

metals, when subjected to very low temperatures, which flowed from his early collaboration with Sir James Dewar in investigating this domain. In recent years he has taken a prominent part in the scientific development of telegraphy by free electric waves.

The Action of Bacillus lactis aërogenes on Glucose and Mannitol.
Part II.—*The Investigation of the 2 : 3 Butanediol and the Acetylmethylcarbinol Formed; the Effect of Free Oxygen on their Production; the Action of B. lactis aërogenes on Fructose.*

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(Communicated by Dr. A. Harden, F.R.S. Received October 22, 1910,—Read January 19, 1911.)

(From the Bio-Chemical Laboratory, Lister Institute.)

A preliminary communication on some of this work has been published.* The present paper deals with the experimental part of the investigation in question, and some new results are recorded. On examining the action of *B. lactis aërogenes* on glucose, it was found that the carbon content of the products isolated was not so great as that of the original glucose employed. A further examination disclosed that a quantity of a neutral liquid was present containing the amount of carbon needed to explain this discrepancy. As, in the preliminary communication, justification was shown for calling this liquid "crude butylene glycol," the use of this convenient name will be continued here. The object of this investigation was to determine as far as possible the nature of the constituents of this material, and the proportions in which they are found therein.

The Conditions of Cultivation of the Organism.

In all the earlier experiments the organism (*B. lactis aërogenes*) was grown anaërobically in a medium containing 1 per cent. of Witte peptone, 2 per cent. of pure glucose, and an excess of chalk. Later, other sugars were used, and sometimes, when using glucose, the percentage of sugar employed was raised to 5, in order to obtain larger yields of one particular product per

* Harden and Walpole, 'Roy. Soc. Proc.' B, 1906, vol. 77, pp. 399—405.

litre of medium treated. The flasks employed were of 1 litre capacity, and each was provided with a mercury trap which permitted the egress of the gases evolved. The fermentation was carried on under strictly anaërobic conditions, and the method of preparing the flasks was that employed by Harden.* While in the incubator they were agitated from time to time to distribute the chalk throughout the medium. The gas evolution was found to be vigorous during the first four days, but after two to four weeks ceased completely, and the quantity of unchanged sugar in the flask was very small indeed.

At first every flask removed was tested by plating on agar, but the practice was frequently omitted and, instead, a culture on sloped agar in a test-tube made and examined. The quantities of the various substances found are stated in the previous paper. In order to isolate the crude butylene glycol, the contents of the flask were first filtered from excess of chalk and then evaporated at 55° under diminished pressure to dryness, and the dry residue, consisting of calcium salts and peptone, extracted with alcohol. The alcohol extracts yielded on fractionation a colourless liquid, boiling at 181° to 183° (corr.) at 760 mm. pressure. The yield was very small, only amounting to about 1 gramme per litre of medium containing 20 grm. of glucose, but it was found possible to increase it by employing a medium containing 5 per cent. of glucose. In this way 8 grm. of the new substance containing 52·8 per cent. of carbon were obtained per litre of medium containing 50 grm. of sugar. This only accounts for about two-thirds of the missing carbon, and a rough estimate of the amount lost during the process of distillation and extraction was therefore made by dissolving 8 grm. of the material in 500 c.c. of a medium containing 5 grm. of Witte peptone, 6 grm. of calcium lactate, and 6·5 grm. of alcohol, and then extracting it in the manner described above. Only 5·2 grm. were recovered, the loss per 500 c.c. being therefore about 2·8 grm., and the loss per litre about 5·6 grm. This brings the total amount produced from 50 grm. of glucose to about 13·6 grm., slightly in excess of that required.

In one case a yield of 10·75 grm. was obtained from 1 litre of medium containing 5 per cent. of glucose. Generally, only 7 to 8 grm. of the "crude glycol" were obtained from each flask of 50 grm. of glucose, in spite of numerous attempts to improve the yield.

I. THE NATURE OF THE "CRUDE GLYCOL."

This substance boils at 181° to 183° (corr.) at ordinary pressure, and at 12 mm. the boiling point is from 83° to 84°·5 C.; it solidifies in the cold

* 'Jenner Inst. Trans.,' 1899, vol. 2, p. 126.

to a transparent mass, which melts indefinitely at about 28° , and shows to a marked degree the phenomena of super-cooling. It is slightly dextro-rotatory, the value for $[\alpha]_D$ for different preparations varying from $+0.46$ to $+0.71$. A sample of crude glycol gave the value $+1.6$ for $[\alpha]_D$ when dissolved to the proportion 22 per cent. in a saturated aqueous solution of borax, while in aqueous solution it was $+0.8$. Dried over quicklime no decomposition apparently takes place at once, though, after several months, discoloration, diminution of viscosity, and development of a peculiar smell occur.

Samples of the natural product gave the following figures on burning:—

	C.	H.
	per cent.	per cent.
I	52.41	10.80
II	52.68	10.92
III	52.62	10.92
IV	52.71	10.93

The figures corresponding to the formula $C_4H_{10}O_2$ are 53.33 for C and 11.11 for H. It will be seen that the presence of a trace of moisture will account for this discrepancy, which will be referred to later.

Desiccation of the "Crude Glycol."

In order to determine the percentage composition of the desiccated natural "glycol" a sample several times redistilled *in vacuo* was distilled on to quicklime, sealed up for several weeks, and then distilled off again at 12 mm. pressure. No difference in boiling point was observed between the four consecutive fractions of several cubic centimetres, each of which was collected and at once hermetically sealed.

Melting-point.—These were now all frozen and very slowly warmed in a large bath from 5° to 30° , the operation taking two hours and the bath being vigorously stirred the while.

Sample I.—At 21° it became pasty and began to liquefy and did so more rapidly than IV. *Sample II* was not further examined. *Sample III* remained solid above $28^{\circ}5$. *Sample IV* became pasty and began to liquefy at 21° , and completely liquefied at 28° .

It will be seen that the figures of Sample III, which had the highest melting point, correspond accurately to the formula $C_4H_{10}O_2$, but are considerably different from those of Nos. I and IV.

The value of $[\alpha]_D$ falls as the material distils.

Composition and Optical Activity of Desiccated "Crude Glycol."

No. of sample.	Wt. taken.	CO ₂ .	H ₂ O.	C.	H.	[α] _D in 35 per cent. aqueous solution.
I	0.1464	0.2836	0.1448	per cent. 52.83	per cent. 10.99	+1.41
III	0.2945	0.5770	0.2925	53.43	11.11	+1.21
IV	0.3190	0.6160	0.3164	52.66	11.09	
	0.3682	0.7147	0.3669	52.94	11.07	+0.92

Molecular Weight of the "Crude Glycol."—A molecular weight determination by Victor Meyer's method gave the value 80.6. The temperature employed was 215°, and 0.0435 gram. of glycol gave 12.6 c.c. of air at 15° and 776 mm. By Beckmann's freezing-point method the value 80 was obtained—

0.1386 gram. "glycol" in 22 c.c. aqueous solution gave $\Delta = 0.145$

0.2079 " " " " $\Delta = 0.307$

The formula weight of $C_4H_{10}O_2 = 90$.

Oxidation of the "Crude Glycol."

(1) *Oxidation in Aqueous Solution by Bromine in the Presence of Light. Formation of Diacetyl.*—To 0.9 gram. of crude glycol dissolved in 50 c.c. of water 3.25 gram. of bromine was added. The whole was well shaken from time to time and exposed to the light from an arc-lamp, care being taken not to allow the temperature of the mixture to rise to any considerable extent. After six hours a dilute aqueous solution of sulphur dioxide was added until the smell of bromine disappeared, and the mixture was saturated with sodium chloride. Distillation under a Young's still-head of 15 discs yielded some drops of a yellow liquid which possessed the characteristic smell of diacetyl and dissolved in excess of water.

Phenylosazone of Diacetyl.—This was prepared in order to confirm the identification of this product of the oxidation of "crude glycol." The solution of diacetyl obtained above was made up to 10 c.c. with water, 3 gram. of phenylhydrazine and 3 c.c. of 50 per cent. acetic acid added, and the whole kept at 100° for one hour. The phenylosazone deposited was recrystallised from alcohol and then melted at 241.5° to 243°.

0.1147 gram. gave 20.6 c.c. N (moist) at 18.5° and 765 mm.

Found N = 20.8 per cent. Calculated for $C_{16}H_{18}N_4$, N = 21.05 per cent.

The osotetrazone prepared from this by oxidation with potassium bichromate and acetic acid melted at 153°. The needles of this substance,

recrystallised from acetone, were flexible, well defined, violet in colour, of great lustre, and melted at 155° to 156° , thus agreeing in every respect with the compound described by Pechmann.*

0.2613 grm. gave 47.25 c.c. N_2 (moist) at 17° and 763. 8 mm.

Found N = 21 per cent. Calculated for $C_{16}H_{16}N_4$, N = 21.4 per cent.

On reduction with phenylhydrazine the osazone melting at 245° is immediately re-obtained. All these reactions were also obtained with a few grammes of diacetyl prepared from methylacetoacetic ester.

Dioxime from Diacetyl.—Two grammes of crude glycol were converted into diacetyl by the method described and after reaction with hydroxylamine hydrochloride, 30 per cent. of the amount of oxime theoretically obtainable was isolated in the pure condition. This melted at $135^{\circ}.5$ to $136^{\circ}.5$, thus agreeing with the dioxime prepared from diacetyl obtained from methylacetoacetic ester. It gave the well-known dimethylglyoxime reaction with small quantities of nickel.† The yield of diacetyl by oxidation of the crude glycol, taken in conjunction with the constancy of boiling point, percentage composition, and molecular weight determination, are conclusive evidence that the substance is nearly pure 2:3-butanediol. The further confirmation of this by actually separating the purified diol from its diphenylurethane is described later (p. 281).

(2) *Oxidation with Semi-normal and Normal Nitric Acid at 100° .*—Among the products of oxidation were found carbon dioxide, acetaldehyde, small quantities of acetylmethylcarbinol and diacetyl, acetic acid, and inactive lactic acid. The last two substances were identified by the composition of their barium and zinc salts respectively:—

Acetic Acid.—

3.535 grm. of barium salt gave 3.1479 grm. of $BaSO_4$.

Equivalent weight of acid is 60.5. Acetic acid, $C_2H_4O_2 = 60$.

Lactic Acid.—

0.4787 grm. crystallised zinc salt gave 0.3908 grm. anhydrous zinc salt and 0.1329 grm. ZnO.

0.1250 grm. crystallised zinc salt gave 0.3908 grm. anhydrous zinc salt and 0.0341 grm. ZnO.

$H_2O = 18.36$ per cent. $ZnO = 34.00$ per cent.

$Zn(C_3H_5O_3)_2 \cdot 3H_2O$ requires $H_2O = 18.15$: $ZnO = 33.45$ per cent.

No oxalate or tartrate was present.

* v. Pechmann, 'Ber.', 1888, vol. 21, p. 2755.

† Tsugaëff, 'Ber.', 1905, vol. 38, pp. 2520—2522.

(3) The crude glycol does not reduce Fehling's solution either in the cold or on heating, though it contains a constituent which reduces ammoniacal silver nitrate* in the cold. This may be removed without any apparent accompanying change to the "crude glycol" by boiling for eight hours with 30-per-cent. caustic soda solution. When the "crude glycol" was boiled at atmospheric pressure for seven hours in contact with air the liquid assumed a brownish tint, which did not increase with time, and $[\alpha]_D$ fell from +0.723 to +0.590. On boiling with animal charcoal in aqueous solution and recovering the glycol, the $[\alpha]_D$ was found to be 0.749. No reaction product could be obtained by heating an aqueous solution of "crude glycol" and phenylhydrazine acetate to 100° for one hour.

A mixture of 10 grm. glycol, 32 grm. water, and 8 grm. concentrated sulphuric acid boiled up for 10 hours apparently underwent no change, the material being recovered unaltered.

The above experiments, beyond showing the general chemical stability of the constituents of the "crude glycol," indicated that it was not a pure chemical substance, but did not provide any information as to the other substances accompanying the 2:3-butanediol therein.

Treatment of "Crude Glycol" with Phenylisocyanate, and Fractionation of the Resulting Phenylurethanes.

20.4 grm. of "crude glycol," 64 c.c. phenylisocyanate, 250 c.c. of pure dry ether were boiled on a water-bath for 12 hours till "bumping," due to deposition of crystals commenced. The mixture was then allowed to cool. The supernatant fluid was poured off, and, together with the dry ethereal washings of the crystals, boiled up again for an additional seven hours. This liquid was concentrated, and, on cooling, the mother liquors containing the excess of phenylisocyanate were removed, and the isocyanate decomposed by alcohol. In some later preparations the excess of phenylisocyanate, together with the most soluble phenylurethanes, was carried on to the next batch. Precautions were taken to prevent access of water during the performance of the above operations. The fractionation of the reaction-products was commenced by recrystallisation from alcohol. Four substances were obtained pure in this way. The mother liquors containing substances more soluble in alcohol were dissolved in benzene, and precipitated fractionally by adding light petroleum.

Altogether, about 105 grm. of "crude glycol" were treated with phenylisocyanate, corresponding to about 383 grm. of mixed phenylurethanes.

From alcohol there were obtained:—

* Tollens, 'Ber.,' vol. 15, p. 1635.

(1) 2:3-*Butyleneglycoldiphenylurethane*.—It is sparingly soluble in cold alcohol, ether and benzene, crystallises in rosettes of colourless needles and melts at 199°·5 (uncorr.). One hundred cubic centimetres of a solution in absolute alcohol saturated at 13° contain 1·02 gm. No evidence of optical activity was found in the pure material. Its hydrolysis will be described later (p. 280). It comprises about 90 per cent. of the whole amount of the reaction-product.

0·2783 gm. gave 0·6716 gm. CO₂ and 0·1557 gm. H₂O.

0·2725 gm. gave 20·2 c.c. N at 23°·5 and 766 mm.

	Found.	Calculated for C ₄ H ₁₀ O ₂ (C ₆ H ₅ CNO) ₂
C	65·79	65·85
H	6·21	6·09
N	8·57	8·53

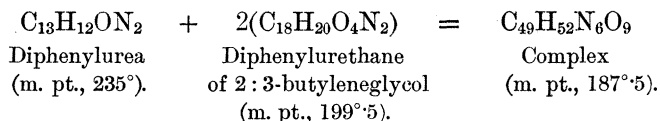
(2) 2:3-*Butyleneglycoldiphenylurethane and Diphenylurea in the Proportion* C₁₃H₁₂ON₂·2C₁₈H₂₀O₄N₂.—When the mother liquors from (1) were concentrated, further crops of crystals were obtained, which, recrystallised from hot alcohol, gave colourless prisms very similar in physical properties to (1), and melting at 187°·5 C. Repeated recrystallisation from alcohol did not alter this melting-point. By the use of benzene as a solvent, however, careful fractionation of this material yielded crops melting at 235° (diphenylurea) and 196° (diphenylurethane of 2:3-butyleneglycol) respectively. On taking pure specimens of these two substances and dissolving them in hot alcohol there was deposited again this complex, melting at 187°·5.

On analysis the following figures were obtained:—

- I. 0·1993 gm. gave 0·4923 gm. CO₂ and 0·1110 gm. H₂O,
 II. 0·1745 gm. „ 0·4320 gm. CO₂ and 0·0994 gm. H₂O.
 I. 0·2809 gm. „ 23·6 c.c. N (moist) at 24°·5 and 765 mm.
 II. 0·2096 gm. „ 16·8 c.c. N (moist) at 23° and 764 mm.

	Found.		Calculated for C ₄₉ H ₅₂ N ₆ O ₉ .
	I.	II.	
C	67·37	67·52	67·7
H	6·20	6·33	6·00
N	9·48	9·25	9·67

These facts, taken together, indicate that these crystals had the composition



The formation of the diphenylurea is doubtless partly due to moisture gaining access to the reaction mixture and present in the "crude glycol."

(3) *2:3-Butylene glycol monophenylurethane*.—In later preparations, the formation of the substance was avoided by using excess of phenylisocyanate and continuing the heating for many hours. It resembles the diurethane very closely in its solubility relationships, but it is, of course, more soluble than this. The melting point of the pure substance recrystallised from alcohol was found to be 100°C. , and a sample of the material treated with phenylisocyanate in benzene solution at its boiling point for eight hours gave an almost theoretical yield of the diphenylurethane (melting point, 199°).

0.0808 grm. gave 0.1876 grm. CO_2 and 0.0572 grm. H_2O .

0.2558 grm. „ 15.00 c.c. N (moist) at 16° and 766 mm.

	Found.	Calculated $\text{C}_{11}\text{H}_{15}\text{NO}_3$.
C	63.32	63.16
H	7.86	17.7
N	6.89	6.65

1.2 grm. of material in 57 c.c. of benzene gave in a 200-mm. tube a rotation of $+0^\circ\cdot 004$. If it is assumed that a rotation of $0^\circ\cdot 01$ is detectable with certainty, the specific rotation for this substance in benzene solution cannot be greater than $+0^\circ\cdot 25$.

Fractionation by means of Benzene and Petroleum.—The alcoholic mother liquors from the three substances described were now concentrated and taken up in benzene. A collection of a quantity of this material was made, and then fractionation effected by precipitation with low boiling-point petroleum ether. After a while the mother liquors became thick, and then the phenylurethane formed by the reaction of phenylisocyanate and the alcohol added was removed by distillation *in vacuo*. The distillate, boiling at about 120° under reduced pressure, consisted of nearly pure phenylurethane, melting after recrystallisation at $49^\circ\cdot 5$. The value given by Paal and Otten* is 52° .

* Paal and Otten, 'Ber.,' vol. 23, p. 2590.

The residue from the distillation was dissolved in benzene, and fractionation by petroleum ether was continued. It became necessary at this stage to take the $[\alpha]_D$, as well as the melting point and weight of each crop, and these crops were then sorted out with respect to their optical activities. It frequently happened that when material that was considered inactive was further fractionated it yielded strongly dextro- and strongly lævo-rotatory substances. The separation at each step was very slight, and a very large number of precipitations was required before any fractions which could be regarded as pure substances were obtained.

The final products of this fractionation of the substances contained in the alcoholic mother liquors mentioned above were:—

(1) A substance of $[\alpha]_D$ about -50 in alcoholic solution, melting at 145°C . The glycol obtained by hydrolysis was strongly dextro-rotatory.

(2) A substance of $[\alpha]_D$ greater than $+34$ and melting higher than 200°C .

These substances were not further examined, owing to the small amount obtainable in the pure condition, as judged by the definiteness of the melting point. One of them is possibly the diphenylurethane of an active 2 : 3-butanediol.

(3) A substance melting at 157° , and optically inactive in alcoholic solution. Analysis:—

0.1056 grm. gave 0.2530 grm. CO_2 and 0.0578 grm. H_2O .

0.1120 grm. „ 8.6 c.c. N. at 12° and 723 mm.

	Found.	Calculated for $\text{C}_4\text{H}_{10}\text{O}_2(\text{C}_6\text{H}_5\text{CNO})_2$.
* C	65.3	65.82
H	6.08	6.09
N	8.62	8.53

0.3 grm. was taken and hydrolysed in the manner described below and the aqueous solution of the resulting glycol oxidised by bromine in the light. On distillation under a long column the characteristic yellowish-green ring of diacetyl was seen to come over and the material was found to possess the peculiar smell of diacetyl. The distillate reduced Fehling's solution at once in the cold and yielded a crystalline osazone. The quantity was so minute that a melting-point determination could not be performed satisfactorily.

The evidence obtained, however, was considered to prove that the inactive diphenylurethane melting at 157° gave on hydrolysis a butanediol which

yielded diacetyl by oxidation with bromine in the light and was therefore the diphenylurethane of a 2:3-butanediol.

Recovery of 2:3 Butanediol from Purified Diphenylurethane, melting at 197° to 199°.

(1) A quantity of the inactive purified diphenylurethane of 2:3 butanediol of melting point 197° to 198°·5 (p. 278) was boiled up with alcoholic caustic potash until a small quantity gave no precipitate with excess of water. Carbon dioxide was then passed in to saturation, the solution evaporated to dryness *in vacuo*, and the glycol dissolved out in alcohol. The alcoholic solution on distillation gave a yellow oily liquid. This remained yellow on redistilling several times, ultimately, however, by repeated boiling with animal charcoal it was obtained colourless. Two combustions of this substance were made—

- I. 0·2449 grm. gave 0·4746 grm. CO₂ and 0·2396 grm. H₂O.
 II. 0·3592 grm. „ 0·6945 grm. CO₂ and 0·3550 grm. H₂O.

	Found.		Calculated for C ₄ H ₁₀ O ₂ .
	I.	II.	
C	52·85	52·75	53·33
H	10·87	10·99	11·11

The boiling point (uncorr.) at atmospheric pressure was 180° to 181°.

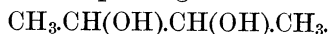
(2) In a second preparation 2·35 grms. were recovered from 14 grms. of diurethane.

0·2322 grm. gave 0·4497 grm. CO₂ and 0·2298 grm. H₂O

	Found.
{ C	52·82
{ H	10·99

This glycol showed a very faint but just recognisable positive rotation.

(3) A sample of diphenylurethane (melting point 199°) had been many times recrystallised with great care until in the mother liquors, when brought to maximum concentration, no optical activity could be observed. Of this 29·1 grm. were hydrolysed as before. 4·1 grm. of 2:3-butanediol were obtained. The value of $[\alpha]_D$ in 10 per cent. dry ether solution was demonstrated to be less than +0·08 and it was considered that this material was the inactive substance corresponding to the formula—



At ordinary temperatures it exists as a hard white mass.

0·2432 grm. gave 0·4691 grm. CO₂ and 0·2424 grm. H₂O.

	Found.	Calculated $C_4H_{10}O_2$.
C	52·6	53·33
H	11·07	11·11

It will be seen that the combustions of the "glycol" prepared from the diphenylurethane consistently give figures for the carbon content which are less than the theoretical. This cannot be put down to errors in the combustion process. It is suggested that a small percentage (about 1 per cent.) of water persistently accompanies the glycol even in its distillation. A sample treated in aqueous solution with bromine in the light gave diacetyl from which the osazone of melting point 241° to 243° was prepared. The identity of this osazone with the corresponding one from the "crude glycol" was established. With iodine and potash an aqueous solution of the glycol gives an immediate precipitate of iodoform.

It is seen that the "crude glycol" has given large quantities of a pure diphenylurethane from which a pure butanediol has been prepared by alkaline hydrolysis. This recovered diol yields diacetyl on oxidation, and this shows conclusively that the butanediol has a straight chain with the two hydroxyl groups in the 2:3-position. On this, and the fact of the large yield of diphenylurethane melting at $199^\circ\cdot5$ when phenylisocyanate acts on the "crude glycol" is based the conclusion that well over 90 per cent. of the "crude glycol" consists of an inactive 2:3-butanediol. From the remaining 10 per cent. only one substance has been definitely isolated and this appears also to be an inactive 2:3-butanediol, but yields a phenylurethane melting at 157° . It is probable that one of these inactive diols is the *r.* or *d.l.* isomer and the other the internally compensated isomer, but no attempt has been made to decide this question.

II. THE EFFECT OF FREE OXYGEN ON THE ACTION OF *B. lactis aërogenes* ON GLUCOSE AND ON 2:3—BUTANEDIOL.

In the experiments described the amount of acetylmethylcarbinol obtained from 50 grm. of glucose was very small and it was desired to increase the yield of this substance. An endeavour to do this by passing oxygen into the inoculated flask during incubation proved successful. It was also found that acetylmethylcarbinol is abundantly formed when the bacillus is cultivated in a solution of the butanediol in 1 per cent. peptone water in a current of oxygen. The oxidation of an alcohol group to a ketone group by an organism in an abundant supply of oxygen is

interesting, and is quite analogous to the bacterial oxidation of mannitol to fructose (Vincent and Delachanal),* sorbitol to sorbose, and glycerol to dihydroxyacetone (Bertrand).†

The Arrangement of the Experiments.

Two similar flasks were filled with the same medium.

In Experiment I $\left\{ \begin{array}{l} \text{Glucose, 50 grm.} \\ \text{Witte peptone, 10 grm.} \\ \text{Chalk, 15 grm.} \end{array} \right\}$ Water to 1 litre.

In Experiment II ... $\left\{ \begin{array}{l} \text{Butyleneglycol, 8 grm.} \\ \text{Witte peptone, 10 grm.} \end{array} \right\}$ Water to 1 litre.

One, after sterilisation, was freed completely from oxygen in the manner described,‡ inoculated, fitted with a mercury trap, and incubated at 37° for 21 days.

The other, also after sterilisation, was inoculated and incubated at 37° for 22 days, but a stream of oxygen from a cylinder fitted with a reducing valve, after passing through two wash bottles containing water and kept at 37°, was bubbled through it the whole time at the rate of about 50 c.c. per minute. The volume of the contents of the flask scarcely changed during the passage of the gas. The oxygen after leaving the flask passed again through wash bottles kept cold by circulation of the main supply (12° C.) and up an inverted condenser. Any acetylmethylcarbinol carried over by the oxygen was thus trapped. In Experiment I the proportion of carbon dioxide in the gas leaving the apparatus never exceeded 2 per cent.

The Examination of the Media after Fermentation.

Experiment I.—The glucose in the residues after distillation was determined by Pavy's method after removal of protein by mercuric nitrate and excess of mercury by caustic soda. The residual sugar in the anaërobic culture was less than 3 grm.; that in the aërobic culture was less than 0.6 grm.

The comparison of the amounts of acetylmethylcarbinol produced in the two cases was conducted as follows. The contents of the two flasks were filtered from unattacked CaCO_3 and insoluble calcium salts and the filtrates distilled until in each case 800 c.c. of distillate were collected. Very small traces of acetylmethylcarbinol can be detected by Fehling's solution, and previous experience with this substance demonstrated that such a distillate

* 'Compt. Rend.,' 1897, vol. 125, pp. 716—717.

† 'Ann. Chim. Phys.,' 1904, 18, vol. 3, pp. 181—288.

‡ 'Jenner Inst. Trans.,' 1899, vol. 2, p. 129.

would contain nearly all the acetylmethylcarbinol of the medium. A number of experiments were then made to determine the volume of distillate in each case which, when made up to 10 c.c. with distilled water and 1 c.c. Fehling's solution, *just* brought about complete reduction in 10 minutes. This was found to be:—

Aërobic flask	0.7 c.c.
Anaërobic flask	3.9 „

There was no other volatile reducing substance discovered in these fermentation products, nor was there any indication of a foreign substance which would vitiate, by its presence, the accuracy of these results. The amount of acetylmethylcarbinol produced in the aërobic fermentation was therefore $\frac{3.9}{0.7} = 5.6$ times that produced in the similar fermentation under anaërobic conditions.

The acetylmethylcarbinol was found to be levo-rotatory, but was not obtained pure enough for a determination of the $[\alpha]_D$.

Experiments with synthetic acetylmethylcarbinol (kindly made by Mrs. D. Norris) show that under the above conditions 1 c.c. of Fehling's solution is reduced by 2.48 mgrm. of the carbinol. The absolute quantities found were therefore approximately,

Aërobic flask	$\frac{800 \times 2.48}{0.7} = 2.83$ gm.
Anaërobic flask	$\frac{800 \times 2.48}{3.9} = 0.51$ „

The extraction of crude 2 : 3-butanediol (boiling point, 181 to 183°) from the two residues of the two cultures was performed in precisely the same manner in each case. In the anaërobic experiment 7.9 gm., and in the aërobic experiment 6.85 gm. were obtained. The values of $[\alpha]_D$ in 10-per-cent. solution in dry ether for these two samples were found to be +1.71 and +1.52 respectively.

Experiment II.—The culture made under anaërobic conditions did not grow. The stopper was therefore removed, the flask reinoculated and the stopper replaced, and the flask was then incubated. Growth then took place.

After the expiration of three weeks both flasks were found to contain pure cultures of *B. lactis aërogenes*. The liquids were distilled exactly as described for Experiment I, and the reducing power to Fehling estimated. This was found to be, for 1 c.c. Fehling solution—

Anaërobic flask	22.4 c.c. approx.
Aërobic flask	3.6 „

The acetylmethylcarbinol was identified by conversion into the osazone of diacetyl.

The relative amounts produced anaërobically and aërobically from 2:3-butanediol were therefore as $1 : \frac{22.4}{3.6} = 1 : 6.2$, which is, curiously enough, nearly the same ratio as that found to exist between the corresponding amounts from glucose, 1:5.6. The absolute amounts were:—

Aërobic flask.....	0.55 grm.
Anaërobic flask.....	0.09 „

III. THE ACTION OF *B. lactis aërogenes* ON FRUCTOSE.

One litre of medium was made up to contain 1 per cent. of peptone, 1.5 per cent. calcium carbonate, and 5.0 per cent. of fructose. The period of incubation at 37° was 37 days. After filtration the volume was 963 c.c., and from this 750 c.c. were distilled. The distillates readily reduced Fehling's solution in the cold, and when extracted with ether after saturation with potassium carbonate a liquid was obtained which gave with phenylhydrazine acetate an osazone melting at 240° C. It was therefore concluded that acetylmethylcarbinol was present. The residue was treated in the manner described for glucose, and the weight of crude glycol obtained was 3.2 grms. The value of $[\alpha]_D$ was 1.32 in 10-per-cent. solution in dry ether.

During the course of this investigation the following substances, in addition to those described, were prepared for purposes of comparison:—

1. *Diphenylurethane of 1:3-butanediol* was obtained from the diol prepared by the reduction of aldol. It crystallises readily from alcohol in colourless prisms, melting at 122° to 123° C.⁴

0.2763 grm. gave 20.2 c.c. N at 14° 8 and 768 mm.

	Found.	Calculated for $C_{18}H_{20}N_2O_4$.
N	8.77	8.53

2. *Diphenylurethane of 1:2 Propanediol*.—The glycol from which it was prepared boiled at 185° to 195° at ordinary pressure. The diphenylurethane is readily soluble in hot alcohol, from which it is for the most part precipitated in colourless silky needles on cooling. These melt at 152° 5 to 153° 5.

0.2571 grm. gave 0.6138 grm. CO₂ and 0.1306 grm. H₂O.

0.2581 grm. „ 19.7 c.c. N (moist) at 770 mm. and 20° C.

	Found.	Calculated for $C_{17}H_{18}N_2O_4$.
C	65·11	64·96
H	5·64	5·73
N	9·01	8·91

My thanks are due to Dr. Harden for advice throughout the investigation.

Summary.

1. The "crude glycol" obtained by the action of *B. lactis aërogenes* on glucose contains two optically inactive 2:3-butanediols, whose diphenylurethanes melt at 199°·5 and 157° respectively. The former constitutes well over 90 per cent. of the material. As no attempt was made to resolve either, it is impossible to say which is the racemic and which the meso- form.

2. Small quantities of the phenylurethanes of other glycols of unknown constitution were obtained from the crude glycol.

3. Acetylmethylcarbinol is formed from 2:3 butanediol by *B. lactis aërogenes* in presence of oxygen, and the yield from glucose is increased by the presence of oxygen.

4. Fructose is decomposed by the bacillus in a similar manner to glucose.

