnosis is readily made after a few observations and confirmed by microscopical examination; sometimes the amount of spores is largely in excess of the mycelium.

The treatment which I have adopted in both varieties is the same and it consists in syringing very thoroughly and using iodoform and boric acid in equal parts. The canal may be swabbed out with camphorated salol, but the drugs used are not of themselves so important as frequent cleansing. It is not necessary to say more than this—that cleanliness and dryness are most efficacious.

Notes on some fungi recovered from the cerumen of the ear from the private notebook of Dr. Row.—Out of the 10 specimens which were kindly collected and sent to me for examination by Lieutenant-Colonel Hatch, I.M.S., only one gave a negative result. From the rest a variety of fungi