

Journals (*continued*).

Meteorologische Zeitschrift. July to December, 1888. Small folio.
Berlin. Oesterreichische Gesellschaft für Meteorologie.

Morskoi Sbornik. 1888. Nos. 6-11. 8vo. *St. Petersburg.*

Compass Observatory, Cronstadt.

Nature. July to December, 1888. Roy. 8vo. *London.*

The Editor.

New York Medical Journal. July to December, 1888. 4to. *New York.*

The Editor.

Notes and Queries. July to December, 1888. 4to. *London.*

The Editor.

The Sun's Surface as observed by James Nasmyth, June 5th, 1864.
 Photographed from the original drawing. Mr. Nasmyth.

January 24, 1889.

Professor G. G. STOKES, D.C.L., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read:—

- I. "On the Influence of Carbonic Anhydride and other Gases on the Development of Micro-organisms." By PERCY F. FRANKLAND, Ph.D., B.Sc. (Lond.), F.I.C., Assoc. Roy. Sch. of Mines, Professor of Chemistry in University College, Dundee. Communicated by Professor T. E. THORPE, Ph.D., F.R.S. Received December 18, 1888.

In consequence of a paper which has appeared in the last number of the '*Zeitschrift für Hygiene*,' by Dr. Carl Fränkel, entitled "Ueber die Einwirkung der Kohlensäure auf die Lebensthätigkeit der Mikroorganismen," I have been led to publish the results of some preliminary experiments on the same subject which I made in the spring of 1886, but which, owing to my attention being at that time devoted to investigations in other directions, I was obliged to put on one side. Although the methods which I adopted in my experiments are essentially different from those which Fränkel has employed, yet the results, so far as they can be compared with his, are on the whole concordant.

In my experiments I used the ordinary methods of plate-cultivation (Esmarch's important modification having not yet been published), the plate-cultivations of the various micro-organisms being then submitted to an atmosphere of different gases in the following manner:—A suitable attenuation of a particular micro-organism was employed, and gelatine plates were poured in the usual way; the different plates, resting one above the other on small glass stages, were placed in a flat porcelain dish and covered over with a glass bell-jar. Mercury was then poured into the dish, thus forming an effectual seal, and sterilised water was poured on to the surface of the mercury. The weight of the bell-jar causes it to sink to a certain depth into the mercury, so that the damp-chamber is in reality cut off from the external air by the mercury, and not by the sterilised water. A piece of sterilised india-rubber tubing is then introduced beneath the mercury, and a current of any particular gas can be passed into the chamber, the excess of gas escaping at the edge of the bell-jar through the mercury and water.

After the air has been driven out of the chamber in this manner, and replaced by any given gas, the tubing is removed, and the dish is kept at the requisite temperature, which in my experiments was about 20° C.

The particular micro-organisms which I used in these experiments were (1) the *Bacillus pyocyaneus*, (2) *Koch's Comma Spirillum*, (3) *Finkler's Comma Spirillum*, which were procured from the Hygienic Institute in Berlin. The different organisms were obtained in a suitable degree of attenuation by mixing them with sterilised water, from which a definite quantity was taken and gelatine plates poured.

In each experiment one plate was placed in a damp-chamber containing ordinary air, whilst a second was exposed in a similar chamber filled with the particular gas under examination. After the lapse of an adequate period of time admitting of their development, the colonies were counted in both cases and the results compared.

I. *Experiments with Hydrogen.*

The hydrogen was generated in a Kipp's apparatus by the action of dilute sulphuric acid on zinc; it was purified by passing it through a saturated solution of caustic soda, and was then conveyed through a sterilised piece of india-rubber tubing and a sterile plug of cotton-wool into the damp-chamber containing the inoculated gelatine plates. The following results were obtained in the use of this gas:—

(a.) With *B. pyocyaneus* (Green Pus).

1st Experiment (March 4th, 1886).

	Air-plates (after 2 days).	H-plates (after 4 days).
Number of colonies from { (a.) 22,412 }	22,500	11,500
1 c.c. of the mixture.. { (b.) 22,651 }		

The appearance presented by the plates developed in the hydrogen-chamber and those developed in air was very different. On the former the colonies were decidedly larger, less sharply defined, fainter in colour, and of more radiated structure than those on the air-plates.

2nd Experiment (March 11th, 1886).

	Air-plates (after 5 days).	H-plates (after 7 days).
Number of colonies from { (a.) 15,515 }	17,200	{ (a'.) 12,365 }
1 c.c. of the mixture.. { (b.) 18,950 }		{ (b'.) 12,262 }

The hydrogen-plates again showed the characteristic appearances mentioned above, many of the surface colonies having reached a diameter of 1 cm.

3rd Experiment (March 29th, 1886).

	Air-plates (after 4 days).	H-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture.....	6124	5600

In this case again the hydrogen-plates had the same characteristic appearance, the colonies on the surface being surrounded by a complete circular zone which exceeded by many diameters the original size of the colony.

From these experiments it is seen that the development of the *Bacillus pyocyaneus* is only slightly affected in an atmosphere of hydrogen; the colonies, however, grow more slowly and present a distinctly different appearance.

(b.) With Koch's *Comma Spirillum*.

1st Experiment (March 15th, 1886).

	Air-plates.	H-plates (after 7 days).
Number of colonies from { (a.) 4183 (after 4 days)		{ (a'.) 6767
1 c.c. of the mixture .. { (b.) 4440 (after 5 days)		{ (b'.) 8260

The colonies on the hydrogen-plates were smaller than those on the

air-plates, and they did not exhibit the characteristic depression on the surface of the gelatine.

2nd Experiment (March 29th, 1886).

	Air-plates (after 4 days).	H-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture.....	100	110

The vitality of Koch's comma spirillum is therefore in no way affected by exposure to an atmosphere of hydrogen, although its development into colonies is considerably retarded.

(c.) With Finkler's *Spirillum*.

1st Experiment (March 15th, 1886).

	Air-plates (after 4 days).	H-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture.....	12,107	6726

The colonies on the hydrogen-plates had the appearance of small milky dots, and caused in many cases a depression on the surface of the gelatine; they resembled, in fact, very young colonies on an ordinary plate culture of these spirilla.

In an atmosphere of hydrogen it would appear that of the three organisms with which I have experimented Koch's comma spirilla were the least prejudicially affected in their vitality.

II. *Experiments with Carbonic Anhydride.*

The gas was prepared in a Kipp's apparatus by the action of dilute hydrochloric acid on marble, and purified by passing it first through a saturated solution of carbonate of soda and then through a sterilised plug of cotton-wool.

The same three micro-organisms were submitted to experiment in the manner previously described.

(a.) With the *B. pyocyaneus*.

1st Experiment (March 4th, 1886).

	Air-plates (after 2 days).	CO ₂ -plates (after 9 days).
Number of colonies from 1 c.c. { of the mixture..... {	22,412 22,651	} 0*

*This plate was then placed in a damp-chamber in an atmosphere of air, and after seven days 2023 colonies were found.

2nd Experiment (March 11th, 1886).

	Air-plates (after 5 days).	CO ₂ -plates (after 8 days).
Number of colonies from 1 c.c. {	(a.) 15,515	(a'.) 0*
of the mixture..... {	(b.) 18,950	(b'.) 0

*On being transferred to a damp-chamber filled with air, there were after three days—

(a') 1288 colonies.

(b') 1150 „

In an atmosphere of carbonic anhydride *B. pyocyaneus* is thus not only prevented from multiplying, but the greater proportion of the bacilli present are destroyed in the course of a few days.

(b.) With Koch's *Comma Spirilla* (March 11th, 1886).

	Air-plates (after 4 days)	CO ₂ -plates (after 8 days).
Number of colonies from {	(a.) 4183 (after 4 days)	(a'.) 0*
1 c.c. of the mixture .. {	(b.) 4440 (after 5 days)	(b'.) 0

*These plates were then transferred to a damp-chamber filled with air, and examined after three days, but no colonies were found.

(c.) With Finkler's *Spirilla* (March 11th, 1886).

	Air-plates (after 4 days).	CO ₂ -plates (after 8 days).
Number of colonies from 1 c.c. {	12,107	{ (a'.) 0*
of the mixture		{ (b'.) 0

*These plates were then transferred to a damp-chamber filled with air, and re-examined after three days, but no colonies were found.

The deleterious effect of carbonic anhydride on the vitality of these organisms is, therefore, far more intense in the case of the Koch and Finkler spirilla than in that of the *Bacillus pyocyaneus*, for not only can no colonies develop in the atmosphere of CO₂, but the spirilla are either destroyed or so weakened during eight days' exposure to this gas that even on being transferred to an ordinary air-chamber no colonies are developed.

III. *Experiments with Carbonic Oxide.*

This gas was prepared from potassium ferrocyanide and strong sulphuric acid, and purified by passing it through a saturated solution of caustic soda and then through a small tower filled with slaked lime, and finally through a plug of sterilised cotton-wool.

The following experiments were made in the manner previously described with the three micro-organisms mentioned :—

(a.) With *B. pyocyaneus*.

1st Experiment (March 19th, 1886).

	Air-plates (after 4 days).	CO-plates (after 8 days).
Number of colonies from 1 c.c. of the mixture.....	{ (a.) 28,952 (b.) 27,794	(a.) 0* (b.) 0

*After three days' exposure to air, there were found on examination—

(a') 20,558 colonies.

(b') 16,142 „

2nd Experiment (March 29th, 1886).

	Air-plates (after 4 days).	CO-plates (after 9 days).
Number of colonies from 1 c.c. of the mixture.....	6124	467*

*After five days' exposure to air, 6333 colonies were found.

3rd Experiment (April 10th, 1886).

	Air-plates (after 4 days).	CO-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture.....	113,978	0*

*In this experiment a dish with pyrogallic acid and caustic potash was placed in the damp-chamber, in order to remove any trace of free oxygen which might be present. After four days' subsequent exposure to air, 100,821 colonies were found.

From the above experiments, it is evident that carbonic oxide exerts a very powerful influence on the vitality of *B. pyocyaneus*, for it effectually stops their development, but that this is only a temporary check to their growth is shown by the fact that on being removed to a damp-chamber containing air, almost the same number of colonies made their appearance as were found in the first instance on the air-exposed plates. The results of the 2nd experiment suggest that in this case there were possibly traces of air still remaining in the chamber.

(b.) With Koch's *Comma Spirilla*.

1st Experiment (March 29th, 1886).

	Air-plates (after 4 days).	CO-plates (after 9 days).
Number of colonies from 1 c.c. of the mixture.....	100	48*

*After five days' exposure to the air, the number of colonies rose to 76.

2nd Experiment (April 10th, 1886).

	Air-plates (after 4 days).	CO-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture.....	$\left\{ \begin{array}{l} (a.) \ 2,800 \\ (b.) \ 52,020 \\ (c.) \ 52,470 \end{array} \right\}$	$\left\{ \begin{array}{l} (a'.) \ 809 \\ (b'.) \ 19,494^* \end{array} \right\}$

*In these experiments pyrogallic acid was employed. The plates were exposed afterwards during four days to the air, but on subsequent examination the number of colonies was not found to have increased.

(c.) With Finkler's *Spirilla* (April 10th, 1886).

	Air-plates (after 3 days).	CO-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture	$\left\{ \begin{array}{l} (a.) \ 4574 \\ (b.) \ 4320 \end{array} \right\}$	2*

*In this experiment pyrogallic acid was employed. After four days' exposure to the air, the number of colonies rose to 501.

In the carbonic oxide atmosphere, therefore, only a fraction of Koch's comma spirilla, and a still smaller fraction of Finkler's spirilla are developed; the subsequent growth on exposure to the air is relatively small, and in the case of Koch's comma spirilla practically *nil*.

IV. *Experiments with Nitrous Oxide, Nitric Oxide, Sulphuretted Hydrogen, and Sulphurous Anhydride.*

Similar experiments were made with these gases. Those plates which were exposed to an atmosphere of nitric oxide, sulphuretted hydrogen, or sulphurous anhydride developed no colonies, neither were any found on subsequently placing the plates in air-chambers. These three micro-organisms are, therefore, rapidly destroyed by the action of these gases.

In the experiments with nitric oxide, the air was first driven out of the damp-chamber with hydrogen in order to prevent the formation of nitrous acid.

The organisms behaved, however, differently in the presence of nitrous oxide; in the chambers which were filled with this gas, and in which pyrogallic acid was also present, the *Bacillus pyocyaneus* developed no colonies, but afterwards on being placed in an air-chamber, almost as many colonies were found as were present in the original control air-plates.

Under similar circumstances, Koch's comma spirilla developed in an atmosphere of nitrous oxide nearly one-third of the colonies

found on the control air-plates, and on being transferred to the air-chamber a further though slight increase was found on re-examination.

In the case of the Finkler spirilla, about one-seventh of the total number of colonies were developed, and on being transferred to the air-chamber a further increase was observed, being about one-fifth of the total number which had grown on the control air-plate.

These results are tabulated below.

Experiments with Nitrous Oxide.

This gas was prepared by heating ammonium nitrate in a retort, and purified by passing it through a small tower filled with slaked lime, also through strong sulphuric acid and sterilised cotton-wool. In all the experiments, a dish containing pyrogallic acid and caustic potash was placed in the damp-chamber.

(a.) With *B. pyocyaneus* (April 10th, 1886).

	Air-plate (after 4 days).	N ₂ O-plate (after 7 days).
Number of colonies from 1 c.c. of the mixture	113,978	0*

*On being transferred to an air-chamber, there were found after four days 89,368 colonies.

(b.) With Koch's *Comma Spirilla* (April 10th, 1886).

	Air-plates (after 4 days).	N ₂ O-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture	$\left\{ \begin{array}{l} (a.) \quad 2,800 \\ (b.) \quad 52,020 \\ (c.) \quad 52,470 \end{array} \right\}$	$\left\{ \begin{array}{l} (a'.) \quad 903* \\ (b'.) \quad 17,496 \end{array} \right\}$

*On being placed in an air-chamber no further colonies were developed on (a') plate, whilst on (b') after four days the number had risen to 23,328.

(c.) With Finkler's *Spirilla* (April 10th, 1886).

	Air-plates (after 3 days).	N ₂ O-plates (after 7 days).
Number of colonies from 1 c.c. of the mixture	$\left\{ \begin{array}{l} (a.) \quad 4574 \\ (b.) \quad 4320 \end{array} \right\}$	649*

*On being transferred to an air-chamber there were found, after two days, 816 colonies.

Nitrous oxide acts, therefore, upon these three micro-organisms much in the same manner as carbonic oxide.

Remarks.

From the above series of experiments, it is at once apparent that the four different gases act very differently towards micro-organisms. Of the four gases employed, hydrogen, carbonic oxide, nitrous oxide, and carbonic anhydride, hydrogen had the least deleterious effect upon those microbes with which I experimented, whilst carbonic anhydride had the most destructive influence. There is, therefore, no longer any doubt, as indeed Liborius and C. Fränkel have already pointed out, that in the anaërobic culture of micro-organisms hydrogen is by far the most suitable medium for the expulsion of air, whilst carbonic anhydride, owing to its markedly deleterious effect upon many forms of bacteria, is not only ill suited, but is in many cases quite unfit for such a purpose.

And although there is no doubt, as Buchner asserts, that all those bacteria which give rise to fermentations attended with an abundant evolution of carbonic anhydride, must also be capable of flourishing in an atmosphere of this gas, yet it by no means follows that these organisms attain their full vitality in such an atmosphere. On the contrary, it is very possible that their anaërobic and fermenting powers only reach their maximum degree of activity when the gaseous products to which they give rise are removed either by a really indifferent gas, such as hydrogen, or by a vacuum.

The results of some experiments on the fermentative activity of yeast by Boussingault ('Compt. Rend.,' vol. 91, p. 37) support this view, for they show that in such a vacuum alcoholic fermentation takes place more actively, and is more quickly completed than at the ordinary pressure of the atmosphere.

As regards the particular behaviour of these three micro-organisms towards carbonic anhydride, the results of my experiments agree almost entirely with those of Fränkel. In both series of experiments it was found that the growth of *B. pyocyaneus* was entirely suspended by the action of this gas, but that on subsequent exposure to air the growth, attended with the formation of the characteristic pigment, commenced.

Again, in both series of experiments, it was observed that carbonic anhydride completely arrested the growth both of Koch's comma spirillum and Finkler's spirillum, but whilst C. Fränkel always succeeded on subsequent exposure to the air in obtaining a growth, although a very feeble one, in my experiments no such secondary growth was observed.

This discrepancy may, however, very possibly arise from the difference in the power of resistance which is often observed in the same

organism in different cultures. Of particular interest is the fact, which is brought out in the quantitative results of the experiments made by both of us, that there is a great variation in the power of resistance possessed by the individual organisms in an ordinary cultivation, and that conditions which exert a rapidly destructive influence on the majority of the microbes, leave the more hardy individuals of the same culture unaffected.

I have already had occasion* to notice a similar result in experiments on the introduction of Koch's comma spirilla and *B. pyocyaneus* into drinking water; in these experiments it was repeatedly observed that the greater proportion of the organisms which were inoculated into the water rapidly died off, whilst a small proportion survived much longer, and, in fact, subsequently exhibited multiplication.

II. "The Spinal Curvature in an Aboriginal Australian." By D. J. CUNNINGHAM, M.D., Trinity College, Dublin. Communicated by Sir W. TURNER, Knt., F.R.S. Received January 14, 1889.

(Abstract.)

1. The lumbo-vertebral index gives no information as to the character and degree of the lumbar curve of the vertebral column. If it did so, we might assume that in the native Australian the lumbar region of the spine was curved so as to present a concavity to the front.

2. To estimate the extent and the degree of the different curves of the column it is necessary to examine fresh spines in which both the vertebral bodies and intervertebral disks may be studied in conjunction with each other.

3. In the spine of the native Australian (described in the extended paper) the secondary curves (*i.e.*, the cervical and the lumbar curves) are strongly accentuated, whilst the primary curves (*i.e.*, dorsal and sacral) are not so marked. In these particulars the Australian spine resembles somewhat the spine of a Chimpanzee.

4. The points of inflexion of the axial curvature of the vertebral column, in the case of the cervico-dorsal transition and the dorso-lumbar transition, are placed differently in the Australian from the corresponding points in the European female and the Chimpanzee.

5. In the European the sacral curve is cut off in the most decided manner from the lumbar curve: not so in the Australian. In the latter the first sacral vertebra just escapes being included in the lumbar curve, and the importance of this is centred in the fact that

* "On the Multiplication of Micro-organisms." 'Roy. Soc. Proc.,' vol. 40, 1886, p. 543.