

orbicularis palpebrarum was very weak. The patient could not firmly shut his eyelids but could only just close them and offered no resistance to attempts at opening them. There was a certain degree of absence and incompleteness of nictitation. The orbicularis oris was very weak. He could not whistle or show his teeth. When he laughed his face assumed a sneering expression owing to the fact that he only raised his upper lip so that the nasolabial furrow did not extend round the corners of the mouth. VIII. Unaffected. IX. Taste unaffected. X. and XI. The patient had no difficulty in swallowing whilst an in-patient although he gave a previous history of that condition. The muscles supplied by the eleventh were, as stated above, weak. XII. He could only protrude the tongue incompletely. There were no fibrillary tremors.

The respiratory and circulatory systems were normal. A blood count showed 5,300,000 red corpuscles and 9600 white corpuscles per cubic millimetre. The pulse was generally about 80 and the respirations were 18 per minute.

The urinary system was normal; the urine was alkaline, turbid from urates, and contained neither albumin nor sugar. The digestive system was normal and the liver and spleen did not appear to be enlarged. His temperature remained normal or rather subnormal. He lost three and a half pounds in weight during the ten days he was in hospital and left with his condition unimproved. He was treated with the usual tonics, iron, arsenic, and strychnine.

It was a most markedly typical case of myasthenia gravis and showed such a rapid progress that in four weeks from a strong healthy man the patient became so extremely weak that he actually fell and later could not raise himself in bed. The onset of the trouble appeared to have followed an attack of influenza and it is possible that this disease may stand in a causal relation to this case. He appeared to have such a difficulty in feeding himself before he came into the hospital that he felt half starved and this may have added somewhat to his rapid increase in weakness. His mental condition was remarkably good and acute; there were a certain depression of spirits and a tendency perhaps to suspicion. Since leaving the hospital he has been under the care of Dr. T. Battersby Jobson of Ilford, who reports on April 11th, 1905, that "he was little better than he was on leaving the hospital."

A SIMPLE TECHNIQUE FOR THE ENUMERATION OF ORGANISMS IN ANY FLUID.

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CRITICAL consideration of the plating-out method of estimating the number of organisms in fluids such as water, milk, sewage, &c., reveals several disadvantages apart from the inaccuracies inseparable from the method. The two chief disadvantages are the multiplicity of manipulations and the quantity of apparatus required, the result being that in routine work the amount of time expended on the process is altogether out of proportion to the value of the information obtained. It will be readily admitted that in actual practice information as to the source of the sample will often indicate the probable number of contained organisms sufficiently well to save the experienced investigator from much unnecessary manipulation; but, on the other hand, cases not infrequently occur in which such information is either not available or if available will not suffice to guide the investigator in the important question as to the limit dilution to be employed. For example, I have received within a fortnight three samples of stored rain water which contained respectively 100, 1600, and 3,000,000 organisms per cubic centimetre. On the basis of these results a rigid application of the method of plating out, as recommended by the committee of experts appointed by the Royal Institute of Public Health,¹ to future samples of stored rain water would necessitate an expenditure of time and energy which could be more profitably employed in other

directions. I would submit that for routine work it will be sufficient if in place of the actual number of organisms per cubic centimetre we can state reasonable limits, such as 10-100, 100-1000, 1000-10,000, 10,000-100,000, &c., within which the actual number of organisms must lie and therefore any method of enumeration which fulfils this requirement and in addition offers considerable advantages over the plating-out method in respect to the number of manipulations and quantity of apparatus required is worthy of consideration on the part of the general body of laboratory workers on public health questions. If a method can be devised which will go further and in addition to the above advantages will indicate with an accuracy equal to, if not greater than that of, the plating-out method the total number of organisms per cubic centimetre in terms of 10, 100, 1000, 10,000, 100,000, &c., then a very strong case can be made out in its favour in opposition to the plating-out method as at present practised. I have therefore felt justified in putting before my fellow workers a method of enumerating organisms in any fluid which I have used in routine work for some months past and which seems to me to fulfil the following requirements: (1) Minimum number of manipulations; (2) minimum quantity of apparatus; and (3) accurate estimation of the total number of organisms in terms of 10, 100, 1000, 10,000, &c.

The method in question is not original, being merely a modification of the technique introduced by my former teacher Dr. A. E. Wright for the estimation of the number of living organisms in a given culture and used by him in connexion with his researches on the blood serum. The technique referred to is described in detail by Dr. Wright in his original papers, to which the reader is referred,² but in order to give an intelligible description of the modification I have adopted it will be necessary for me to repeat some of the subject-matter of those papers. The method is applicable to the quantitative estimation of bacillus coli, streptococcus, and the spores of bacillus enteritidis sporogenes in sewage effluents in addition to the quantitative estimation of organisms in water, milk, &c., but it will be sufficient for the purposes of description to state the details of the method as applied to the quantitative estimation of organisms in water.

DESCRIPTION OF THE METHOD.

The following is the apparatus required: three sterile diluting pipettes; three sterile watch glasses; eight agar slopes numbered from 1 to 8 consecutively; eight gelatin slopes numbered from 1 to 8 consecutively; and a vessel containing sterile water. The pipette which I use and which I have found to be extremely useful as a general service instrument in the laboratory is graduated differently from Dr. Wright's original model. The stem of the pipette is graduated in ten divisions of five cubic millimetres each, the remaining graduation marks indicating 450 cubic millimetres and 500 cubic millimetres respectively. The safety chamber is dispensed with, the shank of the pipette being merely bent to a right angle in the blow-pipe flame. With the exception of these differences the pipette is constructed and graduated according to the directions of Dr. Wright.

The steps of the operation are as follows. 495 cubic millimetres of sterile water are measured into each watch glass. This quantity is obtained by filling the pipette up to the 500 cubic millimetre mark and withholding five cubic millimetres when injecting into the watch glass. From the water to be examined five cubic millimetres are removed with the pipette—care being taken not to soil the pipette above the five cubic millimetre mark—and added to the contents of the first watch glass. This gives a dilution of 1 in 100. The contents of the watch glass are then thoroughly mixed with the pipette and from this dilution 100 cubic millimetres (obtained by filling twice up to the 50 cubic millimetre mark, interspersing with an air bubble) are planted out in the gelatin and agar tubes numbered 3. Similarly, 10 cubic millimetres are planted out in the tubes numbered 4. From the 1 in 100 dilution five cubic millimetres are then transferred to the second watch glass, giving a dilution of 1 in 10,000. With the same pipette 100 cubic millimetres and ten cubic millimetres of the original water are planted out in the tubes numbered 1 and 2 respectively. (This manoeuvre requires a little explanation. If, instead of proceeding as described, tubes 1 and 2 were first seeded and then five cubic millimetres of the original water were added

¹ Journal of State Med. Soc., August, 1904.

² Proceedings of the Royal Society, 1902; THE LANCET, June 1st, 1901, p. 1532.

to the contents of watch glass No. 1 it is obvious that this pipette cannot be used to mix the contents of the watch glass without being re-sterilised, as it has been soiled for a considerable portion of its length with a fluid containing spore-bearing organisms.) The first pipette is then laid aside and another one is taken in hand. The contents of the second watch glass are thoroughly mixed and 100 cubic millimetres and ten cubic millimetres are planted out in the tubes numbered 5 and 6. From the 1 in 10,000 dilution five cubic millimetres are transferred to the third watch glass giving a dilution of 1 in 1,000,000. The third pipette is now taken in hand and after thorough mixing 100 cubic millimetres and ten cubic millimetres of this dilution are planted out in the tubes numbered 7 and 8. The whole of the process described can, of course, be carried out with the aid of a single pipette, provided it be sterilised thoroughly in the flame between each dilution. This, however, occasions considerable loss of time and for rapid working three pipettes will be found essential. The remaining steps of the technique will vary according to the wishes of the investigator. Taking either the gelatin or agar series of tubes the quantity of water (in cubic centimetres) planted out will be as follows: tube 1, 0.1; tube 2, 0.01; tube 3, 0.001; tube 4, 0.0001; tube 5, 0.00001; tube 6, 0.000001; tube 7, 0.0000001; and tube 8, 0.00000001. If it is only desired to ascertain the limits within which the number of organisms per cubic centimetre must lie then the tubes are simply incubated in the vertical position and the results are interpreted as follows. Growth in tube 1, but not in tube 2, would place the number of organisms per cubic centimetre between 10 and 100. Growth in tube 2, but not in tube 3, would place the number of organisms per cubic centimetre between 100 and 1000, and so on, the limits adopted being those in general use for the classification of waters by the number of contained organisms. If it is desired to know the actual number of organisms in terms of 10, 100, 1000, &c., it will be necessary to distribute the water planted out over the surface of the medium and to incubate the tubes in the sloping position in order to prevent the accumulation of organisms at the foot of the slope. If preferred the quantity of water planted out may be spread over the surface of the medium with a glass rod but in actual practice I have found this to be unnecessary as in the case of the larger quantities even distribution can be obtained by manipulation of the tube, whilst in the case of the smaller quantities sufficient distribution can be obtained by means of the pipette at the time of planting out. The following three examples will indicate sufficiently well the results obtainable by this method:—

1. Sample A.

Gelatin at 22° C. for seven days:—		Agar at 37° C. for 48 hours:—	
Tube 1.	Growth (liquefied).	Tube 1.	Growth.
" 2.	"	" 2.	" (2 colonies).
" 3.	"	" 3.	Sterile.
" 4.	" (4 colonies).	" 4.	"
" 5.	Sterile.	" 5.	"
" 6.	"	" 6.	"
" 7.	"	" 7.	"
" 8.	"	" 8.	"
Number of organisms per cubic centimetre of gelatin at 22° C., 40,000.		" agar at 37° C., 200.	

2. Sample B.

Agar at 22° C. for seven days:—		Agar at 37° C. for 48 hours:—	
Tube 1.	Growth.	Tube 1.	Growth.
" 2.	"	" 2.	" (14 colonies).
" 3.	" (1 colony).	" 3.	" (1 colony).
" 4.	Sterile.	" 4.	Sterile.
" 5.	"	" 5.	"
" 6.	"	" 6.	"
" 7.	"	" 7.	"
" 8.	"	" 8.	"
Number of organisms per cubic centimetre of agar at 22° C., 1000.		" 37° C., 1200 (mean	
" between tubes 2 and 3).			

3. Sample C.

Agar at 22° C. for seven days:—	
Tube 1.	Growth.
" 2.	"
" 3.	"
" 4.	" (5 colonies).
Number of organisms per cubic centimetre of agar at 22° C., 50,000.	

In the quantitative estimation of bacillus coli in sewage

effluents one pipette will be sufficient as washing out with boiling water between the dilutions will meet the requirements of the case and the pipette can then be rapidly cooled by washing out with sterile water. With regard to bacillus coli, &c., the special media and methods of incubation used in such cases will, of course, take the place of the ordinary media and methods of incubation used in the mere enumeration of organisms in water.

In conclusion it will be well to consider the advantages and disadvantages of this technique as compared with the plating-out method. The following disadvantages may be urged against the technique:—

1. The diluting pipette being home-made is liable to be inaccurate. I have had a batch of these pipettes tested against a standard one centimetre pipette and it was found that while the relative error as between pipettes was barely appreciable the absolute error in one centimetre was in all cases less than 0.01 centimetre.

2. The method does not admit of microscopic examination of the colonies which develop. This difficulty can easily be overcome by subculture.

Against these disadvantages the following advantages may be set forth:—

1. It relieves the investigator of all anxiety on the score of the limit dilution to be employed when dealing with unknown waters, &c. By the employment of this technique all unpleasant surprises can be avoided. Whether the sample contain 12 organisms per cubic centimetre, or 12,000,000, the number will be estimated with equal accuracy and beyond the possibility of mishaps, such as "rapid liquefaction of the plates," "organisms innumerable in a thousandfold dilution."

2. The technique permits of very rapid working. With the aid of an ordinary coolie I have found that the whole process takes between six and seven minutes.

3. The small quantity of apparatus required.

4. There is practically no waste in this method as all tubes which remain sterile are ready for use again.

5. The pipettes are easily made, inexpensive, and can be sterilised in the flame without fear of breakage.

6. The whole process can be carried out at the water side, a spirit lamp being added to the armamentarium for the purpose of sterilising the pipette.

7. The labour of counting colonies in a Petri dish is entirely dispensed with and consequently all artificial aids to counting, such as counting discs, cease to be necessary.

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SEVEN CASES OF BERI-BERI.

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SINCE my return to the East I have had the opportunity of observing and of carrying out post-mortem examinations upon seven fulminating cases of beri-beri which have occurred in the District Hospital, Taiping. As some of the observations which have been made are of interest and as the disease has of late attracted a certain amount of attention at home it seems to me advisable to publish these cases to the profession in view of the fact that fulminating cases occurring under the conditions of a local epidemic are rare. The cases all occurred in the same ward. All the cases except one occurred in patients who had been for a considerable time under treatment for beri-beri and the one exception was a case which was brought to hospital in the fulminating state but late in the local epidemic, which lasted about one month. All of the patients in the other wards of the hospital, 400 in number, were receiving similar diets to those which were prescribed for the subjects of the cases which fulminated and no patient in the ward where the epidemic occurred who was not admitted in the first instance for beri-beri acquired the disease. (There may be one exception to this statement in the case of a Chinese, aged 38 years, who was admitted on Oct. 17th, 1904, for dysentery. He recovered from his dysentery but developed beri-beri which was diagnosed as such on Dec. 3rd.) The majority of the patients in the ward who were not suffering from beri-beri were phthisical. One end of this ward was used as a reception ward for all cases that were admitted to