THE VASELINE TUBE AND SYRINGE METHOD OF MICRO GAS ANALYSIS OF BACTERIAL CULTURES.

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PLATES 55 TO 57.

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The methods to be described have been evolved not so much because of dissatisfaction with results which could be obtained by methods previously used but rather for reasons of greater convenience and economy. It is for these reasons that the Smith fermentation tube has continued in use long after its limitations have been recognized. The Smith tube was never recommended as a means for accurate study of the gas-forming function of bacteria. It was recommended as an instrument for collecting and approximately measuring and analyzing the gases which are given off by certain cultures when grown in this tube. The result of such measurement and analysis was found to be a means of distinguishing certain broad groups of bacteria. The gas was produced under the conditions imposed by the tube and the gas formula \( \frac{H_2}{CO_2} \) was understood to be valid for those conditions only. Critics of the Smith fermentation tube should not lose sight of these facts.

More efficient and more elaborate forms of apparatus have been devised by various authors with a different purpose in view, namely to collect all the gas which a culture forms, to measure both that given off from the medium and that held by the medium, and to make very accurate analyses of these gases. I refer particularly to the apparatus described by Keyes (1909), Frieber (1913, b), Rogers, Clark and Davis (1914), and Wolf and Harris (1917). These methods are much better adapted for the careful study of the gas-forming activity of a
few cultures than for the comparative study of a large number of strains. Doubtless the apparatus described by Van Slyke and Stadie (1921) could be used for bacterial cultures and should yield more accurate results than the syringe. However, it is believed that the method to be described retains the simplicity of the Smith tube, at least something of the greater accuracy of the more elaborate methods, and some advantages for the bacteriologist possessed by none of the older methods.

During a study of anaerobic bacteria still in progress, use was made of small amounts of media covered by vaseline in test-tubes. It was noticed that as gas was formed by a culture the vaseline was forced up the tube, the vaseline plug remaining perfectly intact and acting as a self-lubricating piston at the temperature of the room or incubator. We have continued to use this form of culture tube in the manner to be described.

Measurement of Gas above the Medium.

A record of the amount of gas present above the medium may be made at any time by marking with a wax pencil on the side of the tube the lower level of the vaseline plug. To measure the amount of gas in the tube a device somewhat like the Frost gasometer for the Smith fermentation tube is used. The gasometer shown in Text-fig. 1 is made of sheet tin or brass. The lower edge is turned at a right angle towards the observer so that a shelf is formed on which rests the bottom of the tube being examined. The base-line of the graduations ruled on the surface of the gasometer is shown as a double line marked 0.0 and placed diagonally with reference to the shelf and above it. The culture tube is pushed along the shelf until the meniscus of the culture medium rests at the base-line. The graduations above the base-line radiate from a point on the base-line at some distance to the right of the figure so that when the tube cuts the base-line vertically the amount of gas between the medium and the vaseline plug can be read as volumes and tenths of a volume of the amount of medium present in the tube, or, if multiplied by 100, as volumes per cent. If the culture produces sufficient gas to force the vaseline up nearly to the cotton plug the amount of gas is recorded, then by directing the flame of a micro burner against the side of the tube the vaseline is
melted and allowed to drop down onto the surface of the medium and to solidify again. This may be done repeatedly in which case the total gas formed above the medium is obtained by addition of the measurements recorded before each melting of the vaseline plug.

Determination of CO$_2$ in the Gas above the Medium.

By means of a Luer tuberculin syringe with a long fine needle$^1$ attached by means of a short length of capillary rubber tubing a sample of gas may be withdrawn and the CO$_2$ determined in the syringe. The technique and necessary equipment are illustrated in Figs. 1 and 2. The various steps of the technique are as follows:

$^1$The needle used was Gauge 20 and 6 inches long, specially made for us by Becton, Dickinson and Co., Rutherford, N. J. There is also needed a small adapter such as that listed by Arthur H. Thomas Co., No. 9419, for 606 Hose End to Luer Slip Needles.
1. Rinse the syringe and needle with dilute acid which is then expelled as completely as possible by forcing air into and out of the syringe a number of times. Push the syringe plunger in as far as it will go, better, to an exact reading at the lower end of the scale on the barrel of the syringe.
2. Dry and sterilize the needle by passing it through the flame a number of times.
3. With the usual precautions to maintain sterility draw the cotton plug from the culture tube and replace it with the needle held between the cotton plug and the side of the tube.
4. Push the needle down until the point passes through the vaseline plug and well into the zone of gas.
5. With the syringe held as shown in Fig. 1 slowly withdraw a measured quantity of gas (something less than 1 cc. so that when step 7 is taken the total gas can be read between the plunger and the meniscus). The vaseline plug will be seen to move down the tube as the gas is withdrawn.
6. Leave the cotton plug in place and pull the needle up until the point remains in the upper part of the vaseline. With a small hot spatula touch the outside of the test-tube opposite the track of the needle in the lower part of the vaseline plug. Leave the spatula in contact with the tube just long enough to close the opening in the vaseline.
7. Withdraw the needle from the tube, immediately stick the end into the dilute acid, and with the syringe held vertically draw up acid until the meniscus can be seen and read against the scale on the lower end of the syringe. Read the exact amount of gas (and air) in the syringe between the plunger and the meniscus of acid.
8. Stick the end of the needle into a 2 or 3 per cent solution of sodium hydroxide. With the apparatus in the position shown in Fig. 2 draw out the plunger until the alkali begins to enter the barrel of the syringe and neutralises the acid there. As carbon dioxide is absorbed more alkali is automatically drawn into the syringe without further use of the plunger. With the end of the needle still in the alkali solution rock the syringe back and forth a number of times to insure complete absorption of the CO₂.
9. Again hold the syringe vertically with the plunger uppermost and read the amount of residual gas in the syringe. The difference between this reading and the one taken in step 7 equals the amount of CO₂ in the sample taken. The percentage of CO₂ in the sample is easily calculated and may be expressed as a gas ratio \( \frac{H₂}{CO₂} \) if so desired.

We have tried a number of slight modifications of the details of the above technique but the above is probably the most perfect. The accuracy of the method is apparently limited only by the fineness of the graduations on the syringe. These should be at most 0.01 cc.
We are able to get almost as accurate results with 0.1 cc. of gas sample as with 1.0 cc. It is possible to determine the CO₂ content of a good sized bubble beneath the vaseline plug or in an agar shake culture. It is an advantage to select a tuberculin syringe having the following characteristics: (a) finely cut graduations, (b) a long slender barrel with considerable space above the graduations, (c) a well fitting plunger of colored glass with a sharp square-cut end which can be accurately read against the graduations on the colorless glass barrel. The carbon dioxide content of the small amount of atmospheric air contained in the needle and capillary rubber tube is a negligible quantity well within the limits of error. If the cultures are allowed to cool down to room temperature before the gas analysis is made and if the acid and alkali solutions are kept at the same temperature the temperature factor is also negligible. Formerly we took the precaution of allowing the end of the needle to be sealed by the vaseline (melted by the hot spatula) as it was withdrawn from the tube and then pricking this seal just as the needle was placed into the acid but it has been found unnecessary. There is no appreciable interchange of air and gas through the needle during the short time it is exposed to the air while being transferred from the culture tube to the dilute acid.

**Determination of Carbonates and CO₂ in the Medium.**

The carbonates and CO₂ in a fluid medium may be determined by means of the same apparatus. The equipment and technique are illustrated in Fig. 3. The steps of the technique are as follows:

1. Rinse the syringe and needle thoroughly with distilled water.
2. Dry and sterilize the needle in the flame.
3. With aseptic precautions place the needle so that it is held between the cotton plug and the side of the tube as for the determination of CO₂ above the medium.
4. Having placed the end of the syringe plunger at one of the graduation marks on the lower end of the barrel push the needle down the side of the tube through the vaseline plug (if vaseline is used) until the point is in the medium.
5. Draw the plunger of the syringe back just 0.1 cc.
6. Withdraw the needle with the same precautions as described in step 6 for the determination of CO₂ above the medium.
7. Draw into the needle about 0.03 cc. of air.
8. Stick the point of the needle into a small vial of capryl alcohol (colored with scarlet R) and draw 0.01 cc. into the needle, followed by 0.01 cc. of air.

9. Draw into the needle 0.05 cc. of 5 per cent sulfuric acid (colored with methyl red).

10. Holding the syringe vertically with the open needle uppermost draw back the plunger just until all of the red capryl alcohol and sulfuric acid are seen to be within the barrel of the syringe.

11. With the index finger of the left hand tightly stop up the end of the syringe where the rubber tube is attached and holding it as shown in Fig. 3 draw down the plunger while with a partial vacuum within the syringe the air leaks by the plunger and passes up through the contents of the syringe in a stream of fine bubbles. When the end of the plunger reaches a position a little beyond the graduations it should be allowed to remain here until the air ceases bubbling.

12. Turn the syringe to a vertical position with the plunger uppermost, release the finger from the rubber tube, push in the plunger to the beginning of the graduation marks, wait a few seconds to allow the fluid to drain down the sides of the syringe, and read the amount of gas and air present between the meniscus and the plunger.

13. Without changing the position of the syringe, needle hanging down, expel most of the fluid until the meniscus reaches the bottom of the syringe. Without admitting any air dip the end of the needle, now filled with fluid, into the sodium hydroxide solution and with the syringe held in the position shown in Fig. 2 draw up some of the alkali. Rock the syringe back and forth a few times. Again expel most of the fluid, holding the syringe in a vertical position and taking care to expel none of the gas. Draw in a fresh portion of sodium hydroxide and rock again.

14. Finally, holding the syringe vertically with needle hanging downward push the plunger in until its end reaches the graduation marks and read the amount of air remaining in the syringe. The difference between this reading and the one taken in step 12 equals the amount of CO₂ extracted from 0.1 cc. of medium, best expressed as volumes or as volumes per cent; e.g., 0.1 cc. of CO₂ from 0.1 cc. of medium equals 1 volume or 100 volumes per cent.

It is our experience that more accurate results are obtained with samples of 0.1 cc. of medium than with larger amounts. This is doubtless due to the fact that in such a small syringe as we use the amount of air that can be passed through the sample and held within the syringe for measurement is limited and although sufficient for complete aeration of 0.1 cc. of medium is hardly sufficient for a larger sample. Without doubt larger samples could be employed in a longer syringe. It is an advantage to color the acid and alkali solutions with indicators, methyl red in the acid and thymol blue in the alkali, so that one may be sure of the reaction of the contents of the syringe at all times.
Accuracy of the Determinations.

The determinations in Table I were made from a single culture of an anaerobic organism in 5 cc. of plain bouillon under vaseline. The results illustrate the possibility of obtaining uniform results with multiple determinations and various methods of expressing the results.

**TABLE I.**

*Multiple Determinations of Carbon Dioxide Produced by an Anaerobic Organism in Plain Bouillon.*

<table>
<thead>
<tr>
<th>Sample, cc</th>
<th>CO₂, per cent</th>
<th>H₂</th>
<th>CO₂</th>
<th>CO₂ (vol. at 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>0.75</td>
<td>59</td>
<td>41</td>
<td>0.43</td>
</tr>
<tr>
<td>0.45</td>
<td>0.45</td>
<td>60</td>
<td>40</td>
<td>0.42</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>39</td>
<td>40</td>
<td>0.42</td>
</tr>
<tr>
<td>0.1</td>
<td>0.1</td>
<td>39</td>
<td>40</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**Determinations of CO₂ above medium.**

<table>
<thead>
<tr>
<th>CO₂ (vol. at 20°C)</th>
<th>0.55 0.5 0.5 0.55 0.5 0.5</th>
</tr>
</thead>
</table>

**Computation of total CO₂.**

<table>
<thead>
<tr>
<th>Average; CO₂ above medium</th>
<th>0.42 vol. at 37°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; CO₂ in medium</td>
<td>0.52 vol. at 20°C, or 0.55 &quot; 37°C</td>
</tr>
</tbody>
</table>

**Total CO₂ per 1.0 vol. of medium**

| 0.97 " 37°C |

*By CO₂ in medium is meant not only CO₂ present as such but also that present in the form of carbonates or carbonic acid.*

To determine how much of the CO₂ present as carbonate might be recovered by the above method from water or from bouillon the following experiment was performed. 0.5066 gm. of sodium oxalate was converted into sodium carbonate by ignition in a platinum crucible. The sodium carbonate was dissolved in 10 cc. of distilled water and 3 cc. of this solution were diluted to 25 cc. with distilled water and with plain bouillon respectively for the determinations to be described.
As a control 3 cc. of distilled water were added to 22 cc. of the bouillon without addition of carbonate. Quadruple determinations were made of the CO₂ recovered from each of the three solutions. The amount of each sample taken was 0.1 cc. The results are presented in Table II.

**Table II.**

**Determinations of Carbon Dioxide in Standard Solutions of Sodium Carbonate in Water and in Bouillon.**

<table>
<thead>
<tr>
<th></th>
<th>Determinations</th>
<th>Average.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ from Na₂CO₃ in water</td>
<td>1.05 1.05 1.05 1.05</td>
<td>1.045 vol. in 1.0 vol. of solution.</td>
</tr>
<tr>
<td>CO₂ &quot; Na₂CO₃ &quot; bouillon</td>
<td>1.02 1.05 1.05 1.05</td>
<td>1.042 vol. in 1.0 vol. of solution.</td>
</tr>
<tr>
<td>CO₂ &quot; H₂O &quot; &quot;</td>
<td>0.02 0.0 0.03 0.0</td>
<td>0.012 vol. in 1.0 vol. of solution.</td>
</tr>
</tbody>
</table>

Calculated theoretical volume of CO₂ in the above solutions of Na₂CO₃ in water and in bouillon = 1.066 volume in 1.0 volume of solution at 20°C. and 765.2 mm. Hg.

**Experiments Illustrating the Technical Possibilities of the Methods.**

Although the conditions under which a culture grows in the vaseline tube are not the same as those in the Smith fermentation tube and one would therefore not expect to obtain identical results in the two tubes, it seemed worth while to compare the results obtained by both methods with a view to interpreting their differences. A number of series of experiments have been performed with this end in view. The following is typical.

A strain of *Bacterium coli* was inoculated into six vaseline tubes and five fermentation tubes containing a certain lot of 2 per cent dextrose bouillon (pH = 7.2). By the 4th day of incubation all tubes had ceased to show changes in gas volume. The results of analysis of the gas above the media on the 4th day are recorded in Table III.

It should be noted that the total volume of gas produced above the medium in the two kinds of tubes cannot be compared because the
gas is measured in different terms. In the fermentation tube the gas collected comes from a diminishing amount of culture as gas formation forces the medium over into the open bulb and the gas is measured in terms of the capacity of the closed arm of the tube. In the vaseline tube the volume of the culture medium remains constant and the gas is measured in terms of volume of the medium. It is for this reason

TABLE III.

Gas Formation by Dextrose Bouillon Cultures of Bacterium coli in Smith Fermentation Tubes and in Vaseline Tubes.

<table>
<thead>
<tr>
<th>Fermentation tubes</th>
<th>Vaseline tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas above medium.</td>
<td>H2 CO2</td>
</tr>
<tr>
<td>per cent</td>
<td>vol.</td>
</tr>
</tbody>
</table>
| 65                 | 53
|                    | 47
| 41                 | 55
|                    | 43
| 46                 | 55
|                    | 45
| 49                 | 51
|                    | 49
| 51                 | 56
|                    | 44

that we prefer to avoid the use of the term "per cent" in stating the volume of gas produced in the vaseline tube. It is difficult to see how one set of values can be translated into terms of the other. Aside from this fact it is noted that the results in the vaseline tubes are somewhat more uniform than those in the fermentation tubes. It is also found that invariably a larger proportion of CO2 is formed under vaseline than in the closed arm of the fermentation tube. The reason for this is, as pointed out by Keyes (1909), that CO2 is much more
soluble in water than is hydrogen and therefore not only does a considerable volume of CO₂ pass into solution in the medium but in the fermentation tube passes through the medium out into the air.

As a means of studying gas production the vaseline tube has many points of similarity to the long agar tube of Burri and Dürgeli (1909) used by Frieber (1913, a and b) for gas analyses. In this tube, however, only solid medium was used and a layer of sterile non-nutrient agar was used instead of vaseline as a seal. The technical possibilities of the vaseline tube are much greater.

**TABLE IV.**

*Gas Formation by Dextrose Bouillon Cultures of Bacterium coli in Vaseline Tubes under Anaerobic and Aerobic Conditions.*

<table>
<thead>
<tr>
<th>Vaseline tubes (without air).</th>
<th>Vaseline tubes (plus 1.0 vol. of air).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas above medium.</td>
<td>CO₂</td>
</tr>
<tr>
<td><strong>vol.</strong></td>
<td><strong>per cent</strong></td>
</tr>
<tr>
<td>0.95</td>
<td>52</td>
</tr>
<tr>
<td>0.9</td>
<td>51</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
</tr>
<tr>
<td>0.9</td>
<td>50</td>
</tr>
</tbody>
</table>

Average...0.91

0.91 × 0.505 = 0.46 vol. of CO₂

1.93 × 0.368 = 0.71 vol. of CO₂

In the methods of all the authors referred to above the cultures were grown under anaerobic conditions. Eldredge and Rogers (1914) and Osterhout (1918) have devised forms of apparatus for studying CO₂ production under aerobic or at least partially aerobic conditions. Some of the results obtained by the use of the tube of Eldredge and Rogers will be referred to later. The vaseline tube also permits the study of gas production under controlled aerobic conditions. After the medium in the tube has been inoculated and the vaseline allowed to solidify on the surface a measured volume of air or other gas may be injected beneath the vaseline plug by means of a syringe and the long needle used for gas analysis. The passage made by the needle in the vaseline is easily closed by means of a warm spatula as the needle...
is withdrawn. In the experiment partially recorded in Table III was also included a series of six similar vaseline tubes of the same medium inoculated with the same strain of *Bacterium coli* but injected with an equal volume of air just after inoculation. Gas analyses were made at the same time as were those recorded in Table III. A comparison of the results with those of the vaseline tubes without air is given in Table IV.

Experiments were carried out with various volumes of air injected under the vaseline plug. With decreasing amounts of air below 1 volume the results gradually approach those obtained under anaerobic conditions. With 2 volumes of air the results were very nearly the same as with 1 volume. Naturally these proportions must be regarded as valid for this culture and medium only.

If from the 1.93 volumes of gas found in the tubes containing air the volume of air injected be subtracted, there remains 0.93 volume of gas produced by the culture, and since 0.71 volumes of CO₂ were present it might be computed that the gas ratio under aerobic conditions was \( \frac{H_2}{CO_2} = \frac{22}{71} \), or \( \frac{24}{76} \) as compared with \( \frac{H_2}{CO_2} = \frac{50}{50} \) under anaerobic conditions. This calculation, however, assumes that the air injected is entirely inert and takes no part in the reaction. This we have reason to believe is not the case.

*Determinations of Oxygen in the Gas above the Medium.*

The oxygen is readily determined in the same sample of gas used for the determination of CO₂ provided no atmospheric air is drawn into the syringe. This may be accomplished by having the space between the syringe plunger and the end of the needle filled with dilute acid instead of air as the needle is thrust through the vaseline plug. Otherwise the CO₂ is determined in the same manner described above and then the needle is dipped into a concentrated aqueous solution of pyrogalllic acid. A little of the pyrogalllic acid solution is drawn up into the syringe and as it mixes with the sodium hydroxide and absorbs the oxygen present more of the pyrogalllic acid is automatically sucked up. The process is slower than the absorption of CO₂ by sodium hydroxide but, with constant rocking of the syringe back and forth, is complete in 2 or 3 minutes and the reading is made.
In another experiment with the same strain of *Bacterium coli* as used for the results given in Tables III and IV, both the CO₂ and the O₂ above the medium were determined and also the CO₂ in the medium. The results are given in Table V.

The outstanding feature of this experiment was the disappearance of the oxygen from the air above the aerobic culture. Assuming that

<table>
<thead>
<tr>
<th>TABLE V.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Further Gas Analysis of Dextrose Bouillon Cultures of Bacterium coli under Anaerobic and Aerobic Conditions.</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vaseline tubes (without air).</th>
<th>Vaseline tubes (plus 1.15 vol. of air).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gas above medium</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>CO₂ in</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Total CO₂</td>
<td>0.75</td>
<td>0.05</td>
</tr>
<tr>
<td>O₂ above medium</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Oxygen determined in atmosphere = 0.2 vol.

this was the case also in the experiment of Table IV, consideration of this factor would increase the gas ratio under aerobic conditions to $\frac{H_2}{CO_2} = \frac{37}{63}$ instead of $\frac{24}{76}$ since the space assumed to be occupied by oxygen was in reality occupied by some gas other than CO₂ or O₂ produced by the culture. The disappearance of a part of the oxygen in the aerobic control tube noted in Table V was probably due to its passing into solution in the air-free medium which had been kept tightly sealed since it was autoclaved. The small amounts of CO₂ found in the controls may or may not be significant. It is not surprising that less CO₂ was found in the medium of the aerobic culture than in the anaerobic culture since the presence of air above the former favored the outward diffusion of CO₂. Taking all the figures into

3 In stating the gas ratio $\left(\frac{H_2}{CO_2}\right)$ we have followed the custom of regarding all of the gas produced by the culture, other than CO₂, as hydrogen.
consideration it appears that *Bacterium coli* not only produces more 
CO₂ under aerobic conditions but a larger proportion of CO₂ with 
respect to H₂ or other gases.

Certain organisms which have been regarded as non-producers of 
gas have been found by special methods to produce appreciable 
amounts of CO₂. Such results are interesting and open up new pos-
sibilities for classification and for physiological study. However, they 
do not invalidate the practical value of formerly used methods for 
distinguishing between so called “gas-producing” and “non-gas-pro-
ducing” organisms under stated conditions of cultivation. It was 
shown by Hesse (1893) that *Bacterium typhosus* and many other 
bacteria ordinarily considered to be non-gas producers do produce an 
appreciable amount of CO₂ and consume O₂ in the process. He calls 
this process the respiratory activity of bacteria.

Ayers, Rupp, and Mudge (1921) used the tube of Eldredge and 
Rogers (1914) to demonstrate CO₂ production by streptococci, and 
Nichols (1921) demonstrated CO₂ production by *Bacterium typhosus* 
by the same means. In this tube cultures are grown under aerobic 
conditions. Since, however, the atmosphere of the tube above the 
medium is kept free of CO₂ by the barium hydroxide solution we would 
expect to find less CO₂ in the medium than when the aerobic vaseline 
tube is used. To compare the results obtained in the vaseline tube 
with those of the Eldredge tube we must therefore consider the CO₂ 
in the medium as well as that above it in the vaseline tube. We have 
done this with two strains of streptococci for which we are indebted 
to Dr. Ayers. Strain X-4 was reported by Ayers, Rupp, and Mudge 
as producing CO₂ from dextrose and Strain 16H-1 was reported to 
produce CO₂ from Bacto-peptone. In Table VI are given the total 
CO₂ determinations of Ayers' strains of streptococci in various media. 
The determinations were made after incubation at 37°C. for 6 days. 
The amount of medium used was 3 cc. in each tube.

The resemblance between the CO₂ determinations of our aerobic 
cultures and those of Ayers, Rupp, and Mudge obtained by an en-
tirely different method is so close that the differences seem insignif-
ient. It is to be noted, however, that under anaerobic conditions 
much less CO₂ is produced in all media. Possibly this explains failure 
to demonstrate gas production by streptococci under the anaerobic 
conditions imposed by other methods.
Using the tube of Eldredge and Rogers, Nichols (1921) demonstrated CO₂ production by *Bacterium typhosus*. He obtained the maximum amount of CO₂ from cultures in 1 per cent glucose extract broth. In 2 per cent glucose veal infusion broth and with a different strain of typhoid bacillus, however, we have obtained even larger amounts of CO₂. The results of our determinations are recorded in Table VII.

### TABLE VII.

**Determination of Total Carbon Dioxide Produced by Streptococci in Vaseline Tubes under Anaerobic and Aerobic Conditions.**

The medium was veal infusion bouillon containing 1 per cent Fairchild's peptone plus the substances indicated above each column.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Vaseline tubes (without air)</th>
<th>Vaseline tubes (plus 1.0 vol. of air)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 per cent Fairchild's peptone</td>
<td>3 per cent Bacto- peptone</td>
</tr>
<tr>
<td>Streptococcus X-4</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>&quot; 16H-1.</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>(0.12)*</td>
<td>(0.33)</td>
</tr>
</tbody>
</table>

* The bold faced figures in parentheses are the results of Ayers, Rupp, and Mudge reduced to the same terms as ours. It should be noted, however, that whereas one of their media contained 4 per cent of Bacto-peptone ours used for comparison contained 3 per cent of Bacto-peptone and 1 per cent of Fairchild's peptone.

There was good growth of the typhoid bacillus under anaerobic conditions, though somewhat better under aerobic conditions. It is again seen that a really significant amount of CO₂ is produced under aerobic conditions only, and that a part but not all of the oxygen was consumed.

It must be pointed out that in interpreting the results of the determination of CO₂ in and above the medium certain very important factors must be taken into consideration. There is a very intimate relationship between temperature, hydrogen ion concentration of the culture, and the proportionate amount of CO₂ in and above the me-
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medium. For instance, if the culture becomes alkaline, as may be the case with certain anaerobes, a large proportion of the CO₂ will be found in the medium as carbonates. If it becomes strongly acid a large proportion of the CO₂ will be found in the gas above the medium. If the culture is transferred from the incubator to the refrigerator the solubility of gases in the medium is appreciably increased. If the vaseline plug is broken so that atmospheric air gains access to the culture, CO₂ begins to pass out of the medium into the air. The latter can be demonstrated easily by breaking the seal of a culture and making periodic determinations of the CO₂ in the medium.

**Table VII.**

_Determination of Carbon Dioxide Produced by Bacterium typhosus in Dextrose Bouillon under Anaerobic and Aerobic Conditions._

<table>
<thead>
<tr>
<th></th>
<th>Vaseline tube (without air)</th>
<th>Vaseline tubes (plus 1.0 vol. of air)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture.</strong></td>
<td>vol.</td>
<td>vol.</td>
<td>vol.</td>
</tr>
<tr>
<td>Gas above medium</td>
<td>0.0</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>CO₂</td>
<td>0.0</td>
<td>0.14</td>
<td>0.03</td>
</tr>
<tr>
<td>CO₂ in</td>
<td>0.1</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Total CO₂</td>
<td>0.1</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>O₂ above medium</td>
<td>0.0</td>
<td>0.06</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Oxygen determined in atmosphere = 0.2 vol.

_Sterilisation, Inoculation, and Cleaning of Vaseline Tubes._

Before summarizing the advantages of the technique described it may be well to describe one or two simple points of technique which serve to make the vaseline tube almost as convenient to handle as an ordinary test-tube.

Autoclaving cannot be relied upon to sterilize vaseline since it is essentially a "dry" substance which the steam does not penetrate. It may be perfectly sterilized without visible alteration along with glassware in the hot air sterilizer at 175–185°C. for 2 hours. After being so sterilized it may be pipetted onto non-sterile medium in the tubes and then autoclaved with the medium. During sterilization air is driven from the medium and if the tubes are promptly cooled...
after coming from the autoclave the medium under the solid vaseline plug is preserved free from air for long periods of time. There is no danger of spilling if tubes are turned over, and the medium may be kept at room or incubator temperature without evaporation. Since there is no water vapor in the tubes above the vaseline there is no tendency for molds to grow through the cotton plugs.

It is not necessary to pass a pipette through the vaseline to inoculate or add anything to the medium in the tube. In fact it may be inoculated with a platinum loop if the vaseline seal is opened as shown in Figs. 4 and 5. The vaseline is melted by directing the flame of a micro burner against the side of the tube. The tube is then slanted in a dish or tray of water as shown in Fig. 4. After the vaseline has hardened as a layer over the slanted surface of the medium the tube is rotated and gently tapped by the fingers, as shown in Fig. 5, until the vaseline over the medium flaps up and adheres to the side of the tube, exposing the medium for inoculation. The seal is again closed by melting the vaseline by means of the flame directed against the outside of the tube in the region of the vaseline. The medium need not be perceptibly heated by the process and with reasonable care tubes rarely crack.

Tubes of discarded cultures are cleaned as follows: The tubes are placed upright in a basket and autoclaved. While they are still hot the cotton plugs are withdrawn, the basket is placed in a deep pail or other vessel somewhat deeper than the tubes, and the tubes, remaining upright, are filled and covered with hot water. The pail of water is heated on a burner while the vaseline rises to the surface whence after cooling it may be recovered if desired. The tubes may then be washed by the usual method.

**SUMMARY.**

There has been described the use of the vaseline tube and the tuberculin syringe for the study of gas production by bacteria.

A comparison is made of some of the results obtained by the use of the method here described, the Smith fermentation tube, and the tube of Eldredge and Rogers.

The reports of CO₂ production by certain streptococci by Ayers, Rupp, and Mudge and by *Bacterium typhosus* by Nichols have been confirmed by the author’s method.
The data presented serve to illustrate the accuracy and technical possibilities of the method. In addition to economy of glassware, medium, and labor, the vaseline tube and syringe method of micro gas analysis possesses the following advantages. (1) Gas produced above either liquid or solid media may be measured and analyzed. (2) The gas produced may be measured in terms of a definite and constant quantity of medium used. (3) The vaseline tube provides a closed system from which gases do not escape into the air. (4) Separate determinations of the CO₂ produced in and above fluid media may be made. (5) Determinations may be made from very small samples of material. (6) Numerous gas analyses of the same culture may be made at various times during the growth of the culture without contaminating or destroying it. (7) Gas production may be observed under both anaerobic and controlled aerobic conditions.

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EXPLANATION OF PLATES.

PLATE 55.

Fig. 1. Withdrawing the sample of gas from beneath the vaseline plug.

PLATE 56.

Fig. 2. Sodium hydroxide solution being drawn into the syringe for the absorption of carbon dioxide.
Fig. 3. The aeration of a sample of culture for the determination of the carbon dioxide in the medium.

PLATE 57.

Fig. 4. The tube of medium slanted but still covered by a layer of vaseline.
Fig. 5. The slanted tube rotated causing the lower end of the vaseline seal to flap up, thus exposing the medium for inoculation.
FIG. 1.

(Brown: Micro gas analysis of bacterial cultures.)
Fig. 2.

Fig. 3.

(Brown: Micro gas analysis of bacterial cultures.)
(Brown: Micro gas analysis of bacterial cultures.)