A SIMPLE METHOD FOR THE CULTIVATION OF ANAEROBIC ORGANISMS.

BY

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THE large number of wounds in the present campaign contaminated with anaerobic organisms has brought into prominence the different methods for the isolation and culture of these organisms.

A simple method for obtaining anaerobic conditions is the following, and I have found that by its employment large numbers of these organisms can be investigated with regard to their cultural characteristics, their carbohydrate reactions, etc., with comparative ease, and the laborious technique involved in carrying out many of the methods in common use avoided.

The sole apparatus required are test tubes of two different sizes for cultures on solid media and three different sizes for liquid media.

The diameters of the test tubes which I have found most convenient have been 1.5 and 2.5 cm. for the first method and 1, 1.5, and 2.5 cm. for the second method.

1. For Solid Media.

The test tubes, 1.5 cm. in diameter, are prepared, plugged, and sterilized with the sloped medium, such as glucose agar, in the usual manner. After inoculation of these media, a corresponding number of the 2.5 cm. test tubes are half filled with a solution of pyrogallic acid;

Method 1.

Method 2.

when liq. potassae has been added the plugs of the culture tubes are removed, and they are inserted, with their upper ends downwards, into the alkaline pyrogallate solution contained in the 2.5 cm. tube. The contained air in the tube containing the culture medium prevents the pyrogallate solution from passing up into the tube for more than a few millimetres, and the inverted tube, by virtue of its contained air, floats upon the pyrogallate solution. A few cubic centimetres of liquid paraffin are then poured upon the surface of the pyrogallate solution, thus

preventing it from becoming inert through absorbing the oxygen of the external air.

This method, which I have now used some hundreds of times, has invariably given excellent results. After incubation is completed the culture tube is lifted out of the pyrogallate solution, wiped with sterile wool, and plugged, after which the culture can be investigated in the usual manner.

2. For Liquid Media.

The smaller 1 cm. diameter test tube is prepared with its liquid medium in the usual way. After inoculation it is inserted into the large 2.5 cm. test tube containing alkaline pyrogallate solution, and after removal of its wool plug the 1.5 cm. test tube is inverted over it until its margin dips into the alkaline pyrogallate solution. A few cubic centimetres of sterile paraffin are then poured upon the surface of the pyrogallate solution, between the wall of the medium-sized tube and that of the larger tube.

For the investigation of the carbohydrate reactions, gas formation, etc., of anaerobic organisms, I have found this an extremely simple and reliable method.

The diagrams give a general idea of the arrangement of the test tubes in the two methods.

THE TREATMENT OF WOUND INFECTIONS BY ANTISEPTICS.

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I SHOULD like to be allowed to say something in support of the view that antiseptics are of value in the treatment of infected wounds. Sir Almroth Wright tells us that they are not necessary because the resistance of the tissues is sufficient to deal with the infection, if the wound is kept by the surgeon in a favourable condition, by adequate drainage and other means; and that, although antiseptics have the power to kill micro-organisms in the laboratory, they cannot act efficiently in contact with the tissues of the body. He tells us that, in judging of the value of antiseptics, we must be guided by laboratory methods, and not by clinical experience, because its results are not "brilliantly successful" or the reverse. He considers that because clinical results do not almost invariably prove the utility of antiseptics we are not to be guided by them, but by laboratory experiments. But is this reasonable? If we have evidence from the use of a drug in a sufficiently large number of cases to enable us to really judge of its action, are we to refuse to believe in its efficacy because it is not "brilliantly successful"? Are we not to judge of the value of vaccine treatment by its clinical results? They can hardly be said to have been "brilliantly successful" for all the various morbid conditions for which they have been used. Why should we not refuse to judge of the value of vaccine treatment because it is not always "brilliantly successful," if we are to refuse to judge of the value of antiseptics on such grounds? There is, indeed, no reason why we should replace antiseptics by vaccines in the treatment of infected wounds, as Sir Almroth Wright says we should do.

And are his laboratory experiments really convincing that antiseptics can do no good in infected wounds? He says they cannot do good at the time of infection because they cannot penetrate "the solid barriers of albuminous substance, provided in the early stages of the wound by exposed muscles and connective tissue." But may they not reach the organisms lying outside these barriers, if applied early enough? Cannot millions of microbes be destroyed by the antiseptic before they have begun to penetrate into the tissues? Even if contact with the albuminous material in the wound reduces the power of the antiseptic by the chemical change which it produces, if sufficient antiseptic is used, and if it is frequently changed and used for a prolonged period, it is not likely to be efficient. If the "barrier of albuminous substance" is formed by the exudation on the surface of the wound, bathing the organisms lying there, and then coagulated into an impenetrable layer by the antiseptic, then it would certainly seem impossible for the antiseptic to destroy the organisms. But it seems to me that we ought to require very strong proof that this was what happened before we abandoned the antiseptic treatment of wounds.

Judging by the results of Dr. Alexis Carrel's work at the hospital at Compiègne,* it seems to me that the clinical results of the treatment of infected wounds with an antiseptic have been most encouraging, and should certainly lead to a wide adoption of his thorough methods. With the result of Carrel's work before us, I venture to think Sir Almroth Wright is not in the least justified in stating that the antiseptic treatment of wounds has completely broken down. In the instances it has done so this has happened probably because the surgeon had no chance of dealing with the wound for many hours after it was inflicted, or used an antiseptic in an imperfect way—perhaps just douched the wound with it, and then washed it away at once with saline for fear of its supposed injurious action on the tissues. In cases in which the wound has been well cleaned, and the antiseptic of sufficient strength, and freely and for a sufficient time applied, within a few hours of the injury, experience both in civil and military

* See abstract of his paper in BRITISH MEDICAL JOURNAL, October 23rd, 1915, p. 609. The investigations which resulted in the use of sodium hypochlorite as an antiseptic were carried out by Professor Lorrain Smith and others at Edinburgh (BRITISH MEDICAL JOURNAL, July 24th, 1915, p. 129) and by Dr. Dakin in France (BRITISH MEDICAL JOURNAL, August 28th, 1915, p. 318), and were subsidized by the Medical Research Committee of this country.

practice has shown that it has been efficient in preventing sepsis.

I very much doubt if we can rid an infected wound of organisms by douching it out with saline. If we take a mixture of chalk and water and throw it into a wound, and allow it to remain there a short time, we cannot expect to get even such gross particles as the chalk away from the wound by such means, much less millions of microbes. Just as we could only get rid of the deposited chalk by applying to the wound an acid solution, so as to act chemically on the chalk, so we can only get rid of the microbes by killing them with an antiseptic. Carrel truly says we cannot clean a wound of microbes even by a swab, for as well might we expect to clean a greasy bottle by such means. But, if we cannot mechanically remove the organisms, surely the right thing to do is to apply an antiseptic to kill them.

I admit that it does not seem so reasonable to apply antiseptics to an already suppurating wound as to one just infected, because of the difficulty, if not impossibility, of the antiseptic penetrating the living tissues, into which the organisms have already penetrated; and, as Sir Almroth Wright points out, if you by irrigation wash out the pus, why trouble to try and kill the microbes in that pus? But here again the question seems to me to arise, Can we irrigate out all the microbes in the wound (as distinct from those which have penetrated into its walls), and may we not be wise in employing an antiseptic to kill those we cannot? Is it not possible that some antiseptics may have some power of penetrating into the living wall of the wound? Here, again, Carrel's experience in treating suppurating wounds seems to me quite good clinical evidence of the advantage of using hypochlorous acid or hypochlorite of soda in the treatment of suppurating wounds.

Now, Sir Almroth Wright tells us that the question as to the value of antiseptics cannot be answered by clinical observation, yet, all the same, he does seem to attach importance to such evidence when it is the result of the observation of "experienced and very competent observers." But though he now admits that we may judge of the value of therapeutic measures used in connexion with the healing of wounds by clinical evidence, he tries to show that even these "experienced and competent observers" have been deceived, for he says their good results may have been due to using the antiseptic with a "hypertonic menstruum," the washing out of the wound by peroxide of hydrogen, or the use of antiseptics in the form of fomentation. But the use of such things without the antiseptic has probably not been found by these "experienced and very competent observers" to have the same beneficial action on the wound as the particular antiseptic they consider has been of value.

Let me give one striking example of the value of clinical evidence. It does not refer to the beneficial action of antiseptics but to their harmlessness. It has generally been maintained that antiseptics damage the living tissues, possibly because of experimental evidence, but I do not know of it; but perhaps simply because certain antiseptics have caused irritation of the skin. Mr. Bond of Leicester has recently shown¹ that even quite strong antiseptics can be applied to wounds without interfering with their healing. But we have even some laboratory evidence on this matter, which may appeal to those who think clinical evidence unreliable. The late Mr. Lockwood, writing some years ago of the unreasonable dread of the entrance of antiseptics into wounds, said he had made sections of the tissues from wounds to which antiseptics had been applied, and could find no evidence of damage to them.

The sterilization of the infected wounds of war is a matter of such vast importance to us at the present time that I would plead that further attempts on the lines practised and advocated by Carrel should be made, and that we should not rest content to depend on the resistance of the tissues—a resistance which very often completely fails, the failure leading to the most serious condition of sepsis in the wound, and often costing the wounded soldier his life. Nor, I would urge, should we depend on irrigation with saline to rid a recently infected wound of organisms, but should freely apply, for a considerable period, either hypochlorous acid in 0.5 per cent. or hypochlorite of soda solution; and these antiseptics in

a strength potent to kill micro-organisms will do no harm to the tissues.

I think Sir Almroth Wright's suggestion that physicians should now take charge of wounds will be read with some astonishment. Does he think that a surgeon's only business is with the knife? Can it be possible that he thinks they will be too free in making counter openings for drainage? Or does he think that the surgeon will have a greater tendency to do harm by inflicting on the wound a douching with some antiseptic, because he will be less readily converted to his views on the sufficiency of tissue resistance and the value of only using saline? Is it necessary to remind Sir Almroth Wright that even physicians use drugs which they believe to be internal antiseptics, and that the faith some of them have in the good results of vaccine treatment is very feeble, and that he must not trust them too far? But, after all, it is clear from Sir Almroth Wright's last lecture that he does really use antiseptics himself, for if a 5 per cent. salt solution can prevent the growth of pyogenic organisms what is it but a feeble antiseptic? I fear the knowledge that saline solution is an antiseptic will come as a rude shock to "aseptic" surgeons. They will discover that they have really been using a deadly antiseptic in the wounds all the time, even though, in the strength they have used it, too weak to have any antiseptic power. But it may, by accident, have been used as strong as 2 per cent., and then it would undoubtedly be antiseptic.

REFERENCE.

¹ BRITISH MEDICAL JOURNAL, vol. i, 1915, p. 405.

Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

TREATMENT OF "FLAGELLATE-DIARRHOEA" AND OF KALA-AZAR.

SEVERAL authors have recently called attention in the BRITISH MEDICAL JOURNAL to the presence of flagellates in cases of diarrhoea, and discussed their etiological rôle. When present in a small number such flagellates are probably harmless. In the tropics they are found in the stools of more than 25 per cent. of apparently healthy persons after a saline purge. When present, however, in enormous numbers, as in the two cases which I published in the BRITISH MEDICAL JOURNAL of November 11th, 1905, the probabilities are that they play a part in the causation of the intestinal symptoms.

I can confirm Low, Wenyon, Ledingham, and Penfold's experience that ipecacuanha and emetine have no effect on such parasites. I recommend instead the use of methylene blue. This drug has a powerful effect on flagellates. I may quote a paragraph from my paper on the resistance of the intestinal flagellates to various chemical substances¹:

The action of different chemicals on the parasites was tried repeatedly. Without referring in particular to all the experiments made, I may say that methylene blue solutions are without doubt the most active—much more so for instance than quinine solutions. Mixing one loopful of the stools with one loopful of 1 in 1,000 solution of methylene blue and observing the preparation microscopically, the flagellates (*Trichomonas* and *Cercomonas*) are seen to take up the colour, and stop almost instantly their movements, losing their ordinary shape and becoming globular without exhibiting any flagella or undulating membrane.

I give methylene blue by the mouth, in cachets containing 2 or 3 grains of the drug, three times a day, and also by intestinal irrigation (1 in 5,000 or 1 in 3,000). The patient must be informed that his stools and urine will become blue. The treatment has induced in most of my cases a rapid decrease or complete disappearance of the flagellates within a very few days. In a few obstinate cases the administration of the drug may have to be persisted in for a long period of time. In such a contingency the methylene blue should be discontinued from time to time to prevent the formation of methylene blue concretions in the intestine.

In connexion with Major Mackie's interesting paper on the treatment of kala-azar with tartar emetic, published

¹ BRITISH MEDICAL JOURNAL, November 11th, 1905.