THE UNIVERSITY OF MINNESOTA



GRADUATE SCHOOL

Report

of

Committee on Examination

This is to certify that we the undersigned, as a committee of the Graduate School, have given Beryl Sparks Green final oral examination for the degree of Master of Arts. We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

May 24 1921

[signature] Chairman

Arthur P. Henrici

E. C. Rosenow

THE UNIVERSITY OF MINNESOTA

GRADUATE SCHOOL

Report

of

Committee on Thesis

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Beryl Sparks Green for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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May 24 1921

# The Oxygen Content of Media in Relation to Surface Tension and Bacterial Growth

A Thesis presented tothe Faculty of the

Graduate School of

THE UNIVERSITY OF MINNESOTA

by

Beryl Sparks Green

in Partial Fulfillment of the requirements for the degree of

Master of Arts

1921

# The Oxygen Content of Media in Relation to Surface Tension and Bacterial Growth.

The following investigation was undertaken to determine whether the change effected in bacterial growth by a lowering of the surface tension of the medium, was due to an increased absorption of oxygen or to some other factor.

Larson (1) lowered the surface tension of nutrient broth by the addition of castor oil soap. This gave a clear solution and the tension remained constant. He found that when B. Subtilis was grown on such a medium it no longer produced a pellicle but grew down in the bottom of the medium. Spore formation was greatly reduced. B. Tetani grew well under ordinary aerobic conditions when the surface tension of the medium was sufficiently lowered. B. Chauvei failed to show any considerable growth on a medium of lowered tention. Most bacteria seemed to grow best when the tension had not been depressed below 53 to 55 dynes, altho some organisms, the staphylococcus, B. Coli, B. typhosus and B. paratyphosus showed some growth when the tension was as low as 32 dynes. The pneumococci tended to disintegrate when grown on a medium of low tension. Larson (1) suggested that bacteria which normally formed a pellicle might be enabled to grow in the bottom of the medium when the tension was lowered because of an increased oxygen supply or because the surface tension reducing substances, collected normally at the surface and thruout the medium or lowered tension,

(1) Larson, Journal of Inf. Diseases, Vol. 25, No. I, July 1919, 41 - 46.

might be necessary for their metabolism.

This paper is an attempt to determine if the growth at the bottom of the medium was due to an increased $O_2$ supply and if there is any relation between the surface tension of a liquid and its ability to absorb $O_2$.

A great deal of work has been done on the absorption of atmospheric gases by water. The absorption coefficients have been determined for distilled water, rain water and sea water. The actual gaseous content of sea water has received a great deal of attention.

Henry's (2) law for the absorption of a gas by a liquid states that for a given amount of fluid the amount of gas absorbed or dissolved is proportional to the pressure of the gas. Bunsen (3) in his investigations, confirmed this law. When a gas comes in contact with a fluid, absorption begins at once until the surface of the liquid takes up as much gas as possible. Into the interior of the liquid the absorbed gas enters by diffusion, thus decreasing the amount in the surface and making it possible for the surface layer to absorb more gas. This continues until saturation is complete, and as diffusion is slow, the time needed for saturation is long. In order to reach the saturation point quickly, a shaking of the gas and liquid together is preferable.

There are many types of apparatus for measuring the absorp-

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(2) Henry, Phil. Trans. 1803.

(3) Bunsen, Annals der Chemie und Pharm. 93, 31, 1885.

tion of a gas by a liquid. Bunsen (3) and Heidenhain and Meyer (4) have given us good types. Absorption is best carried out over mercury as its chemical activity, when clean, is negligible.

In 1855 Bunsen (3) and his pupils instigated the absorption of many gases , including air, by water. Bunsen did most of his work on the solubility of gases in his absorptiometer, shaking known volumes of gas and water and noting the decrease in the volume of the gas, due to absorption by the liquid. This method can be used only with pure gases and Bunsen therefor investigated air, by making a number of determinations of the absorption coefficient of nitrogen and by boiling out water, saturated with air, at 0°, 13°, and 20°C. From this data and his accurate knowledge of the composition of air, he calculated the absorption coefficient of air and of oxygen. He concluded that the composition of the air, dissolved in water at various temperatures was always constant. Tornoe (5) was the first to paint out the fact that as the temperature increased the amount of oxygen absorbed decreased. This was later confirmed by Dittmar (6), Winkler (7) and Bohr and Bock (8). The differences at various temperatures are, however, not great.

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(4) Meyer, Zeitsch. Elecktro Chemie, 15, 249-52, 1909.

(5) Tornoe, Norwegian North Atlantic Exp. 1876-8, C Vol. I, Chemistry.

(6) Dittmar, "The Challenger" Exp. Chemistry and Physics.

(7) Winkler, Bestimmung des im Wassergelösten Sauerstoffes, Ber., 21, 2843, 1888.

(8) Bohr and Bock, Annals d. Physik. u. Chem. XLIV, N.F., 319, 1891.

The absorption coefficient and so the solubility of a gas is not related to pressure but is dependent on the nature of the substance and on the temperature. As yet we have no simple relation between the nature of the fluid solvent and the solubility of a gas in the same. Also the absorption coefficients of a gas in different solvents are not proportional to one another as Bunsen (3) showed for alcohol and water, and Guiewosz and Wolfisz (9) found for petroleum. The absorption of hydrogen by water as well as oxygen and carbon monoxide by alcohol is according to Bunsen unaffected by temperature. The following table is given by Bunsen and his pupils for the absorption coefficient of oxygen in water and in alcohol.

| Temperature | Ab. Coeff. in $H_2O$ | Ab. Coeff. in Alcohol |
|---|---|---|
| 0°C | .04114 | .2840 |
| 5 | .03628 | .2840 |
| 10 | .03250 | .2840 |
| 15 | .02989 | .2840 |
| 20 | .02838 | .2840 |

Winkler (7) gives the following table for the absorption coefficient of oxygen in water as worked out by his own excellent iodometric process for oxygen.

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(9) Guieqosz and Wolfisz, Zeitsch. f. Phy. Chem. 1, 70, 1887.

| Temperature | Ab. Coeff. of $O_2$ in $H_2O$ |
|---|---|
| 0 | .0489 |
| 5 | .0429 |
| 10 | .0380 |
| 15 | .0342 |
| 20 | .0310 |
| 25 | .0284 |
| 30 | .0262 |

Dalton (10) explained the phenomena of absorption as of a purely mechanical nature since they were proportional to the pressure and chemical combinations were not. He described the entrance of the particles of gas into the space between the molecules of the fluid, but he could not explain why different gases would go into the same fluid in different amounts. Today, since we know the effect of pressure on the state of chemical combination, Dalton's chief argument for his conception of gas absorption, is not decisive. The idea of a simple entrance of the gas into the space between the molecules is contrary to the decrease of absorption with an increase in temperature, since an increase in gas volume should also give an increase in intramolecular space. Ostwald (11) explains the theory of gas absorption as follows: On the basis of the molecular theory of fluid states, we gain ideas which are also valuable in the understanding of gas absorption. One can conceive of the existance of forces of attraction between the molecules of the absorbed gas and

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(10) Dalton, Mem. Phil. Soc. 271, 1805.

(11) Ostwald, Chemie - Stöchiometrie, 612.

and those of the fluid, which hold fast a certain portion of the gas molecules, which are in contact with the surface of the fluid, until a like force is established between those molecules entering the fluid and then because of their kinetic energy, again leaving it. Under these conditions, the amount of gas remaining in the fluid must be proportional to the number of molecules leaving. If the temperature is raised and the gas kept under the same pressure so that the entire active force of the molecular impact on the surface of the fluid is unchanged, then the number of outgoing molecules is smaller in the same proportion as the active force is greater; that is, the first varies in inverse ratio to the absolute temperature. This explains the decrease in the absorption coefficient as the temperature is raised.

The values of oxygen and nitrogen dissolved in distilled water are much higher than those for sea water. The ratio $O_2:N_2$ appears to be the same but the values for sea water are about 20% lower, varying with the salinity of the water. Hamberg (12) investigated the effect of such variation on the nitrogen content, while Clowes and Briggs (13) determined the oxygen content of various mixtures of salt and of fresh water by Winkler's method. Hamberg (12) gives the following table for the volume of nitrogen at normal temperature and pressure in salt water.

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(12) Hamberg, Beiträge zur Chemie des Meerwassers, 10, No. 13, 1885, and Jour. f. prakt. Chem. 33, 433, 1886.

(13) Clowes and Briggs, Jour. Soc. of Chem. Ind., 23, 1904, 358.

| Temp. | 0% NaCl | 1% NaCl | 2% NaCl | 3% NaCl | 3.5% NaCl |
|---|---|---|---|---|---|
| 0°C | 19.14 | 17.71 | 16.45 | 15.34 | 14.85 |
| 5 | 16.93 | 15.76 | 14.76 | 13.75 | 13.32 |
| 10 | 15.14 | 14.16 | 13.26 | 12.44 | 12.06 |
| 15 | 13.73 | 12.88 | 12.10 | 11.38 | 11.04 |
| 20 | 12.63 | 11.87 | 11.17 | 10.54 | 10.25 |
| 25 | 11.81 | 11.09 | 10.45 | 9.88 | 9.62 |

Coste (14) in commenting on the results obtained for the absorption of gases by sea water, says that the principal effect of the salts dissolved in sea water is to decrease the amount of dissolved gases. Ostwald (15) has shown that if the concentration of the salt solution follows are arithmetical progression, the absorption coefficient follows a geometrical progression. The higher the percentage of salt in a solution, the less gas it will absorb. Experiments with acids show variations as a high percentage concentration is reached. In the case of salts in solution, there is an additive effect of both ions on the absorption coefficient. The nitrates have the least effect on absorption, then follows the chlorides and the sulfates. Of the positive ions, the ammonium salts show the least effect, then potassium and sodium.

Setschenow (16) worked with the absorption of carbon monoxide by mixtures of water and sulfuric acid and water and lactic

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(14) Coste, Jour. Soc. Chem. Ind. 36, 846, 1917.

(15) Ostwald, Chemie II, 968.

(16) Setschenow, Mem. Ac. Petersb. 22, 102, 1876.

acid. He found that by mixing the two substances the absorption coefficient decreased. The curve of the absorption coefficient resulting from the mixture always remained under the combined curve of the two pure fluids alone. His results follow:

Sulfuric acid at 17°C.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Pts. $H_2O$ by wgt. | .000 | .032 | .080 | .155 | .270 | .914 | 1.000 |
| Ab. Coeff. | .932 | .852. | .719 | .666 | .705 | .887 | .961 |

Lactic acid at 15.2°C.

| | | | | | |
|---|---|---|---|---|---|
| Pts. $H_2O$ by Vol. | .000 | .50 | .75 | .875 | 1.00 |
| Ab. Coeff. | 1.438 | .956 | .935 | .970 | 1.000 |

It will be seen that he finds a point in the concentration of minimum absorption in both cases. Müller (17) investigated the absorption of carbon monoxide by mixtures of alcohol and water. He found a minimum absorption at 30% by weight of alcohol. Lubarsch (18) working independently of Setschenow (16) with mixtures of sulfuric acid and water found the point of minimum absorption of both carbon dioxide and nitric oxide to be at the same concentration of sulfuric acid. He also used mixtures of alcohol and water and found the minimum absorption at 29% by weight of alcohol. Lubarsch's results with mixtures of alcohol and water follow:

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(17) Müller, Wied, Ann., 37, 24, 1889.

(18) Lübarsch, Wied. Ann. 37, 1889.

Alcohol, $O_2$, $H_2$, CO.

| % by wgt. | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Alcohol | .00 | 9.09 | 16.67 | 23.08 | 28.57 | 33.33 | 50.0 | 66.67 | 80.0 |
| Ab. Coeff $O_2$ | 2.98 | 3.78 | 2.63 | 2.52 | 2.49 | 2.67 | 3.5 | 4.95 | 5.66 |
| $H_2$ | 1.93 | 1.43 | 1.29 | 1.17 | 1.04 | 1.17 | 2.02 | 2.55 | - |
| CO | 2.41 | 1.87 | 1.77 | 1.68 | 1.50 | 1.94 | 3.02 | - | - |

The minima of absorption lay at the same point, which is 28.57% by weight of alcohol. The absorption coefficients of the different gases, however, are not proportional to one another.

Gerhard Just (19) did a great deal of work on the absorption of carbon dioxide, hydrogen, nitrogen, and carbon monoxide by organic liquids or solvents. In all cases he found glycerine to absorb the least gas, in some cases, the amount absorbed being too small for measurement. Water came second in the list and the best absorbants were the acetates. Skirrow (20) using binary organic mixtures investigated their ability to absorb carbon monoxide. He used varying combinations of benzol, naphthalin, anilin, toluol, nitrobenzol, and acetone, one, an easily volatilized substance and the other a substance not easily volatilized. In no case did he find maxima and minima of absorption but a steadily decreasing ability to absorb carbon monoxide. He then tried mixtures of two substances, both easily volatilized, as acetone, chloroform, benzol and alcohol and compared their ability to absorb carbon monoxide with their vapor pressure. He concluded that there was no direct relationship between the vapor pressure of a mixture and its ability to absorb gases. He then compared the curves of gas

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(19) Just, Zeit. Phy. Chem. 37, 342.

(20) Skirrow, Zeit. Phy. Chem. 41, 139.

absorption with the curves of surface tension and found that when the surface tension reached a minimum the absorptive ability reached a maximum.

Timofejew (21) investigated the absorption by water and by alcohol of hydrogen and oxygen. He found a decrease in absorption with an increase in temperature, and gave the absorption coefficients as follows:

| Temp. | Gas | Solvent | Ab. Coeff. |
|---|---|---|---|
| 20°C | $H_2$ | $H_2O$ | .01837 |
| 18.8° | $H_2$ | Alcohol | .07399 |
| 12.6° | $O_2$ | $H_2O$ | .036011 |
| 20°C | $O_2$ | Alcohol | .22007 |

Christoff (22) investigated the absorption of carbon dioxide by aqueous salt solutions and binary fluid mixtures. He used normal salt solutions and found that both the cation and anion exerted a strong effect in decreasing the absorptive ability. Using mixtures of sulfuric acid and water he compared the absorptive ability with the surface tension. The surface tension values were those of Whatmough (23). His results are given in the following table.

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(21) Timofejew, Zeit. Phy. Chem., 6, 141, 1890.

(22) Christoff, Zeit. Phy. Chem., 53, 321, 1905.

(23) Whatmough, Zeit. Phys. Chem., 39, 164, 1901.

| Solvent | Amt. $CO_2$ absorbed | Surface tension |
|---|---|---|
| Water | .1381 | 75.57 |
| 2.5% $H_2SO_4$ | .1282 | - |
| 5. % " | .1179 | - |
| 10 % " | .0833 | 76.14 |
| 20 % " | .0755 | 77.23 |
| 30 % " | .0751 | 78.63 |
| 40 % " | .0713 | 79.88 |
| 45 % " | .0725 | 80.62 |
| 70 % " | .0918 | 78.46 |
| 90 % " | .1433 | 68.58 |

From this table it can be seen that as the surface tension increases the absorption of carbon dioxide decreases. The minimum absorption is at 40% sulfuric acid and the maximum surface tension at 45% sulfuric acid. The same relation was noted between the surface tension and the absorptive ability of mixtures of acetic acid and tetrachlormethan. Mixtures of ethylchloride and carbon disulfide gave a surface tension curve of parabolic form, while the absorption curve of the same mixture was a straight line, showing a steadily decreasing ability to absorb carbon dioxide as the concentration of carbon disulfide increased. Christoff (24) further investigated the relationship between surface tension and absorptive ability. He worked out the absorption coefficients of hydrogen, nitrogen, oxygen, methane, carbon monoxide and air in concentrated sulfuric acid. The values were found to be about the same as those

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(24) Christoff, Zeit. Phy. Chem., 55, 622, 1906.

for water, with the exception of hydrogen, which was absorbed only one half as much by sulfuric acid as by water. The following table gives his results compared with Bunsen's coefficients for water and alcohol and Guiewosz's and Wolfisz's coefficients for petroleum.

| Gas | 95.6% $H_2SO_4$ | $H_2O$ | Alcohol | Petroleum |
|---|---|---|---|---|
| $H_2$ | .01020 | .01930 | .06668 | .0582 |
| $N_2$ | .01555 | .01403 | .1204 | .117 |
| CO | .02164 | .02312 | .2041 | .123 |
| $O_2$ | .02945 | .02838 | .2840 | .202 |
| $CH_4$ | .03072 | .03499 | .4710 | .131 |
| $CO_2$ | .9230 | .90140 | 2.9465 | 1.170 |
| Air | .01703 | .01704 | -- | -- |

For mixtures of sulfuric acid and water he found as before that when the surface tension reached a maximum the absorptive ability reached a minimum. Using pure liquids and carbon monoxide the following results were obtained:

| Liquid | Surface Tension | Absorption Coefficient |
|---|---|---|
| Water | 75.57 | .02192 |
| Anilin | 45.90 | .053 |
| Nitrobenzol | 44.85 | .093 |
| Benzol | 28.94 | .174 |
| Toluol | 28.54 | .182 |
| Acetic acid | 28.08 | .173 |
| Chloroform | 27.33 | .207 |
| Alcohol | 24.11 | .2044 |
| Acetone | 23.76 | .238 |

He concluded that if one arranged pure liquids in the order of decreasing surface tensions the absorption coefficients would follow in the order of increasing values. Later Christoff (25) used ethyl-ether and determined the absorption coefficients for hydrogen, nitrogen, carbon monoxide, oxygen, methane, carbon dioxide, ammonia, and air. He found the following values at 0°C.

| Gas | Absorption Coefficient in Ether |
|---|---|
| $H_2$ | .1115 |
| $N_2$ | .2580 |
| CO | .3618 |
| $O_2$ | .4235 |
| $CH_4$ | 1.066 |
| $CO_2$ | 7.330 |
| $NH_3$ | 17.13 |
| Air | .290 |

Comparing these with Bunsen's figures for alcohol and Winkler's for water we get the following:

| Gas | Water | Alcohol | Ether |
|---|---|---|---|
| $H_2$ | .02148 | .06925 | .1115 |
| $N_2$ | .02348 | .12634 | .2580 |
| CO | .03537 | .20443 | .3618 |
| $O_2$ | .04890 | .28397 | .4235 |
| $CH_4$ | .05563 | .52259 | 1.066 |
| $CO_2$ | 1.713 | 4.3295 | 7.330 |

Thus it will be seen that for pure liquids as the surface tension decreases the absorption coefficient increases. This general law

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(25) Christoff, Zeit. Phy. Chem. 79, 456, 1912.

holds with a few exceptions. Just (19) found glycerine to absorb very small amounts of the different gases, in all cases less than the amounts absorbed by water. Still the surface tension of glycerine is less than that of water, glycerine having a tension of 64.53 dynes at 20°C, while water has a tension of 72.53 dynes at 20°C. In my own results acetone has a higher surface tension than does alcohol, while at the same time it absorbs more oxygen.

The following is a report of the work undertaken to investigate the oxygen content of bacterial culture media and how it was affected by the addition of castor oil soap, by putting a layer of sterile oil on top, and by adding a piece of sterile tissue. These three phases of the work will be taken up separately. The first to be discussed, will be the effect on the absorptive ability of a biological fluid, of the addition of soap, which lowers very considerably the surface tension of the fluid.

The apparatus used was that of Van Slyke for the analysis of blood gas. Descriptions of the same are given by Van Slyke (26). It is simple and consists essentially of a 50 cc. pipette, provided with three way stop cocks at the top and bottom and connected with a mercury bulb. The upper stem of the pipette has a 1 cc. scale divided into .02 divisions. Below the bottom stop cock is attached a small bulbous chamber for drawing off the fluid after extraction of the gases and a small tube, thru which mercury enters and

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(26) Van Slyke, Soc. Exp. Biol. and Med. 12, 13, 165. - 14, 15, 84. Jour. Biol. Chem. 30, 345.

releases the vacuum. The upper stop cock is connected with a cup for the entrance of fluids and a small capillary tube for removing fluids. The bottom of the apparatus is connected, by strong rubber tubing, with a leveling bulb filled with mercury. The stop cocks should be kept well greased and held tightly in place with elastic bands. Before each determination the apparatus should be tested for leakage. This is done by filling the pipette and the capillaries of the upper stop cock with mercury, by raising the leveling bulb. Then the upper stop cock is closed and the mercury bulb lowered, thus producing a Torricellian vacuum. If the apparatus is tight and free from gases, the mercury will hit the upper stop cock with a sharp metallic click when the leveling bulb is again raised. If there is air in the apparatus, it will serve as a cushion and no click will be heard. In this case the apparatus must be evacuated again and again until the click is heard.

In making a determination 5 cc. of the material to be used were run into the cup. This was allowed to run slowly down into the pipette by lowering the leveling bulb. When the last trace had left the cup and after making certain that no bubbles has entered the pipette, the upper stop cock was closed. The bulb was then lowered until only a small amount of mercury was left above the lower stop cock, then that was closed and the 5 cc. of fluid were shaken in the vacuum thus formed. The fluid was then drawn down into the lower chamber and mercury was allowed to fill the pipette. The extracted gas collected in the upper end of the pipette and was removed. The operation was then repeated until no traces of gas were extracted upon shaking the fluid in the vacuum. Then thru the capillary tube at the top oxygen from a tank was let into the pipette. The volume of oxygen was recorded, then the 5 cc. of fluid from the lower chamber were run into the pipette and the oxygen and the fluid were shaken together until the reading was constant. The decrease in the volume of gas, due to absorption by the liquid was then noted. The original volume of gas was read at atmospheric pressure by having the level of the mercury in the bulb on a plane with that in the pipette. The final reading was taken with the level of the mercury in the bulb on a plane with the upper end of the column of oxygen in the pipette. This was a pressure greater than one atmosphere, but was kept constant thruout the experiments so that the results are comparative and not absolute. The temperature was 20°C $\pm$ 1°C.

The materials used included water, alcohol, acetone, ether, glacial acetic acid, olive oil, Douglas salad oil, saturated salt solutions, broth and peptone. Turpentine, xylol and benzol were found to so increase the rate of the oxidation of the mercury that it was impossible to get a constant reading. Castor oil soap was added to salt solutions, nutrient broth and to water and the absorptive ability of such mixtures was compared with that of the original. The lowest tension obtained by the addition of soap was 32 dynes.

The surface tension was measured by the method of pulling off a ring from the surface of the liquid. The apparatus used was that of DuNuoy, which is a modification of the Searles torsion wire apparatus. It was standardized according to the method of Robert G. Green, at this time unpublished, to give at 20°C a surface tension of 72.5 dynes to water, 64.5 dynes to glycerine, 22.0 dynes to alcohol and 16.5 dynes to ether.

Pure liquids were used first, the surface tension was taken, and then the amount of oxygen which they would absorb was determined. Many determinations were made on each liquid and the results checked each time. Two sets of apparatus were used and the same liquid used in each. To determine the effect of the amount of gas and fluid used each time, one determination on ether was performed, having 3 cc. fluid and 2 cc. oxygen in one apparatus and 5 cc. fluid and 3.0 cc. oxygen in the other apparatus. The first absorbed 1.62 cc. oxygen and the second 2.71 cc. oxygen, which gave an absorption per cc. of .540 cc. oxygen in the first and .542 cc. oxygen in the second. In all further experiments 5 cc. of the fluid were used routinely. The following table gives the surface tension of the pure fluids used and the amount of oxygen absorbed by 5 cc. of the fluid.

| Fluids | Surface Tension | $CCO_2$ absorbed. |
|---|---|---|
| Ether | 16.5 dynes | 2.70 |
| Alcohol | 22.0 | 1.40 |
| Acetone | 23.4 | 1.76 |
| Gl. Acetic Acid | 28.0 | 1.28 |
| Salad Oil | 32.5 | .98 |
| Olive Oil | 34.0 | .91 |
| Water | 72.5 | .34 |

It will be seen from these figures that as the surface tension increases the absorptive ability decreases, as was found by Skirrow (20) and Christoff (24) The exception noted above, that of alcohol and acetone, is clear from these figures. Christoff (24) also finds that acetone absorbs more gas than alcohol but he gives

24.11 dynes as the surface tension of alcohol and that reverses the order of the two substances in his series, and thus the sequence holds. However, the breaking point of the film, as read on the dial of the Du Nuoy apparatus is higher for acetone than for alcohol and therefore the surface tension is greater. The general law then, as formulated by Christoff for the relation of surface tension to absorptive ability does not hold in the case of glycerine and water and alcohol and acetone.

Saturated solutions of sodium chloride and potassium carbonate were next used. The surface tension increased and the absorptive ability decreased as the following table shows:

| Solutions | Surface Tension | $CCO_2$ absorbed. |
|---|---|---|
| Saturated NaCl | 78.8 dynes | .24 |
| " $K_2CO_3$ | 85.5 " | .20 |

Soap was now added to these solutions and the following results were obtained:

| Solutions | Surface Tension | $CCO_2$ absorbed. |
|---|---|---|
| Saturated NaCl + Soap & Glycerine | 54.5 dynes | .24 |
| Saturated $K_2CO_3$ + Soap | 32.0 " | .20 |

All workers have found that increased salinity lowered absorptive ability thus making the gaseous content of sea water about 20% lower than that of distilled water. The ability of a salt solution then, to absorb gases is dependent on its salinity and not on its surface tension. A saturated solution of potassium carbonate with a surface tension of 85.5 dynes absorbed just as much oxygen as the same solution with its tension lowered by the addition of soap to 32.0 dynes.

The following table shows the surface tension of some aqueous solutions and the amount of oxygen absorbed by 5 cc. of such solutions. The tensions range from 32.0 to 55.0 dynes and the amount of oxygen absorbed is a constant. This constant will be seen to be the same as that for distilled water alone.

| Solution | Surface Tension | CC$O_2$ absorbed. |
|---|---|---|
| Water | 72.5 | .34 |
| Broth | 55.0 | .34 |
| Peptone | 54.5 | .34 |
| 1% Dextrine | 52.0 | .34 |
| Broth and Soap | 40.2 | .34 |
| Water and Soap | 32.0 | .34 |

Distilled water with a tension of 72.5 dynes absorbs just as much oxygen as a solution of distilled water and soap, which has a surface tension of 32.0 dynes. Therefore the absorptive ability of a solvent is not a function of its surface tension. Skirrow (20 found that there was no direct relationship between the vapour pressure of a mixture and the ability of that mixture to absorb gases. There is likewise no relation between the specific gravity of a substance and its ability to absorb gases as the following figures show.

| Fluid | Specific Gravity | Ab. Coeff. |
|---|---|---|
| Water | 1.0 | .02192 |
| Chloroform | 1.4878 | .207 |
| Alcohol | .7906 | .2044 |

The ability then of a solvent to absorb gases is dependent ohly on the nature of the solvent and on the temperature. We have no simple relation, as yet, between the nature of the solvent and its ability to absorb gases. We have found no direct relationship between the physical properties of a solvent and its capacity for taking up gases.

From the foregoing it will be seen that the effect produced on bacterial growth, by a lowering of the surface tension of the medium is not due to an increased absorption of oxygen, but to some other factor. The oxygen content of bacterial media is not effected by the addition of soap. The changes noted by Larson (1) cannot be accounted for on the basis of an increased oxygen supply but must be due to some change in the distribution of the substances needed for bacterial metabolism. This would account for pellicle formers being able to grow thruout the medium of lowered tension and for anaerobes growing under ordinary aerobic conditions, when the tension was lowered. We have always considered B. subtilis as a fine example of an obligate aerobe, assuming that it grew only at the surface of broth because it needed an abundant supply of oxygen. The fact that it grew thruout the medium of lowered tension as does B. coli and others, which we have considered facultative anaerobes, shows that its oxygen requirement is not its chief reason for growing in a pellicle at the surface. Possibly the lipoids of the cell, in normal broth, prevent the wetting of the cell and the tension holds the cells up. When soap is added to the broth, the lipoids, being miscible in soap, no longer prevent wetting of the cell and water creeps up on the cell, and as the tension is also less, the cells sink, thus producing turbidity of the medium and no pellicle.

The second point to be discussed is the effect of a layer of sterile oil, on the oxygen content of media, which has been boiled to drive off all air and then quickly cooled. We have long cultivated anaerobes by planting them in a medium which had been freshly boiled, quickly cooled and then covered with a layer of sterile oil. This layer of oil was popularly supposed to keep oxygen from entering the medium below and thus to produce anaerobiosis.

In 1803 Guiewosz and Wolfisz (9), after much experimental work on the absorption coefficients of petroleum, concluded that it was illusory to try and protect aqueous fluids from the action of the air by a layer of petroleum. They used a Hempel gas burette connected with an absorption vessel. The petroleum was boiled and cooled and put in the evacuated absorption vessel. The burette was filled with the gas to be used. Then 20 cc. of petroleum were run into the burette, driving out the same amount of gas. The whole apparatus was kept in a large water bath. The volume of absorbed gas divided by the volume of petroleum gave the absorption coefficient B of the gas. Their results at 20°C and 10°C are given in the following table.

| Gas | Temperature | B. or Ab. Coeff. Average |
|---|---|---|
| $H_2$ | 20°C | .0582 |
| " | 10°C | .0652 |
| $N_2$ | 20°C | .117 |
| " | 10°C | .135 |
| $O_2$ | 20°C | .202 |
| " | 10°C | .229 |
| $NO_2$ | 20°C | 2.11 |
| " | 10°C | 2.49 |
| $CO_2$ | 20°C | 1.17 |
| " | 10°C | 1.31 |
| CO | 20°C | .123 |
| " | 10°C | .134 |
| Ethylene | 20°C | .142 |
| | 10°C | .164 |
| Methane | 20°C | .131 |
| | 10°C | .144 |

The next table shows the comparison with water at 20°C., as well as the ratio between the values obtained at 20°C and 10°C. This ratio is fairly constant and shows that the amounts of gases absorbed at different temperatures by petroleum are proportional one to another.

| Gas | 20°C | 10°C | Ratio | $H_2O$ at 20°C. |
|---|---|---|---|---|
| $H_2$ | .0582 | .0652 | 1.12 | .0193 |
| $N_2$ | .117 | .135 | 1.15 | .0140 |
| $O_2$ | .202 | .229 | 1.13 | .0284 |
| $NO_2$ | 2.11 | 2.49 | 1.18 | .670 |
| $CO_2$ | 1.17 | 1.31 | 1.12 | .901 |
| CO | .123 | .134 | 1.09 | .0231 |
| Ethylene | .142 | .164 | 1.15 | .149 |
| Methane | .131 | .114 | 1.10 | .0350 |

According to these workers oxygen is more than seven times as soluble in petroleum as it is in water. From the results obtained they seem justified in concluding that a layer of petroleum would not protect aqueous fluids from the action of the air. In all cases with the exception of ethylene the absorption coefficient for petroleum was greater than for water. Vernon (27) worked on the solubility of air in fats and its relation to caisson disease. The fats used were olive oil, cod liver oil and lard. The solubility of gases in olive oil and cod liver oil was measured at 15°C and 37°C, while that in lard was measured at 45°C. At room temperature oil was shaken violently with air in a bottle for several minutes and then allowed to stand until all the air bubbles had risen to the surface. It was then weighed and about 40 to 50 grams were sucked up into the vacuous flask of a Geisslers mercury pump. This flask contained 70 to 100 cc. of .5% sulfuric acid which had previously

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(27) Vernon, Proc. Royal Society, 79, B., 366.

been boiled for an hour so as to get rid of all traces of air. The mixture of oil and water was then boiled for half an hour, liberating practically all the gas in the first few minutes. The gas was then pumped off and analyzed in a Haldanes gas analysis apparatus. The oil was boiled with dilute acid in order to get all of the carbon dioxide, both combined and in solution. The results obtained are given below.

100 cc. olive oil contain in cc.

| Gas | 15°C | | | Average | | 37°C | | Average |
|---|---|---|---|---|---|---|---|---|
| $O_2$ | 2.20 | 2.23 | 2.42 | 2.28 | 2.33 | 2.36 | 2.30 | 2.33 |
| $N_2$ | 5.23 | 5.30 | 5.27 | 5.26 | 5.19 | 5.23 | 5.15 | 5.19 |
| $CO_2$ | .19 | .24 | .16 | .20 | .17 | .13 | .18 | .16 |

100 cc. cod liver oil contain in cc.

| Gas | 15°C | | | Average | | 37°C | | Average |
|---|---|---|---|---|---|---|---|---|
| $O_2$ | 2.34 | 2.31 | 2.22 | 2.29 | 2.21 | 2.22 | 2.22 | 2.22 |
| $N_2$ | 4.95 | 5.15 | 5.07 | 5.06 | 5.05 | 5.10 | 5.08 | 5.08 |
| $CO_2$ | .19 | .25 | .19 | .21 | .18 | .24 | .20 | .21 |

Considering only mean values, the solubility at 37°C is within the limits of experimental error the same as the solubility at 15°C. The same is true for the solubility of lard at 45°C. Nitrogen varies slightly, but oxygen is practically constant thruout, as can be seen from the following figures.

100 cc. Lard at 45°C contain in cc.

| Gas | | | | Mean |
|---|---|---|---|---|
| $O_2$ | 2.33 | 2.40 | 2.35 | 2.33 |
| $N_2$ | 5.05 | 5.09 | 5.18 | 5.11 |
| $CO_2$ | .12 | .12 | .15 | .13 |

The proportion of unsaturated acids present in a fat seems to have little or no influence upon its solvent powers for oxygen or nitrogen. At 15°C oxygen is 3.1 and nitrogen 3.7 times more soluble in oil than in water. At 37°C oxygen is 4.5 and nitrogen 5.3 times more soluble in oil than in water.

The results of Vernon (27) and Guiewosz and Wolfisz (9) show that oxygen is more soluble in oil than in water. Still anaerobes will grow under a layer of oil when they will not grow under ordinary aerobic conditions. Larson (1) suggested that the oil lowered the surface tension and as a result the surface tension lowering substances were distributed thru out the medium where they became available for the metabolism of the organism. Under normal conditions these substances are collected at the surface, where, because of the high oxygen tension they are unavailable for the metabolic requirements of the anaerobe. This would explain the fact that B. Tetani grew well under otherwise ordinary aerobic conditions, if the tension were sufficiently lowered.

A simple experiment was performed to test the rate of absorption of oxygen thru a layer of oil. A series of test tubes were set up and into these 10 cc. of normal sodium hydroxide were pipetted. Two tubes were left as controls and the others were covered with layers of oil of varying thicknesses. Then thru the oil, 2 cc. of an aqueous solution of pyrogallol were carefully introduced with a pipette drawn out to a capillary tube, with ~~every~~ nut force to insure thoro mixing, but without carrying down any bubbles of air. A darkening ring indicated the absorption of oxygen and the width of the ring was measured from time to time, in centimeters. The oils used were paraffin oil, olive oil and castor oil. Paraffin oil did not react with sodium hydroxide and gave a clean sharp interface. Olive oil and castor oil showed a marked turbidity at the interface. All of the tubes showed an initial darkening ring immediately, evidently due to the absorption by the alkaline pyrogallol of the oxygen from the layer of oil in immediate contact with the pyrogallol. The further widening of the ring was slow. Evidently, although oil does absorb more oxygen than water, the rate of diffusion of the oxygen thru the oil is very slow. The controls always showed complete darkening within twelve hours and some much sooner. In the case of paraffin oil, the thickness of the layer of oil had no effect on the rate of absorption of oxygen. With olive oil and castor oil, the thinner the layer of oil the more rapid was the absorption of oxygen. This can be seen from the following tables.

Paraffin Oil

| Tubes | Oil in Cm. | 1 hr. | 4 hr. | 10 hr. | 25 hr. | 48 hr. | 124 hr. |
|---|---|---|---|---|---|---|---|
| 1 | 4.0 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 2 | 3.0 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 3 | 2.4 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 4 | 2.2 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 5 | 1.5 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 6 | 1.4 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 7 | .9 | .2 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 8 | .4 | .25 | .5 | .6 | 1.2 | 2.2 | 3.3 |
| 9 | 0 | 3.0 | Com. | -- | -- | -- | -- |
| 10 | 0 | 6.2 | Com. | -- | -- | -- | -- |

Olive Oil

| Tubes | Oil in Cm. | 6 hr. | 19 hr. | 30 hr. | 45 hr. | 68 hr. | 144 hr. |
|---|---|---|---|---|---|---|---|
| 1 | 3.2 | Trace | Trace | Trace | Trace | Trace | .5 |
| 2 | 2.6 | " | " | " | " | " | .7 |
| 3 | 2.3 | " | " | " | " | " | .7 |
| 4 | 1.6 | " | " | " | " | .8 | 1.25 |
| 5 | 1.2 | " | .3 | .4 | .6 | 1.0 | 2.0 |
| 6 | .8 | " | .5 | .6 | 1.25 | 1.4 | 2.9 |
| 7 | .7 | " | .6 | .65 | 1.3 | 1.5 | 2.9 |
| 8 | .4 | " | 1.0 | 1.2 | 1.8 | 2.0 | 3.0 |
| 9 | .2 | .5 | 1.2 | 1.7 | 2.1 | 2.5 | 4.0 |
| 10 | 0 | 6.3 | Com | -- | -- | -- | -- |
| 11 | 0 | 5.8 | Com | -- | -- | -- | -- |
| 12 | 0 | 6.5 | Com | -- | -- | -- | -- |

Castor Oil.

| Tubes | Oil in Cm. | 12 min. | 23 hr. | 124 hr. |
|---|---|---|---|---|
| 1 | 4.0 | Trace | Trace | Trace |
| 2 | 3.35 | " | " | " |
| 3 | 2.2 | " | " | " |
| 4 | 1.6 | " | " | " |
| 5 | 1.2 | " | " | .5 |
| 6 | .6 | " | " | 2.9 |
| 7 | 0 | 5.0 | Complete | -- |

The results show that the rate of diffusion thru olive oil and castor oil must be exceptionally slow. That thru paraffin oil is much more rapid. If a thick enough layer of castor oil or olive oil is used oxygen can be kept out for some time. It enters eventually but is very preceptibly retarded by a layer of oil. This period of retardation is more than sufficient for the growth of anaerobes. They can live in a liberal supply of oxygen but do not multiply rapidly. They probably reach their highest rate of growth long before oxygen has penetrated the entire tube. Paraffin oil, while not holding back the oxygen as efficiently as olive oil or castor oil, does prevent its entrance for a length of time amply sufficient for the development of anaerobes. Protection then of aqueous liquids, from the action of the air, by a layer of oil, is not as illusory as it would seem to be, when the relative absorption coefficients of air and oil are compared, due to the very slow rate of diffusion of gases thru oil. A very satisfactory state of anaerobiosis then can be produced by boiling the medium, cooling it quickly and covering with a layer of sterile oil.

The third point in this discussion is the effect on oxygen content of media of the addition of a piece of sterile tissue. An attempt was made to answer the question of how sterile tissue in culture media made possible the growth of anaerobes. Was oxygen actually absorbed by the tissue and if so, to what extent? Tissue was taken from a freshly killed guinea pig and boiled for from 2 to 5 minutes. This coagulated the blood and the proteins and made the pieces firm and easy to handle. They were then washed in running water until all the small particles were carried away. The tissues used included lung, fat, muscle, kidney, liver and heart.

Calibrated bottles holding 132 cc. water at 20°C were filled from a carboy, allowing about three times the volume of water to overflow the bottle, thus assuring no bubbles, and an even concentration of oxygen. Two determinations were made, one using practically oxygen saturated water and the other, air saturated water. After a set of fourteen bottles had been filled, three were picked at random for controls, and one of these was closed immediately, one during the middle of the operation and one at the end. The pieces of tissue were then dropped into the bottles and the bottles closed and put in a water bath at 19°C. The carboys were saturated at 20°C and by keeping the bottles at 19°C no bubbles of oxygen formed in the bottles. They were then left for 20 hours and at the end of that time, the tissue was removed and the bottles were analyzed by the Winkler method, using 2 cc. of manganous chloride solution, 2 cc. sodium hydroxide plus potassium iodide solution and 5 cc. of concentrated hydrochloric acid. The iodine was titrated with sodium thiosulfate solution, using arrowroot starch paste as the indicator. The results are given in the following table.

Oxygen consumed per Gm. wgt. in cc. Thio in 20 hours.

| Tissue | Oxygen Contration | Oxygen Consumed. |
|---|---|---|
| Heart | High | 1.52 |
| " | Low | 1.40 |
| Lung | High | 1.16 |
| " | Low | 1.11 |
| Kidney | High | 1.219 |
| " | Low | 1.29 |
| Liver | High | 1.003 |
| Muscle | Low | .50 |
| Fat | Low | .54 |

The results show that a considerable amount of oxygen is absorbed by the tissue and that practically the same amount is taken from a high concentration of oxygen as from a low concentration. The heart tissue seems to be most efficient in consuming oxygen and the muscular tissue of the thigh the least efficient. Liver peptone then is an excellent medium for the growth of anaerobes, since the liver not only supplies nutritive material for the metabolism of the organism but should assist in lowering the oxygen tension of the medium.

## Summary

The change in bacterial growth effected by the lowering of the surface tension of the medium is not due to an increased oxygen supply.

The addition of soap, which lessens the surface tension of a medium, does not increase its ability to absorb oxygen.

The ability of a solvent to absorb gases is not directly related to the surface tension, the vapour pressure or the specific gravity of the solvent.

While oils do absorb more oxygen than water, due to the slow rate of diffusion of oxygen thru oils, aqueous solutions can be kept practically oxygen free, by a layer of oil, for some time.

Sterile tissue does absorb some oxygen and thus assists in producing a state of anaerobiosis.