The Enzymes of B. coli communis. Part V.—(a) *Anaerobic Growth followed by Anaerobic and Aerobic Fermentation.* (b) *The Effects of Aeration during the Fermentation.*

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(Communicated by Prof. F. G. Hopkins, F.R.S.—Received February 1, 1921.)

In the present communications the effect of oxygen upon the fermentation of glucose and upon the growth of the bacteria, in so far as this affects fermentation, is considered. To this end the organisms have been grown both aerobically and anaerobically, and subsequently made to ferment glucose, both aerobically and anaerobically, with the object of comparing the products of decomposition in the two cases. There are clearly two problems: firstly, the effect of exposure to oxygen during growth upon the subsequent fermentation, whether aerobic or anaerobic, and, secondly, the effect of oxygen admitted during the fermentation. The first question relates to the part played by oxygen in the formation of enzymes, the second to the part played by oxygen in their action on carbohydrates. The first question is considered, though in but a preliminary way, in Section A, the second, more fully, in Section B.

Section A.

Object of the Experiments.

Two results were aimed at in these experiments. Firstly, to compare the products of fermentation of glucose anaerobically, after anaerobic growth, with the products of fermentation anaerobically after previous growth aerobically. And, secondly, to obtain information as to the effect of introducing oxygen during the fermentation itself. This latter consideration, however, though brought to notice by these experiments, is considered only incidentally here because it forms the subject of Section B. In the present section we wish to direct attention particularly to those differences which exist between the fermentation after anaerobic and aerobic growth, not upon the effect of aeration during the fermentation. To point out the difference which previous growth aerobically or anaerobically has made, several analyses from previous experiments are included in Table IV side by side with the completely anaerobic experiments of Tables I, II, and III.

Technique.

Growth of the Organisms anaerobically prior to Fermentation.

Winchester quarts containing agar were inoculated with *B. coli communis* which had been grown anaerobically on broth in an atmosphere of hydrogen. The procedure was to sterilise the whole apparatus, consisting of Winchester quart containing agar and side tube containing broth. The apparatus was cooled in nitrogen, and when cold the side tubes containing broth were inoculated. The bottles were placed side by side in a large incubator and connected in series; hydrogen was then passed through the whole series, and in this way the broth in the side tube of each bottle was anaerobic during the growth of the bacteria. Strict anaerobiosis was maintained by exhausting at the pump from time to time and allowing fresh hydrogen to enter. After seventy-eight hours the broth of the side tubes was allowed to run into the bottles and the growth on agar continued for forty-eight hours. To remove the bacterial growth, a vacuum was created in the bottles and saline solution, such as has been used in all previous experiments of this series, was allowed to enter. The saline emulsion of bacteria was removed to the fermentation flask by previously exhausting the latter of air and allowing the hydrogen to enter the Winchester quarts. The hydrogen was subsequently pumped out of the fermentation flasks by boiling the contained fluids *in vacuo*, and nitrogen was admitted. The fermentation was allowed to proceed anaerobically, so that the whole of the operation of growth of the bacteria and of fermentation by them of the sugar was carried out with rigorous exclusion of air. The operations were found exceedingly difficult.

As regards the fermentations themselves, the technique was that described in Part III of this series, with the addition of an arrangement for regulating the admission of oxygen or air at a measured rate where aerobic fermentations were desired.

Experiment I.—The bacteria were grown anaerobically on agar and approximately equal portions of bacterial emulsion were added to each of three flasks containing sugar, water, and some chalk. The fermentation was allowed to continue forty-eight hours at 37° C. Experiment N was completely anaerobic, A received two litres of air, O two litres of oxygen during the fermentation.

The analysis of the fermentation products gave the following results, expressed as percentages upon the sugar employed:—

Table I.—Fermentation of Glucose by *B. coli communis* grown anaerobically. The fermentations carried out in Nitrogen (N), Air (A), and Oxygen (O), respectively.

Products.	Products as percentages of glucose employed.		
	N.	A.	O.
Hydrogen	0·61	0·50	0·40
Carbon dioxide	27·33	24·17	20·00
Formic acid	16·68	12·33	0·08
Acetic acid	8·67	9·50	18·15
Succinic acid	9·83	10·33	12·33
Lactic acid	Nil	12·00	8·83
Alcohol	7·33	8·66	9·00
Total	70·45	77·49	68·19

It will be seen that there is a gradual diminution of CO_2 production with increasing oxygenation, and a still greater diminution of formic acid. The effect of admitting oxygen during the fermentation, however, will be discussed more fully in Section B of this communication.

Most striking of all is the absence of lactic acid from the completely anaerobic fermentation, and the reappearance of this acid with aeration. It is possible that the missing 30 per cent. of glucose has in part been converted into some mother substance of lactic acid. In Part III of this series an experiment is described in which samples of the fermentation fluid were removed for analysis every twelve hours. At the end of the first period of twelve hours which had been one of rapid death of the bacteria introduced, no lactic acid was found, in the second twelve hours although the glucose disappeared as evidenced by Fehling's Solution, the products recovered only represented a small fraction of the glucose consumed, but in the third period, from twenty-four to forty-eight hours, all the missing glucose reappeared as lactic acid. It would thus seem that the transformation of glucose into some non-reducing carbohydrate is a preliminary reaction to lactic acid formation, and this probably accounts for what has happened in Experiment I.

The results of Table I are represented pictorially in fig. 1A.

Experiment II.—The bacteria were grown anaerobically as before. In B the fermentation was completely anaerobic while in C air was admitted.

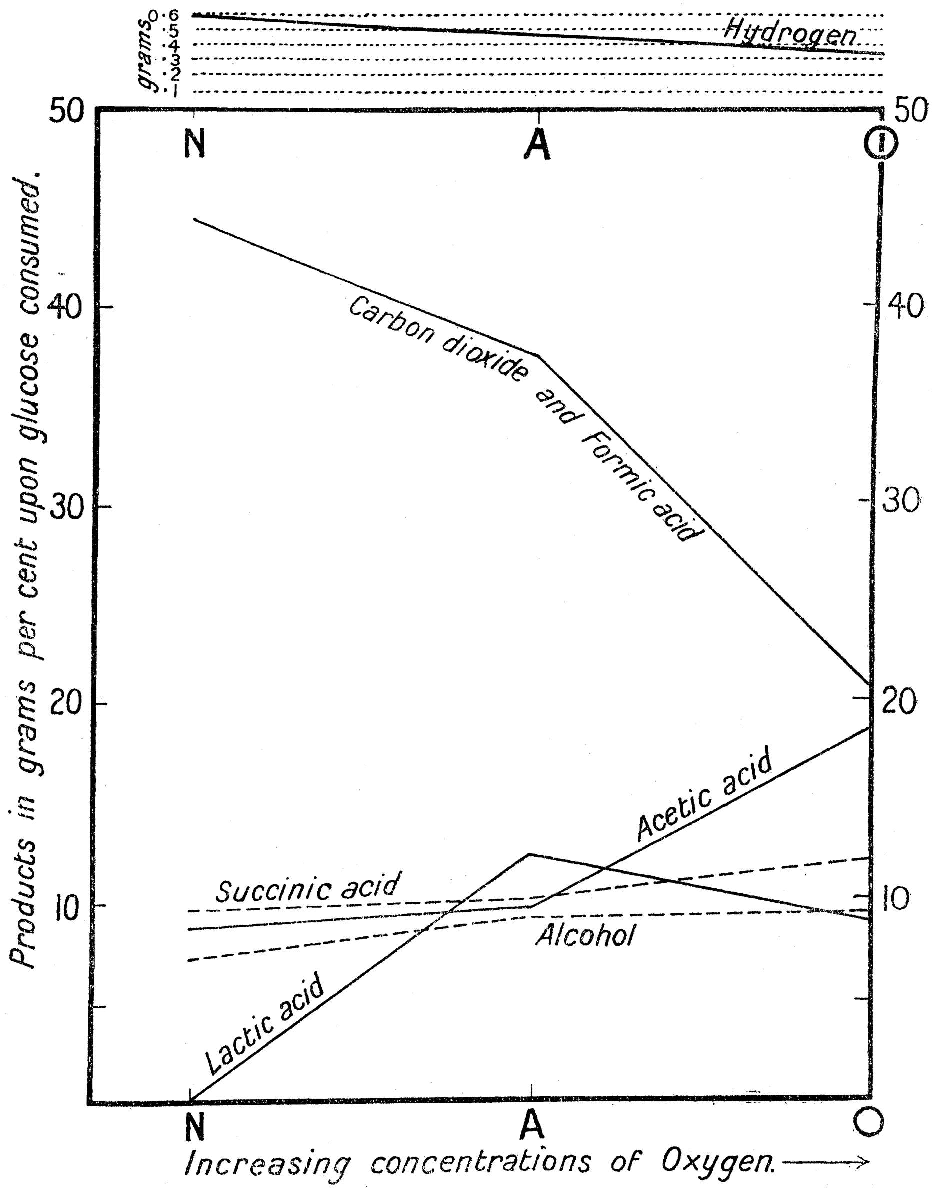

Fig. 1a.—Effect of oxygen upon fermentation of glucose by *B. coli communis*, grown anaerobically; fermentation carried out in nitrogen (N), air (A), and oxygen (O).

In this case some lactic acid has been formed, but when it is recollected that normally this bacterium produces from 20 to 30 per cent. or more of lactic acid it will be seen that the previous anaerobic growth has led to a very marked diminution in lactic acid production. It is to be noted also that

succinic acid has been markedly diminished, even more so than lactic acid and in the place of both acetic acid appears.

Table II.—Fermentation Anaerobic (B) and Aerobic (C) after Anaerobic Growth.

Products.	Products expressed as percentages of the glucose consumed.	
	B.	C
Hydrogen	Not estimated.	Not estimated.
Carbon dioxide	27·30	26·42
Formic acid	12·64	4·96
Acetic acid	34·38	44·76
Lactic acid	8·44	8·20
Succinic acid	0·58	2·46
Alcohol	15·66	12·36
Total	99·00	97·16

In Experiment II a very large amount of bacteria was introduced and the aeration during the fermentation seems to have made but little difference. A third experiment was made similar to the previous one but with less emulsion of bacteria. D was fermented anaerobically; E received a little air at the beginning of the fermentation.

Table III.—Fermentation Anaerobic (D) and Aerobic (E) after Anaerobic Growth.

Products.	Percentages of products upon the glucose consumed.	
	D.	E.
Hydrogen	0·36	0·18
Carbon dioxide	37·32	23·03
Formic acid	8·75	5·18
Acetic acid	41·07	57·68
Lactic acid	1·25	2·68
Succinic acid	Nil	Nil
Alcohol	10·00	5·00
Total	98·75	93·75

Here again lactic acid is practically absent as in Experiment I, and succinic acid is completely absent, confirming Experiment II. Thus two striking phenomena are to be observed when the fermentation of glucose is carried out

by *B. coli* previously grown anaerobically; the one is the absence of lactic acid and the other the absence of succinic acid from the products of decomposition. In place of them acetic acid appears.

In order to make clearer the effect which aerobic and anaerobic growth has upon the subsequent fermenting power of the bacteria, Table IV is included wherein the anaerobic fermentations described in this communication are compared with typical anaerobic fermentations which have been carried out with bacteria grown aerobically as described in Parts II, III and IV of this series.

Table IV.—A Comparison of the Products of the Anaerobic Fermentation of Glucose by *B. coli*, grown previously Anaerobically and Aerobically.

Products.	Products as percentages of the glucose consumed.*						
	Bacteria grown aerobically.			Bacteria grown anaerobically.			
Hydrogen	0·33	0·21	0·35	0·61	—	0·36	0·18
Carbon dioxide	16·37	15·24	19·38	27·33	27·30	37·32	23·03
Formic acid	2·76	12·29	5·22	16·68	12·64	8·75	5·18
Acetic acid	19·34	22·02	18·56	8·67	34·38	41·07	57·68
Lactic acid	28·47	25·46	16·93	Nil	8·44	1·25	2·68
Succinic acid	20·32	11·23	17·36	9·83	0·58	Nil	Nil
Alcohol	11·04	13·36	20·06	7·33	15·66	10·00	5·00
Total	98·63	99·81	97·86	70·45	99·00	98·75	93·75

* Fermentations all anaerobic.

Note on the Relation of Oxygen to the Growth of B. coli communis.

The organism we have been using has frequently been described as a facultative anaerobe. It is easy to see that the growth is much more extensive on agar in air than in hydrogen. Under comparable conditions we obtained 70 mgrm. of *B. coli* (dry weight) after anaerobic growth on agar, and 280 mgrm. after aerobic growth. In the absence of oxygen, *B. coli* grows better in a fluid nitrogenous medium than on an agar surface. It has already been shown (Part III) that when an emulsion of bacteria is mixed with glucose solution the bacteria while carrying out a rapid fermentation of the glucose (1 grm. per hour per gramme of bacillus) are rapidly dying, and in this death process no lactic acid is formed. Subsequently a new generation of bacteria replaces the old and these give rise to lactic acid. *B. coli* would thus appear to have two distinct forms of metabolism. When the organisms have had an immediate past history of life in air they require oxygen for their continued existence in this form; if they must ferment anaerobically they die

rapidly in doing so, but some of the individuals adapt themselves to the anaerobic conditions and probably this means that they possess a mechanism for using combined oxygen. This is discussed later. The results described in this communication must be considered in the light of these remarks for doubtless the increase of lactic acid following admission of oxygen in Experiment I is to be referred to an increased vitality of the bacterial culture, *i.e.*, either the bacteria increased in numbers, or they took longer to die. On the other hand it may be that oxygen is necessary under certain circumstances for the action of the enzyme mechanism which produces lactic acid, but this seems unlikely for reasons stated in the discussion later.

Discussion of Results.

B. coli communis grows better in air than in an atmosphere of hydrogen and ferments glucose more vigorously anaerobically after growth in air than after anaerobic growth. The type of fermentation differs according as the immediate past history of the organism has been aerobic or anaerobic. In the latter case, no lactic acid or only a very slight yield is obtained, and also succinic acid tends to be absent.

The manner in which oxygen affects the fermenting powers of the bacteria subsequently is not obvious, but it seems likely that the oxygen has acted as a stimulus to reproduction, so that when subsequently fermentation takes place multiplication of cells occurs, whereas when this stimulus is absent, as in the anaerobic past history, the fermentation occurs without such reproduction. This view accords well with the observations recorded in Part III of this series where only during a period of cell multiplication was lactic acid produced.

Oxygen in the free state is not necessary for lactic acid production if the bacteria are allowed to multiply in a nitrogenous medium such as peptone, but in the case of bacteria grown previously anaerobically on agar the stimulus of oxygen seems necessary for the production of lactic acid. It is probable that the oxygen acts by increasing cell multiplication, but this has not been proved.

Summing up then, lactic acid production is associated with rapid multiplication of cells, and the cause of lactic acid increase when oxygen is admitted is probably due to stimulation of growth.

Death of the cells and conditions of depressed vitality are associated with the production of carbon dioxide and acetic acid or alcohol.

These results may possibly explain the phenomenon of increased growth of yeast due to preliminary aeration. Horace Brown put forward as an explanation the idea that oxygen was stored up, and subsequent reproduction

was proportional to this oxygen charge. But all evidence seems against the idea that any appreciable amount of oxygen is stored up. It is therefore suggested that the effect of oxygen is that of a stimulus to growth and ferment production, and the effect of such stimulus in modifying the subsequent fermentation has been seen in the experiments described in this communication. A little air introduced at the beginning can give rise to a very marked effect upon the whole subsequent course of the fermentation. In the last two experiments described in this communication, namely D and E, the conditions were practically the same, except that by accident air was momentarily admitted to E, there has followed a large increase in acetic acid and diminution of carbon dioxide.

Again it is frequently observed that fermentations which refuse to begin can be made to do so by the introduction of a little air. Pakes has also observed this with formic acid fermentations by various bacteria. Lately we have observed the same, both with formic acid fermentations and with glycerol fermentations.

Oxygen thus appears to be a stimulus to fermentation production by certain bacteria as by yeast. The effect long outlasts the stimulus and is not proportional in any chemical sense to it. This is probably the explanation of the oxygen charge of Horace Brown.

Conclusions of Section A.

Anaerobic fermentation of glucose by an emulsion of *B. coli communis* proceeds differently according as the organisms have been grown previously with or without oxygen.

When the immediate past history has been anaerobic, the fermentation under anaerobic conditions yields very little or no lactic acid and greatly diminishes succinic acid. In place of these, acetic acid appears in large proportion. Admission of oxygen during the fermentation leads to lactic acid production. The fact that acetic acid replaces succinic acid entirely gives additional proof of the close relationship of these two substances.

The results also confirm the conclusions of the earlier sections as to the independence of the lactic acid, acetic acid, and probably of the carbon dioxide fermentations.

Section B.

In this Section the effect of admitting oxygen during the fermentation is considered in more detail. Eight experiments are described. In the first four, a solution of peptone and glucose was fermented by the introduction of a small seeding of bacteria. In the second four, an emulsion of bacteria,

previously grown on agar, was employed. The eight experiments constitute four pairs, in each of which there is an aerobic and an anaerobic fermentation. In the former case, about 2 litres of air were admitted in forty-eight hours. About 5 grm. of glucose was fermented in each experiment. Further experiments, carried out with more efficient aeration, would be of interest.

Experimental.

In the first four analyses a solution of glucose and peptone was fermented. In A and B the glucose was present when the flasks were inoculated. In C and D the glucose was not added until twenty-four hours after the inoculation of the peptone.

Experiment I.—The solution contained:—peptone, 5 grm.; glucose, 5 grm.; saline,* 500 c.c.; chalk, 5 grm.

After seventy-two hours the glucose was found completely fermented. The results of the analyses are recorded in Table I.

Table I.—Products Expressed as Percentages of Glucose Consumed.

Products.	A (anaerobic).		B (aerobic).	
Hydrogen	0·23	35·70	0·13	24·51
Carbon dioxide	34·22		15·90	
Formic acid	1·25		8·48	
Acetic acid	8·91		17·57	
Lactic acid	10·68		37·57	
Succinic acid	3·94		4·73	
Alcohol	22·45		10·17	
Total	81·68		94·45	

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid, diminution of alcohol;† and to increase of lactic acid and increase of acetic acid.

Experiment II.—A second pair of fermentations was carried out at the same time, and with the same sowing of bacteria as in Experiment I; but the glucose was not added until twenty-four hours from the time of

* Saline solution contained 0·6 per cent. K_2SO_4 and 0·1 per cent. $MgSO_4$.

† It is probable that in this analysis it is not that the oxygen has diminished the alcohol, for 10 per cent. is quite an average yield under normal circumstances. Some other factor has led to an increase of alcohol in A above the normal. This large yield of alcohol is evidently to be correlated with the great diminution of formic acid, and it shows that alcohol is formed in relation to the utilisation of hydrogen.

inoculation. Other conditions were precisely the same as in the previous experiment.

Table II.—Products Expressed as Percentages of Glucose Consumed.

Products.	C (anaerobic).		D (aerobic).	
Hydrogen	0·69	39·74	0·23	18·98
Carbon dioxide	25·83		14·74	
Formic acid	13·22		4·01	
Acetic acid	11·95		43·39	
Lactic acid	19·52		11·69	
Succinic acid	5·61		5·64	
Alcohol	15·34		14·22	
Total	92·18		93·92	

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid; and to increase of acetic acid.

Experiment III.—The next four analyses represent fermentations of glucose by any emulsion of bacteria, previously grown aerobically on agar. E and F were fermented anaerobically, G and H aerobically.

The solutions contained:—glucose, 6 grm.; chalk, 5 grm.; saline, 400 c.c.; bacterial emulsion, 100 c.c.*

Table III.—Products Expressed as Percentages of Glucose Consumed.

Products.	E (anaerobic).	F (anaerobic).	G (aerobic).	H (aerobic).
Hydrogen	0·49	0·41	0·31	0·18
Carbon dioxide	16·19	17·28	6·55	13·12
Formic acid	10·74	11·01	7·02	5·57
Acetic acid	21·11	9·50	16·74	11·49
Lactic acid	23·16	20·33	25·24	30·23
Succinic acid	11·87	11·49	18·23	16·30
Alcohol	9·06	10·30	10·41	10·17
Total	92·62	90·32	84·50	87·06

These analyses show that the introduction of oxygen during the fermentation leads to:—diminution of hydrogen, diminution of carbon dioxide, diminution of formic acid; and to increase of lactic acid and increase of succinic acid.

* Emulsion contained 0·1 grm. bacteria (dry weight) and 0·3 grm. soluble organic matter.

In Table IV, these results are put together in order from left to right of decreasing values for CO_2 + formic acid. For the moment, we wish to direct attention merely to those changes which have resulted from the introduction of air during the fermentation; changes due to other factors we will discuss later.

Table IV.—Products of the Decomposition of Glucose by *B. coli communis* Expressed as Percentages of Glucose Consumed. Experiments arranged from left to right in order of decreasing values of (CO_2 + Formic Acid).

Products.	C.	A.	F.	E.	B.	D.	H.	G.
Hydrogen	0·69	0·23	0·41	0·49	0·13	0·23	0·18	0·31
Carbon dioxide	25·83	34·22	17·28	16·19	15·90	14·74	13·12	6·55
Formic acid	13·22	1·25	11·01	10·74	8·48	4·01	5·57	7·02
Acetic acid	11·95	8·91	19·50	21·11	17·57	43·39	11·49	16·74
Lactic acid	19·52	10·68	20·33	23·16	37·57	11·69	30·23	25·24
Succinic acid	5·61	3·94	11·49	11·87	4·73	5·64	16·30	18·23
Alcohol	15·36	22·45	10·30	9·06	10·17	14·22	10·17	10·41
Total	92·18	81·68	90·32	92·62	94·45	93·92	87·06	84·50

By arranging the products in the order as seen in Table IV, the first thing that is apparent is that all the anaerobic experiments fall on the left, and all the aerobic experiments on the right of the middle line, so that we may say that the most characteristic effect of aeration during fermentation is a diminution in formic acid and the gaseous products, CO_2 and H_2, which for many reasons we have been led to consider are derived from formic acid. Corresponding with this diminution in the products containing one carbon atom there is an increase either of a product with three C atoms (lactic acid) or of two C atoms or a multiple of two (acetic acid, succinic acid). The variations in the products are made clearer by representing the results pictorially as in fig. 1B.

The following points may be noted with regard to fig. 1B :—

(1) The experiments have been arranged in order of decreasing values of CO_2 + formic acid, and no consideration has been taken as to the conditions under which the various experiments were carried out. Nevertheless, it will be seen that all the anaerobic fermentations become grouped on the left and all the aerobic experiments on the right of the figure.

(2) The curve representing alcohol shows very little fluctuation in spite of the great changes in acetic acid, and clearly aeration has but little effect on the alcohol production, though it markedly increases the production of acetic acid.

(3) The two most variable products are lactic acid and acetic acid. And it is remarkable that whereas on the left-hand side of the figure these curves do not cross one another, on the right they do so very frequently. From this it is clear that there is much more constancy between the products derived from glucose by anaerobic than by aerobic fermentation. With aerobic fermentation it would appear that slight variation in the conditions may very greatly affect the resulting proportions between lactic and acetic acid. Bearing in mind what has already been stated in §3 of this series, that variation in the proportions of the products is often to be correlated

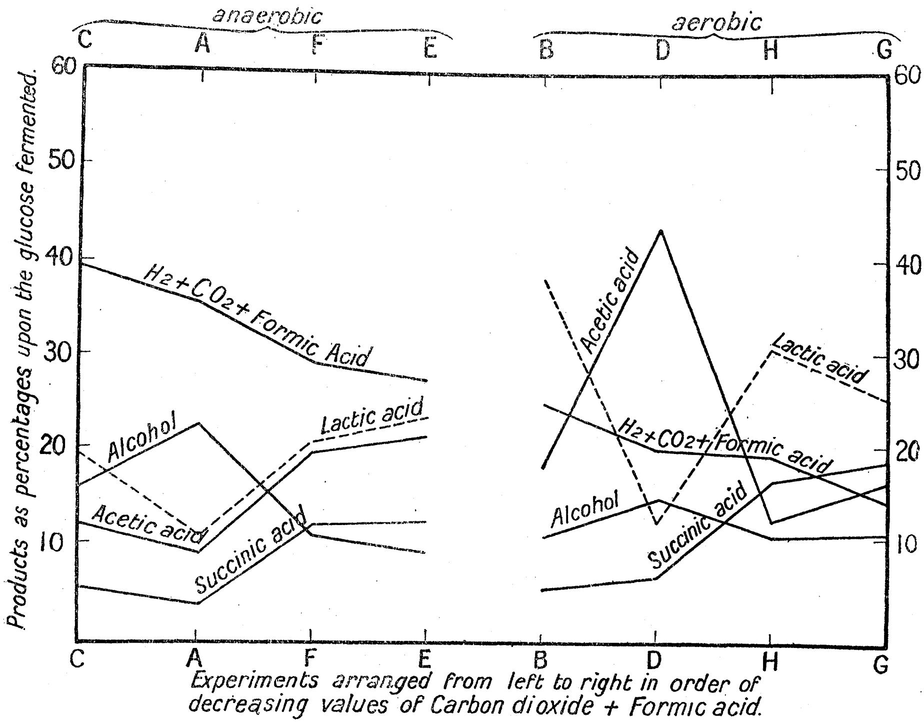

FIG. 1B.—Products of decomposition of glucose by *B. coli communis*.

with variation in the proportion between living and dead or dying cells, it would seem that the most probable explanation of this intercrossing of the curves on the right of the figure is that oxygen has altered this proportion between living and dying cells. And that either the oxygen has caused a multiplication of the cells added in the emulsion, or else at least that it has prevented them dying as rapidly as in the anaerobic fermentations. This is all equivalent to saying that the aerobic fermentations are more complicated by the factor of bacterial life than are the anaerobic fermentation.

(4) The figure shows fairly clearly that there are three main lines of

decomposition of the glucose, viz., into products of one, two and three C atoms respectively.

These three groups are primarily represented by formic acid, alcohol + acetic acid, and lactic acid. Secondary reactions may occur, giving rise to succinic acid, or, by an interaction of nascent H from Group 1 with Group 2, the proportion of alcohol to acetic acid may be raised.

In fig. 2 the products of the decomposition of glucose have been plotted as percentages upon the portion of the glucose which has not been turned into lactic acid. That is to say, the lactic acid in each case has been

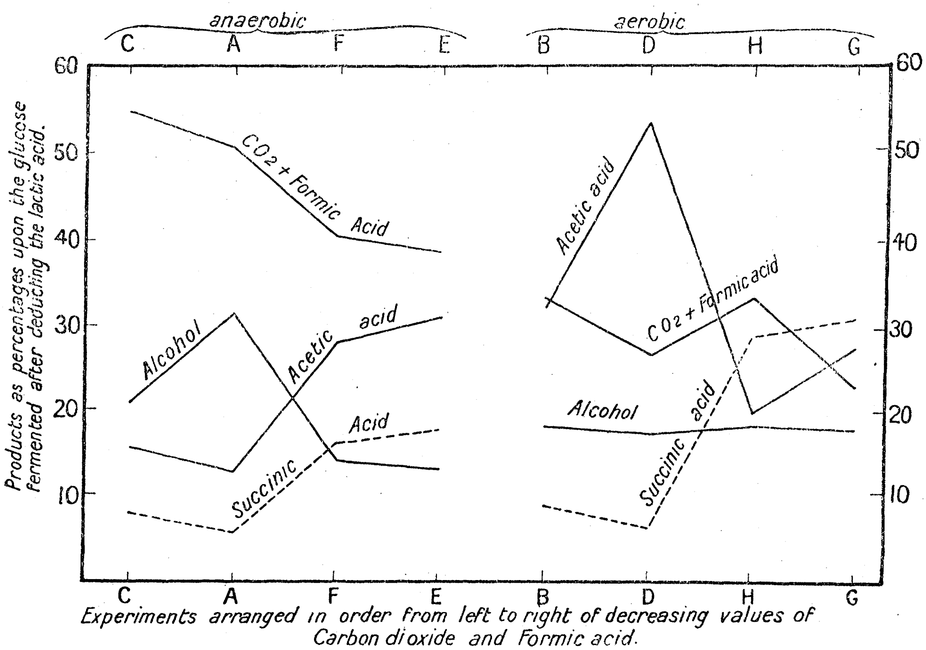

FIG. 2.—Products of decomposition of glucose by *B. coli communis*, after deducting portion changed to lactic acid.

deducted from the weight of glucose consumed, and the products have been recalculated as percentages upon the remainder. The object of this treatment is to do away with variations in the products which result merely secondarily from variations in the lactic acid. One of the immediate results of replotting the products in this manner is to draw attention to the constancy of alcohol under aerobic conditions. It will be seen that in the figures on the left representing anaerobic conditions alcohol shows considerable fluctuations and always in an opposite direction to acetic acid. This relationship has been observed frequently before and can only indicate that the two are derived from the same precursor. The mechanism by which the variations

occur depends upon the production of nascent hydrogen, either from formic acid or its precursor. It would appear, therefore, that the effect of oxygen is to inhibit the mechanism for the utilisation of nascent hydrogen. Clearly also, since the total formic acid CO_2 and H_2 is diminished, it is likely that there is less hydrogen available. These experiments would seem to prove conclusively, therefore, that a constant amount of alcohol is produced by the decomposition of *B. coli* on glucose by a fermentation in which nascent hydrogen plays no part, but that an increase in this alcohol production frequently occurs under circumstances where nascent hydrogen is operative. Likewise for acetic acid there is a tendency for a certain constant production in equimolecular proportion to the alcohol, but nascent hydrogen may diminish and oxygen increase its yield. If we try to explain the mechanism by which alcohol and acetic acid arise, we seem to be led again to the Cannizaro reaction.

Calculation of the Oxygen in the Products of Anaerobic and Aerobic Fermentation as compared to the Oxygen in the Glucose fermented.

It is surprising to find that if the oxygen is calculated for each of the products of the fermentation, more is found than corresponds to the glucose fermented, and corresponding with this gain in oxygen, there is a loss of carbon and slight loss of hydrogen. The gain of oxygen and loss of carbon is much more marked in the anaerobic than it is in the aerobic fermentation.

We give the results of two anaerobic and two aerobic experiments calculated out to show these facts in Table V. No account is taken of lactic acid and acetic acid, since these have the same empirical formula as glucose.

As explanation, we can only suggest that water has been added on in the reaction. Further work must be carried out to explain the facts with certainty.

It may be noted that this gain of oxygen and loss of carbon in the products analysed is not observed in the present analyses only, but in all the previous ones. The results given by Harden earlier show the same relationships, also in the cases we have calculated, and, as a matter of fact, in his first paper on this subject, Harden postulated water as taking part in the reactions and entering into the final products.

Conclusions to Section B.

The effect of introducing oxygen in the fermentation of glucose by *B. coli communis* is to increase the lactic, acetic, and succinic acids, and to diminish the hydrogen, carbon dioxide, and formic acid, but to leave the alcohol unchanged.

Table V.—Products of the Aerobic and Anaerobic Fermentation of Glucose by *B. coli communis*. Calculation of the elementary composition of the products to illustrate the gain of oxygen, especially marked in anaerobic fermentations.

Products.	Anaerobic.					
	C.	H.	O.	C.	H.	O.
Hydrogen	—	0·021	—	—	0·018	—
Carbon dioxide	0·189	—	0·503	0·208	—	0·553
Formic acid	0·120	0·020	0·319	0·127	0·021	0·337
Succinic acid	0·207	0·026	0·275	0·206	0·026	0·274
Alcohol	0·202	0·050	0·237	0·237	0·059	0·277
Total	0·718	0·117	1·222	0·778	0·124	1·441
Calculated for glucose	0·822	0·137	1·095	0·890	0·148	1·185
Calculated on basis of the carbon	0·718	0·120	0·056	0·778	0·130	1·037

Products.	Aerobic.					
	C.	H.	O.	C.	H.	O.
Hydrogen	—	0·014	—	—	0·008	—
Carbon dioxide	0·080	—	0·215	0·161	—	0·426
Formic acid	0·082	0·014	0·220	0·065	0·011	0·173
Succinic acid	0·334	0·042	0·445	0·297	0·037	0·395
Alcohol	0·245	0·061	0·163	0·238	0·059	0·158
Total	0·741	0·131	1·043	0·761	0·115	1·152
Calculated for glucose	0·766	0·127	1·022	0·811	0·135	1·080
Calculated on basis of the carbon	0·741	0·124	0·988	0·761	0·127	1·013

Under anaerobic conditions greater variations occur in the proportion of alcohol to acetic acid than under aerobic conditions, and it would appear that one of the effects of the introduction of oxygen during the fermentation is to inhibit the mechanism of auto-reduction, which is responsible for the variations in alcohol when such occur.

Contrary to expectation, the products of aerobic fermentation contain not more, but less, oxygen than the corresponding products of anaerobic fermentation of glucose; but there is a gain of oxygen in both cases upon the original glucose. If, as seems likely, this extra oxygen comes from the water,

then it would appear that one of the effects of the introduction of oxygen is to diminish the part played by water in the reactions.

In conclusion we wish to express our thanks to Prof. F. Gowland Hopkins, in whose laboratory this work was done.

Anthocyanins and Anthocyanidins. Part IV.—*Observations on: (a) Anthocyan Colours in Flowers, and (b) the Formation of Anthocyans in Plants.*

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(Communicated by Prof. F. Keeble, F.R.S.—Received February 2, 1921.)

In a recent paper, Shibata, Shibata and Kasiwagi,* dealing with the question of the colours observed in flowers, and the condition of the anthocyan pigments as they exist in plants, arrived at conclusions that differ considerably from those of Willstätter and of Everest. In view of this, and of the fact that Shibata, Shibata and Kasiwagi's paper has passed into reviews, the present authors feel that the publication of some observations that they have made, both before the publication of the paper referred to, and since receiving it, as confirmation of the present authors' results and extension of the field, will not be out of place.

Results of experiments upon the formation of anthocyan pigments are also described, and important conclusions drawn from them.

The Constitution of the Blue Anthocyan Pigments in Flowers.

Willstätter and Everest† as the result of their examination of the pigments of the cornflower and of preliminary investigations upon other flowers, concluded that the blue colour in the cornflower was due to an alkali, or alkali-earth, salt of a phenolic substance which was violet in the free state, and which was also capable of forming red oxonium salts with acids. This simple explanation of the main colour changes being due to changes in the condition of the cell sap in the plants concerned, has been elaborated by Willstätter and Mallison‡ to show how such a supposition, coupled with the

* 'J. Amer. Chem. Soc.,' vol. 41, p. 208 (1919).
† 'Ann.,' vol. 401, p. 189 (1913).
‡ 'Ann.,' vol. 408, p. 147 (1915).