THE CHANGES PRODUCED BY THE GROWTH OF BACTERIA IN THE MOLECULAR CONCENTRATION AND ELECTRICAL CONDUCTIVITY OF CULTURE MEDIA.*

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PLATES V–VIII.

By determining from time to time the freezing point of a liquid in which bacteria are growing it is possible to estimate the changes that take place in the molecular concentration.†

By determining the electrical conductivity a measure of the number of dissociated ions in unit volume of the solution may be obtained. The amount by which the freezing point is depressed below the freezing point of distilled water is directly proportional to the molecular concentration when the dissolved substances are incapable of hydrolytic dissociation, like cane sugar or proteids for instance. In the case of dissociable substances, like sodium chloride, the same is true when we reckon each ion as a molecule. The relation between the electrical conductivity and the concentration of the electrolytes is not so simple, since the specific conductivity of a solution depends not only on the number of dissociated molecules in a given volume, but also on the velocities of the ions. But there is always a relation, and when this can be established for a particular case, it can be used to calculate the concentration. For example, when only one electrolyte is present, the concentration can be at once determined from the electrical conductivity by interpolating values in a table showing the relation between the conductivity of the substance and the amount of it in solution. If more than one electrolyte be present, the electrolytes may be such as have approximately equal ionic veloc-

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† The molecular concentration may be defined as the number of gramme-molecules of the dissolved substance in a litre of the solution.

ities. In this case the electrical conductivity can be taken as directly proportional to the total concentration of the electrolytes. If more than one electrolyte is present and the ionic velocities are widely different, then a quantitative determination of one or more of the electrolytes may be made by chemical methods, and the concentration of those that remain be deduced from the electrical conductivity. And even in complex solutions, when without actually knowing the ionic velocities we know the limits between which they must lie, we can often determine from the electrical conductivity whether the number of dissociated molecules in a given volume is increasing or diminishing, and, within certain limits of accuracy, by how much it is increasing or diminishing. This calculation can be controlled and the limits of error narrowed by taking account of the total molecular concentration (including that of the dissociated ions) deduced from observations on the freezing point. For instance, if we found that certain bacteria growing in solution of albumin caused a diminution in the freezing point without any change in the electrical conductivity, this would indicate that the large albumin molecules were being broken down into smaller, but as yet non-dissociable, molecules, possibly into peptones. If, later on, we found that while the molecular concentration, as indicated by the lowering of the freezing point, was still increasing, the electrical conductivity also began to increase, we should have to conclude that in the course of the decomposition the point had now been reached where some of the comparatively small molecules were at length dissociable. If the molecular concentration and the electrical conductivity were found both to increase from the beginning of the bacterial growth, it would be proved that the decomposition even at the beginning went as far as the production of the dissociable electrolytic molecules.

With the view of obtaining some preliminary notion of the magnitude of the changes which were to be expected, I made a series of experiments on the growth of ordinary putrefactive bacteria in blood and serum, and of pure cultures of certain bacteria in solutions of Witte's "peptone." The freezing-point determinations were made by means of a modification of Beckmann's apparatus, and the electrical resistance was measured by the telephone method of Kohlrausch. It

will be seen from the subjoined specimens of the results that very striking changes are observed both in the total molecular concentration and in the electrical conductivity, which, upon the whole, run a parallel course. Thus, in Experiment I, dog's defibrinated blood and serum were allowed to putrefy for 6 weeks in a bath at a temperature of 37° to 39° C. The electrical conductivity of the blood in that time was increased more than eleven-fold, and the freezing point was depressed in proportion. There was a similar change in the serum, although the maximum was much less. Curves plotted from the results of this experiment are shown in Plate V.

In Experiment II, three specimens of blood taken at the same time from the carotid artery of a dog were allowed to putrefy first at the air temperature, then at 35° C., and then at 40° to 43° C. Of course in all the experiments evaporation was prevented. The course of the decomposition is shown in the curves of Plate VI, which represent the electrical conductivity. It is abruptly hastened by raising the temperature to 35° C. Ultimately the conductivity reaches a maximum and then remains practically constant for an indefinite period. At the time when the maximum was reached the blood was seen to be crowded with bacteria which had formed spores. It is to be presumed that at this point the conditions had become unfavorable for the growth of the bacteria, owing to the increase of osmotic pressure due to the accumulation of the products of their activity or to some specific toxic action of these products and the using up of the nutritive material. A specimen of the blood at this stage was diluted with its own volume of water so as to diminish the concentration of the waste-products, and a renewed period of growth, indicated by a steadily mounting electrical conductivity, was again observed. It may seem strange that bacteria should be able to grow at all in a liquid whose osmotic pressure is 8 or 10 times as great as that of normal blood-serum or the cell-sap of ordinary vegetable cells. But Wladimiroff * has shown that the osmotic pressure in the interior of bacteria is extraordinarily great, more than 3 times that of the cells of the higher animals. And if the bacterial cell-wall is freely permeable to any of

* *Zeitsch. f. physikalische Chemie*, vii, 529.

the decomposition products, as, for example, the red blood corpuscles are to urea, these products, although they would diminish the freezing point would not exert any osmotic pressure on the cell-wall.

Experiments III and IV show the result of observations on the growth of B. subtilis, B. proteus Zenkeri, B. proteus vulgaris, B. Friedländer, B. coli communis, Bacillus of hog-cholera, and B. lactis aërogenes. The cultures were all grown in a solution of Witte's peptone. Plates VII and VIII represent in graphic form the results of Experiment III.

As to the nature of the electrolytes produced by the bacteria, we know already numerous bodies, especially among the decomposition products of putrefying proteids, which are capable of acting as ions, for example, such organic kations as the amines and ammonium, and such anions as the acids of the fatty series.

I shall not further discuss these results at present. They are enough to show that in such observations we have a practical method of measuring the amount of decomposition produced by, and therefore the rate of growth of, bacteria. It is possible that sufficiently great and constant differences might be observed between different kinds of bacteria when grown in the same or in different liquid media to enable us to use the method as a supplement to our present means of diagnosing between nearly related forms. And for this purpose, since the curves of concentration and conductivity run practically parallel to each other, it may be that measurements of the electrical conductivity alone might suffice. These would have the great advantage over measurements of the freezing point that they could be carried out in tubes furnished with platinum electrodes fused through the glass, or perhaps passing through the plug, and the necessity of opening the tubes would thus be obviated.

It is hoped that further observations, now in progress, may cast light upon this and some of the other interesting questions arising out of this research.

Tabulated Results.

The conductivity (λ) is given in reciprocal ohms $\times 10^8$ and reduced to 5° C. In the column headed Δ the lowering of the freezing point is given in degrees centigrade.

EXPERIMENT I.—March 30, 1898. PUTREFACTION.

		Δ.	$\lambda_{(5°)} \times 10^8$.				Δ.	$\lambda_{(5°)} \times 10^8$.	
Mar. 30.	Dog's defibrinated blood (fr.). (Sp. gr. 1057.0; ash 1.544%.)	.628	33.80	Air temperature.	Mar. 30.	Serum from clot of same blood. (Sp. gr. 1022.3; ash 0.925%.)	.628	82.44	
Apr. 2.			34.16	" "					
" 6.			31.41	" "					
" 11.			28.35	" "	Apr. 2.				Put in bath at 37°–39° C.
Mar. 30.	Same dog's defib. blood (fresh).	.628	33.80	" "	" 5.		.759		
Apr. 2.			34.16	" "	" 6.		.834	97.99	
" 5.		.632		Now put in bath at 37°–39° C.	" 8.		.832	104.24	
" 6.			55.47		" 11.		1.308	150.63	
" 8.		1.271	81.77	At 2.30 p. m.	" 16.		2.229	220.43	
" 8.			83.25	At 5.25 p. m.	" 25.		2.652	232.57	
" 11.		2.043	130.00		May 15.		2.814	277.05	
" 13.		2.784	154.10						
" 16.		3.506	202.97						
" 25.		7.417	337.10						
" 27.			342.57						
May 15.		8.1 (?)	371.15						
Apr. 25.	The defibrinated blood which had been kept in bath + 1 volume water.	3.072	232.57						
May 15.	" "	4.234	335.76	Kept in bath since Apr. 25.					
" 15.	The defibrinated blood which had been kept in bath + 3 volumes water.	1.974	218.53						

EXPERIMENT II.

Jan. 4, 1898.

PUTREFACTION. DOG'S DEFIBRINATED BLOOD. THREE SAMPLES, A, B AND C, FROM THE SAME DOG. A AND B IN STERILE FLASKS; C, NOT.

	$\lambda_{(5°)} \times 10^8$. A.	$\lambda_{(5°)} \times 10^8$. B.	$\lambda_{(5°)} \times 10^8$. C.	
Jan. 4.			42.82	
" 9.	56.58		37.61	A was opened for the first time to-day, and is laked.
" 11.	60.21		45.02	A and C are both completely laked.
" 13.	65.67	63.66	48.32	B was opened for the first time to-day, and is completely laked.
" 15.	75.44	66.09	58.01	
" 20.	90.21	79.96	68.88	
" 28.	101.81	93.12	77.28	
Feb. 1.	126.75			A was put in bath at 30°–36° C. 30 hours before this measurement.
" 4.	128.68	99.41	82.71	A, B and C were put in bath at 35° C. to-day.
" 5.	139.64		90.37	
" 7.	183.70	143.47	102.80	Temperature of bath now raised to 40°–43° C.
" 8.	232.57	162.50	115.75	
" 9.	274.05	178.52	124.45	
" 10.	308.02	214.47	147.65	
" 16.	359.58	314.13	232.57	
" 24.	356.04			
" 25.	359.58	381.78	318.87	
" 26.	363.70	390.00		
" 28.	364.23	393.02	338.00	
Mar. 2.	360.09	397.96	343.03	
" 3.		403.02	345.37	
" 7.	359.07	397.96	347.26	
" 11.	349.65			
" 22.		399.85	340.27	
Apr. 27.	382.93	396.09	343.03	

From Jan. 4 to Jan. 31, A, B and C were all kept at room temperature. When A and B were first opened (on Jan. 9 and 13, respectively) neither was found completely sterile. A rod-shaped bacterium was present in both, but there was no putrid odor, although this was very marked in C. After being opened A and B were thoroughly exposed to the air and allowed to putrefy like C.

EXPERIMENT III.

A STERILE SOLUTION OF WITTE'S PEPTONE WAS PREPARED. ON APRIL 11, 1898, 6 TUBES WERE INOCULATED WITH BACILLUS SUBTILIS AND 4 TUBES WITH BACILLUS PROTEUS ZENKERI.

		Δ.	$\lambda_{(5°)} \times 10^8$.	
1898. Apr. 5.	Sterile peptone solution.	.124	13.68	Ash .0876 grm. / Solids 4.3052 grm. } in 100 cc.
" 11.	Sterile peptone solution (after further sterilization in steam sterilizer.)	.101	11.25	
" 11. 1 p. m.	Inoculated 6 test-tubes with B. subtilis and 4 with B. proteus Zenkeri.			
" 13. 4 p. m.	B. subtilis No. 1.	.104	12.12	At 35° C. since inoculation. Considerable growth.
	B. proteus Zenkeri No. 1.	.114	11.53	At 35° C. Less growth.
" 16.	B. subtilis No. 2.	.121	13.65	At 35° C. for 3 days; then room temperature since.
	B. proteus Zenkeri No. 4.	.104	12.27	" "
" 27.	B. subtilis No. 3.	.152	16.42	In bath at 40° C. since April 20.
May 15.	B. subtilis No. 4.	.571	51.57	In bath at 40° C. since April 27.
" 15.	B. proteus Zenkeri No. 3.	.134	13.37	At room temperature.
" 15.	B. proteus Zenkeri No. 2.	.125	12.56	At room temperature. Very little growth.
" 15.	B. subtilis No. 5.	.231	21.91	At room temperature since inoculation. Fair growth.
" 15.	B. subtilis No. 6.	.219	19.03	At room temperature since inoculation. Fair growth.
Apr. 13.	B. subtilis No. 1.	.104	12.12	Now exposed to air and put in bath at 40° C.
" 16.	B. subtilis No. 1.	.589	65.57	Far more growth than in unopened tubes.
" 25.	B. subtilis No. 1.	1.267	144.45	
May 15.	B. subtilis No. 1.	1.482	171.05	
" 15.	B. subtilis No. 3.	1.197	142.98	Exposed to air on April 27. In bath at 40° C. since.
Apr. 13	B. proteus Zenkeri No. 1.	.114	11.53	Exposed to air after opening and put in bath at 40° C.
" 16.	B. proteus Zenkeri No. 1.	.530	59.97	
" 25.	B. proteus Zenkeri No. 1.	1.093	132.86	

EXPERIMENT IV.—April 18, 1898, 1 P. M.

TWO TUBES OF THE SAME SOLUTION OF WITTE'S PEPTONE WERE INOCULATED WITH CULTURES OF THE FOLLOWING BACTERIA. ALL WERE PUT IN THERMOSTAT AT 35° C., EXCEPT B. SUBTILIS AND B. PROTEUS VULGARIS, WHICH WERE LEFT AT ROOM TEMPERATURE (10°–15° C.). BUT ON APRIL 27 B. SUBTILIS WAS PUT IN BATH AT 40° AND KEPT THERE TILL MAY 15.

	B. subtilis.		B. proteus vulgaris.		B. Friedländer.		B. coli communis.		B. of hog-cholera.		B. lactis aërogenes.	
	Δ.	$\lambda_{(5°)}\times10^8$.	Δ.	$\lambda_{(5°)}\times10^8$.	Δ.	$\lambda_{(5°)}\times10^8$.	Δ.	$\lambda_{(5°)}\times10^8$.	Δ.	$\lambda_{(5°)}\times10^8$.	Δ.	$\lambda_{(5°)}\times10^8$.
Apr. 22, 2.35 p.m.					.154	17.95	.181	16.98	.142	17.40	.181	20.76
Apr. 27, 11.50 a.m.			.156	20.15								
May 12.					.207	20.05	.187	19.69	.167	16.52	.246	25.59
May 15.	.318	36.31	1.343*	158.52								

Control peptone tube in thermostat till May 12, when $\Delta = .148$ and $\lambda_{(5°)} \times 10^8 = 16.10$.

* Test-tube opened on April 27 and left in bath at 40° C. with ordinary cork till May 15.

DESCRIPTION OF PLATES V-VIII.

PLATE V.

Curves plotted from the results of Experiment I to show the changes in the molecular concentration of putrefying blood and serum.

FB, freezing point of blood; *FS* of serum; *CB*, conductivity of blood; *CS* of serum. *A* shows the course of the curve of conductivity in the defibrinated blood kept at air temperature. On April 2 the blood and serum were put in a bath, the temperature of which varied between 37° and 39°.

PLATE VI.

Curves plotted from the results of Experiment II to show the changes in the electrical conductivity of putrefying blood.

A, *B*, *C*, curves of three samples of blood taken at the same time from the carotid of a dog.

a shows the point at which (on Jan. 28) *A* was put into bath at 30°-36° *C*; *b*, the point at which (on Feb. 1) it was taken out of the bath. The dotted line passing through the three curves and labelled "Bath" shows the point where (on Feb. 4) *A*, *B* and *C* were put into bath at 35°, where they were afterwards kept. *I* is a curve from a specimen of blood from another dog, the numerical results of which are not quoted.

PLATE VII.

Shows the changes in the conductivity of the contents of five tubes (Nos. 1, 3, 4, 5 and 6) inoculated with Bacillus subtilis on April 11. The curves are plotted from the results of Experiment III.

PLATE VIII.

Shows the changes in the freezing point of the contents of the same tubes.

On April 13, No. 1 was freely exposed to the air and then put in a bath at 40°, corked tightly with an ordinary cork.

On April 27, No. 3 was treated in the same way.

No. 4 was put in the bath at 40° on April 27 and kept there till May 15, when it was opened.

Nos. 5 and 6 were kept at the room temperature.

The curves of conductivity (Plate VII) and molecular concentration, it will be seen, run nearly parallel to each other.