period, education, removal from obviously unsuitable environment, and all that constitutes "after-care." It may not be essential to send all patients away for treatment at this stage, but wherever initial symptoms are accompanied or followed by impairment of general health a period of residence in a sanatorium is probably the most efficient means of restoring condition and imparting valuable knowledge. These incipient cases retain their working capacity for very long periods after discharge from sanatoria, in whose records they constitute a considerable proportion of the cases classed together as "tubercle bacilli not found," and whose after-histories are so good. Other methods of treatment can show a high percentage of successful records in this type.

Where similar results follow such widely varying procedures as residence in sanatoria, regular attendance to receive medicine, or the inoculation of various kinds and doses of tuberculin, it seems probable that these results must be ascribed to the factors common to all such forms of treatment—namely, the warning, supervision, and greater or less degree of education and after-care which the patient receives. Recognised and supervised the incipient case does well, unrecognised and assured that nothing is amiss a considerable number certainly do very badly.

In touching upon these causes of "sanatorium wastage" I have emphasised, necessarily, the gloomy aspect of the problem. Nevertheless, I am convinced that given certain essentials—viz., early diagnosis, reasonable treatment, and a chance to spend his future working life in a proper environment—certain attributes of this class of patient make him the most profitable subject for sanatorium treatment.

THE CONDITIONS OF GROWTH OF *BACILLUS WELCHII* IN THE PRESENCE OF OXYGEN.

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Bacillus welchii is classed among the strict anaerobes, and in working with this organism much care is usually taken to make the conditions as anaerobic as possible for its growth. Certain statements in the literature have for a long time made us suspicious of this classification, and in the following notes are presented some observations in this direction.

The matter of the presence of air was taken up many years ago by Tarozzi, and Ori and Wroszek, who claimed that the addition of small pieces of various substances, animal and vegetable, was sufficient to induce the organism to grow in the presence of air. A certain amount of discussion followed as to whether it was necessary that the material should be living or dead when introduced into the culture medium. Rosenthal claimed that these organisms could be grown in the presence of air provided that they had been adapted to this mode of growth by preliminary treatment.

Recently Wright has repeated the experiments of the previous investigators and has confirmed them. On these he has based certain theories regarding treatment. The idea is advanced of "niduses of lowered bacteriotropic pressure."

What appears to have escaped the observation of these investigators is the fact that given a simple culture medium of suitable character, the *Bacillus perfringens* grows perfectly well under those conditions which bacteriologists speak of as aerobic, provided the medium is freshly sterilised. This can be done without the addition of any adventitious substance such as potato, liver, apple, or platinum black. The original difficulty in getting the organism to grow under aerobic conditions was probably due to the relative unsuitability of the culture media which were employed.

The strains of *B. perfringens* which have been used in the present work are those which have been used by us in other series of investigations. They have retained all the cultural characteristics of the organism, and, what is possibly more important, their chemical behaviour has remained absolutely constant. We have every reason to believe that they represent quite normal strains of this organism. As will be seen, we have controlled these results with other strains, among

them one from the Institut Pasteur, Paris, with identical results.

The culture medium used was a peptone solution made by the digestion of casein, to which glucose was added, and adjusted to a definite hydrogen-ion concentration (acidity) with the hydrogen electrode. Experiments which we have recently made, and which are reported in the *Journal of Pathology and Bacteriology*, show the important influence of this single factor—the reaction of the medium—on the growth of these bacteria.

The content of "peptone" was about 2 per cent. The amount of culture medium used was 10 c.c., contained in ordinary bacteriological test-tubes, $5/8 \times 6$ inches. The inoculation was made with three drops of a 15-hour old culture on milk, which was seeded from a 6-hour old culture on cooked meat. The inoculation was made with a fine capillary pipette, so that the amount of fluid introduced was, roughly, 0.1 c.c.

Preliminary Experiment.

A preliminary experiment gave the following results:—

Tubes of glucose peptone containing 10 c.c. of medium and provided with Durham tubes, were inoculated and incubated at 37° C. for 24 hours. All these tubes showed a vigorous fermentation. The Durham tubes were filled with gas.

As the Durham tubes introduced a complication, by providing that a portion of the medium should be more or less sheltered from direct contact with the air, a second set of tubes was used.

These were the ordinary bacteriological test-tubes described above, containing 2 per cent. glucose peptone. The area of fluid exposed to the air was about 2 square centimetres. A similar set contained Durham tubes. A third set was so arranged that they could be incubated in a sloping position and so expose a larger surface to the air. A fourth set consisted of quite large tubes, 1 in. in diameter, containing the same amount of culture fluid. These were also grown in a sloping position.

In the small tubes containing Durham tubes the bacteria might be expected to find conditions which were more or less anaerobic. In the small tubes incubated in the upright position there would be less chance of the bacteria avoiding contact with the air. The chances for contact with air would be increased with the small and large tubes in the sloping position.

Four types of media were used in these experiments: (1) 2 per cent. glucose peptone; (2) 2 per cent. lactose peptone; (3) 2 per cent. lactulose peptone; and (4) 2 per cent. maltose peptone.

Three different specimens of *Bacillus perfringens* were used. The first set was inoculated with *B. perfringens*, K 95, 16 hours old, on milk; the second set with *B. perfringens*, K 94, 16 hours old, on milk; and the third set with *B. perfringens*, K 90, 49 hours old, on cooked meat.

The bacillus fermented the different sugars equally well, so far as could be judged by the gas production.

TABLE I.

	Total number of tubes	Number fermented.	Number unfermented.	Per cent. fermented.
With Durham tubes ...	11	11	0	100
Without „ „ ...	12	8	4	66
Small slopes ...	7	3	4	43
Large „ „ ...	3	0	3	0

From these experiments (see Table I.) it is clear that *B. perfringens* is not absolutely anaerobic. It prefers anaerobic conditions, but can develop when air is present. The greater the amount of air that has access to it the less favourable are the conditions of growth.

Further Experiments.

In order to test further the ability of *B. perfringens* to grow under aerobic conditions another set of experiments was arranged.

Tubes were prepared containing 2 per cent. glucose peptone at a favourable reaction for growth. The reaction was $P_H = 6.6$. Each tube contained 10 c.c. of medium.

There were three sets of tubes, each set being inoculated with a different culture of the bacillus. Each set consisted of:—

1. A small tube containing a Durham tube.
2. A small tube without a Durham tube.
3. A large (1 inch) tube containing a Durham tube.
4. A large (1 inch) tube without a Durham tube.
5. A large (1 inch) tube, incubated in a sloping position.

6. A large (1 inch) tube, incubated in a sloping position and shaken frequently so as to change the air in contact with the surface.

Series A was inoculated with K 97, Series B with K 98, and Series C with K 98 (in each case 24 hours old on cooked meat). The tubes were examined three days after inoculation.

TABLE II.

No. of tube.	Series A.	Series B.	Series C.	No. of tubes fermented.
1	+	+	+	3:3
2	+	+	+	3:3
3	+	+	+	3:3
4	-	-	-	0:3
5	-	-	-	0:3
6	-	-	-	0:3

+ Indicates growth; - indicates no growth.

These experiments show that *B. welchii* prefers anaerobic conditions to those where air is present. When, however, the conditions are nominally aerobic but the organism can find a certain amount of shelter from oxygen, either by reason of the depth of the layer of liquid or other cause, development will take place. Once development has begun the dissolved oxygen of the liquid is swept away in the evolved hydrogen and carbon dioxide produced by the fermentation.

In order to ascertain whether *B. perfringens* could develop in a steady stream of air, the following experiment was devised.

A litre flask was taken and provided with a three-holed rubber stopper. Through one hole passed a tube on the outer end of which was a cotton-wool air filter. The end inside the flask was drawn out into a fine capillary. Through the second hole passed the inoculation tube, and through the third hole passed the air-delivery tube. 500 c.c. of the glucose peptone were placed in the flask, which was sterilised in the ordinary way. After sterilisation all joints were made tight with paraffin wax. The delivery tube was closed off with a sterile-air filter, and the contents of the flask inoculated with a young culture of *B. perfringens*. After inoculation the flask was incubated in a water bath at 37° C. The air filter attached to the delivery tube was then connected to an aspirator by which sterile air could be passed through the medium by way of the capillary. The air-supply was so regulated that about 300 c.c. of air passed through the medium per hour.

After 24 hours the medium was examined to ascertain whether fermentation had taken place. The liquid was only slightly clouded. The reaction of the liquid, as determined by the hydrogen electrode, was very slightly more acid. Analysis of the outgoing air showed it to contain more carbon dioxide than ordinary air. The percentage of oxygen was somewhat low, indicating the presence of a gas such as hydrogen. The liquid had fermented, although the fermentation was of a very feeble nature.

Data.—500 c.c. of glucose peptone, sterilised at 110° C. for 45 minutes. Incubated at 37° C.

Air drawn through at the rate of 300 c.c. per hour.

Absolute reaction before inoculation, $P_H = 6.7$.

Absolute reaction after fermentation, $P_H = 6.4$.

A control experiment made with the same apparatus, using 750 c.c. of nutrient medium, gave practically the same result. The amount of carbon dioxide formed during the 24 hours was about 50 c.c.

A glucose peptone experiment done under strictly anaerobic conditions will yield about 750 c.c. of gas per litre, and the fermented medium will have a reaction of about $P_H = 4.5$.

Experiments with Other Strains.

In order to control the above results obtained with a single strain of *B. perfringens* which we had subjected to frequent transfers, we investigated five different strains of *B. perfringens* to ascertain if their behaviour towards air was the same. These strains were taken from cultures given us by Captain H. Henry, R.A.M.C., and included a strain marked "Pasteur," furnished by the Institut Pasteur of Paris.

The method of testing them was to prepare sets of tubes containing 2 per cent. glucose peptone water. The tubes were so selected that the bacteria, after inoculation, were submitted to conditions becoming less and less anaerobic. The different types of tubes used were:—

1. Small tube; with Durham tube; surface exposed to air, 2 sq. cm.

2. Small tube; no Durham tube; surface exposed to air, 2 sq. cm.

3. Large tube; with Durham tube; surface exposed to air, 7 sq. cm.

4. Large tube; no Durham tube; surface exposed to air, 7 sq. cm.

5. Large tube; incubated in a sloping position.

Five sets of such tubes were prepared, and each set was inoculated with three drops of one of the five cultures, three hours after these arrived in the laboratory. They were then incubated for 24 hours, after which time examination was made for growth.

Series A was inoculated with strain labelled "King, 10/5"; Series B with strain labelled "Pasteur, 10/5"; Series C with strain labelled "Benson, 10/5"; Series D with strain labelled "Bryant, 10/5"; Series E with strain labelled "Wills, 10/5." The results are given in Table III.

TABLE III.

Tube number.	Series.					No. of tubes fermented.
	A	B	C	D	E	
1. Small tube, with Durham.	+	+	+	+	+	5:5
2. Small tube, without Durham.	+	+	+	+	+	5:5
3. Large tube, with Durham.	+	+	+	+	+	5:5
4. Large tube, without Durham.	+	-	+	+	+	4:5
5. Large tube, grown sloped.	-	-	-	-	?	1:5

+ Indicates growth; - indicates no growth.

It is seen from the above that the condition favouring growth is protection from air and a consequent low concentration of oxygen in the nutrient media. There is no essential difference in the behaviour of these organisms from the original ones with which we have worked. The strain which corresponds in every particular to our own is that of the Pasteur Institute.

It would appear, therefore, that with a medium favourable for growth the development of the organism is determined by a suitably low concentration of oxygen.

Remarks.

The solubility of oxygen in water is such that 100 c.c. of water saturated with oxygen gas dissolve about 2.5 c.c. at 37° C. (Fox). At 100° C. the solubility is decreased, so that only 1.7 c.c. are in solution. With air, which has a partial pressure of 20 per cent. of oxygen, only 0.5 c.c. is in solution, while at 100° C. that amount is probably about 0.37 c.c. This is not taking into account any decrease of solubility due to salts or soluble nitrogen compounds in the medium.

The situation, therefore, is this: that during sterilisation less than 0.37 c.c. of oxygen per 100 c.c. has been left in the medium. As the surface of liquid exposed to the air is small, and as convection currents of air and liquid are slight, the amount of oxygen taken up by the liquid from the air is very small. Hence the concentration of oxygen in the lower layers of the tubes is such that it does not interfere with the growth of *B. welchii*. As growth proceeds and gas is evolved, oxygen is driven out of the upper layers, so that finally growth takes place in all parts of the tube.

That a higher concentration of oxygen than that remaining after sterilisation inhibits growth is shown by a simple experiment.

If we saturate the medium with oxygen before inoculation no growth should take place. In order to test this, tubes were prepared of the same diameter containing the same medium. Under aseptic conditions oxygen was bubbled through the sterile medium. The oxygenated and the control tubes were inoculated at the same time. The oxygen tubes produced no growth, whilst in the untreated tubes there was a vigorous growth.

Wright makes the statement that potato bouillon gives a much less vigorous growth when stale than when freshly prepared. The explanation for this is, in our opinion, that the "stale" tubes have had time to absorb sufficient oxygen from the air to inhibit growth.

It would appear, therefore, that with a medium favourable for growth the development of the organism is determined by a suitably low concentration of oxygen.

It is possible to test the conception of a region of diminished bacteriotropic pressure by doing away with this region. For this purpose we take the same medium, which

under certain conditions of oxygen tension does not grow the bacillus of Welch, and under lower oxygen tension produces a vigorous growth. This medium is inoculated, and during incubation a constant stream of nitrogen, containing 1 per cent. of oxygen, is bubbled through. The bubbling of a similar current of air containing 20 per cent. of oxygen inhibited growth. In the nitrogen tube a very vigorous growth takes place. Although we have prevented any segregation of bacteria, this has in no way inhibited growth, although a slight increase in oxygen tension does so.

Wright states that one

"must abandon the traditional view that the growth of anaerobes pivots on the presence or absence of oxygen and that the therapeutic principle of combating anaerobes by combating anaerobic conditions cannot be regarded in any sense as adequate."

This is a paradoxical statement which at once arrests and holds the attention, but the practical fact to be borne in mind is that the anaerobe is at once inhibited in its growth by the increase of oxygen tension above a very low level, and that its so-called aerobic growth depends on the establishment of conditions which maintain that low tension.

In connexion with the conditions which are usually associated with anaerobic technique it would appear that bacteriologists have been deluded for a long time into thinking that by covering a culture medium with a layer of paraffin or lanoline the aqueous layer is protected from the gases of the atmosphere. Vernon, as quoted by Bayliss in his "Principles of General Physiology," found that oxygen was 4.5 times more soluble in fats and oils than in water. Bayliss directs attention to the futility of attempting to protect aqueous solutions from the gases of the atmosphere by layers of oil, as is done in Hesse's method. The anaerobic conditions desired can be just as well obtained by employing *high narrow* tubes of freshly sterilised media.

We have made a certain number of experiments which confirm Bayliss's statement in every particular. Sloped tubes covered with paraffin do not grow the bacillus any better than do similar tubes unprotected by the fatty layer. If the paraffin layer is of any real value, it probably plays its part in diminishing convection currents in the aqueous layer.

Summary.

With a properly prepared culture medium, *B. welchii* will grow freely in open narrow tubes. The addition of any solid matter is not necessary to produce this apparently aerobic growth.

No segregation of organisms is necessary, for the bacillus grows vigorously when nitrogen containing 1 per cent. of oxygen is bubbled continuously through the tubes, but the organism is very sensitive to, and its growth is inhibited by higher proportions of, oxygen. For this reason the so-called aerobic growth is best obtained in narrow tubes of freshly sterilised fluid from which the air has been partly expelled by the heat of sterilisation.

TREATMENT OF WOUNDS BY A SOLUTION OF SOAP IN THE CASUALTY CLEARING STATION.

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A SHORT time ago Colonel Cuthbert Wallace, A.M.S., brought to our notice that some French surgeons were using soap solutions in the treatment of wounds, and suggested that we should try this method.

During the last few months we have used this dressing on 368 cases. During the same period we have treated similar cases with other antiseptics, including eusol, hydrogen peroxide, and B.I.P.P., with the result that we are using more soap, and less of the other antiseptics.

A sample of common yellow soap was analysed and found to contain per cent.: Water, 24.6; fatty acids, 63.0; combined alkali, 4.6; free alkali, nil; impurities, 7.8. This was considered suitable, and it was found possible to make a 2½ per cent. solution of it.

The first cases tried were small open muscle wounds in which there was no gas gangrene. After being opened up, and metal and cloth removed, the wounds were dressed with sterile gauze soaked in this solution. The dressings were left undisturbed for two, three, or four days, provided there was no rise in temperature. At the first dressing the most striking features were less pain, the unusually clean appearance of the wound, absence of pus, and the redness of the whole of the tissues—the muscle in particular.

Encouraged by the success in regard to small wounds, we tried the same treatment in larger and deeper wounds, using Carrel's tubes in the ordinary way. Here we found a difficulty with the common soap solution; it was found to be too gelatinous to flow at all well down the tubes. A 2½ per cent. solution of the B.P. soft green soap was then tried, and found to be quite satisfactory.

The results continued to be most encouraging, and compound fractures of the bones of the hands and feet, also the long bones, including the femur, were so treated successfully. Cases of penetrating wounds of the knee-joint were treated by this method, the joints being irrigated and the capsule and external wounds closed. The cases did very well.

In cases of gas gangrene the results were usually good, but in one case (buttock) a gelatinous film or glaze was formed over the affected muscles, and the treatment had to be discontinued in favour of hydrogen peroxide.

In the case where a man had similar wounds of both calves the soap solution with Carrel's tubes was tried on one side, and another antiseptic, also with Carrel's tubes, on the other. Both wounds did well, but the side treated with soap was not dressed as frequently as the other side, and when the patient went to the base his soap-dressed wound looked more like a granulating wound, and more like one that it would be safe to close than the other.

The points claimed for the soap solution dressings are that they clean up a wound quickly, the dressings are much less painful than ordinary dressings, there is a saving of labour as the dressings need only be changed every three or four days, the solution is easily procured, easily made, and cheap.

Soap Dressings.

Superficial wounds	73	Joints	30
Uncomplicated severe wounds involving muscle	160	Amputations	14
Compound fractures—		Vessels	17
Fractures	83	Nerves	6
Partial fractures	22		

Notes of Cases.

CASE 1.—Gunshot wound of left arm, compound comminuted fracture of humerus. Date of wound, July 1st, 1917. Re-dressed eusol, dressing requiring surgical treatment. Injection of antitetanic serum, first, 750 units, on the 1st. Morphine gr. ½ 12 o'clock same day. Reig. C.E. At 3 P.M., pre-operation hypodermic injection of atropine gr. 1/100, operation by Captain Dixon; anaesthetic, closed ether, Captain Lloyd. A large portion of the biceps and triceps muscles was excised. A large piece of isolated bone was removed as well as dirt and khaki cloth. Six Carrel tubes were inserted; soap dressing; Thomas splint. The temperature rose to 102.8° F. on the evening of the second day, was not above 100° after the third day, and fell to normal by the seventh day.

CASE 2.—Date of wound, June 28th, 1917. Compound comminuted fracture of left humerus. 29th: Antitetanic serum, 500 units; morphine gr. 1/4 and atropine gr. 1/100 at 2.30 A.M. Operation by Captain Dixon at 3.30 A.M. Anaesthetic, closed ether, Captain Lloyd. Loose bone removed; hæmorrhage arrested; Thomas splint; soap dressing: three Carrel tubes. The temperature slightly subnormal after operation, gradually rose to 102.8° F. by the 30th, thereafter ranging from 102° to just above normal until July 8th. On the latter date it was 100.4° in the evening.

CASE 3.—Gunshot wound of left leg; compound comminuted fracture of tibia and fibula. Operation on June 20th, 1917. Wound opened up. Three pieces of shrapnel removed, also a piece of isolated bone. Wound swabbed out with soap solution and sutured. Soap solution. On the 20th the temperature was 100.6° F. After this it ranged between 100.4° and normal, being 100° on the 27th and normal on the 28th.

CASE 4.—Multiple shell wounds; compound comminuted fracture femur; under care of Captain Bates. Patient admitted at 1.30 P.M. on 28th; temperature 100°. Morphine 1/4 gr. hypodermically. Operation; Carrel-Dakin tubes, soap solution. On the 29th the temperature rose to 101.8° and on the 30th to 103.6°; oleum ricini given. Temperature normal on morning of 31st, rising to 102.6° in evening. Wound dressed. From 1st to 4th temperature swinging from 102.2° to 100°. On 4th wound dressed. On the 5th temperature lower; urine tested and found normal. On the 7th wound dressed; antitetanic serum 500 units; temperature 100.8°. Then up to the 18th the range was between 100° and subnormal; and on the 11th patient taken to theatre at 6 P.M. Morph. tart.

CASE 5.—Compound comminuted fracture of right femur; secondary closure; soap solution; under the care of Captain Bates. The temperature on May 24th was subnormal; 25th–29th, from 101.4° to 99°; May 30th–June 12th, 99.8° to 97.4°; June 13th, 100.4°; 14th–25th, 99° to 96.8°; 26th–28th, 99.4° to 97°.