Conclusions.

1. Antelope may remain in apparently perfect health for a year after having been infected with a human strain of *T. gambiense.*

2. One antelope was still capable of infecting clean laboratory-bred *G. palpalis* 315 days after it had been infected.

3. A small quantity of blood taken from one antelope 327 days after its infection was proved by inoculation into a white rat to be infective.

4. As the interval after the infection of the antelope increases their infectivity, as tested by "cycle" transmission experiments, dissection of flies which have fed upon them, and by the injection of the buck's blood into susceptible animals, appears to diminish.

5. A duiker was infected with a human strain of *T. gambiense* by feeding infected *G. palpalis* upon it.

The Bacterial Production of Acetylmethylcarbinol and 2.3-Butylene Glycol from Various Substances.

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In working out the action of *B. lactis aërogenes* on glucose quantitatively, Harden and Walpole (1) found that, in addition to the products already noted in the action of *B. coli communis* on glucose (2), a small quantity of acetylmethylcarbinol, $CH_3{\cdot}CH(OH){\cdot}CO{\cdot}CH_3$, and a considerable proportion of 2.3-butylene glycol, $CH_3{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_3$, were formed, the latter corresponding to about 33 per cent. of the carbon of the sugar fermented. The production of acetylmethylcarbinol by the action of *Tyrothrix tenuis*, *B. subtilis* and *B. mesentericus vulgatus*, and similar organisms on glucose, had been previously noted by Grimbert (3) and by Desmots (4).

The presence of acetylmethylcarbinol is of especial interest, as it has been shown to be the substance responsible for the Voges and Proskauer reaction (5). In view of this fact, and of the interest attaching to this mode of decomposition of glucose, it becomes a matter of some importance to discover what substances will give rise to acetylmethylcarbinol and butylene glycol during fermentation, and also which bacteria are capable of producing these two compounds. *B. lactis aërogenes* and *B. cloacæ* have been shown to

produce both substances from glucose, mannitol, and fructose (6, 7, 8); hence, in the first place, the action of these organisms was studied, and similar experiments were later carried out with *B. coli communis*, but with negative results.

It was of especial interest to discover whether these substances could be produced from carbon compounds less complex than the hexoses, and a variety of simpler substances containing fewer carbon atoms were therefore tried, *e.g.*, glycerol, ethylene glycol, malic acid, etc.

The formation of acetylmethylcarbinol or butylene glycol from substances containing three or fewer carbon atoms would necessarily involve a carbon synthesis which would be of considerable theoretical interest. An instance of this kind, the production of butyric acid and butyl alcohol from lactic acid and from glycerol in the butyric fermentation, has long been known (9, 10). To explain this, it has been supposed that acetaldehyde is first formed and then serves as the source of the butyl alcohol and butyric acid. The aldehyde may be supposed to undergo an aldol condensation followed by reduction, with or without subsequent oxidation:

$$2CH_3{\cdot}CHO = CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO.$$

$$CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO + 4H = CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}OH + H_2O.$$

It seems possible that the production of acetylmethylcarbinol and butylene glycol may be due to a somewhat similar course of events, which may be represented as follows, although these reactions have not, hitherto, been observed in the laboratory:—

$$2CH_3{\cdot}CHO + 2H = CH_3{\cdot}CH(OH){\cdot}CH(OH){\cdot}CH_3.$$

$$2CH_3{\cdot}CHO = CH_3{\cdot}CO{\cdot}CH(OH){\cdot}CH_3.$$

Experiments to test this hypothesis were made, with the result that the production of the glycol by bacterial action from acetaldehyde was conclusively established, although the mechanism of this production has not yet been ascertained.

It by no means necessarily follows, however, that in the fermentation of glucose the butylene glycol is actually produced from preformed acetaldehyde.

1. *Experimental Methods.*

As a general rule the culture medium consisted of 1 per cent. Witte's peptone water containing 5 per cent. of the substance under investigation. Sufficient chalk was added to neutralise any acid formed during fermentation, and after inoculation the culture was grown for three weeks at 37° C. under anaërobic conditions, the flask being fitted with a mercury trap to allow the escape of any gases.

Among the products of the reaction, acetylmethylcarbinol and butylene glycol only have been estimated quantitatively. A more complete investigation has, however, been made in the case of glycerol, and the results obtained will form the subject of another communication.

To ascertain whether the carbinol and glycol were derived from the acetaldehyde, sugar or other substances in question, and not simply from a constituent of the peptone, control experiments were carried out with peptone water alone as culture medium. In no case could the slightest trace of either substance be detected.

2. *Detection and Estimation of Acetylmethylcarbinol and Butylene Glycol.*

As some of the substances employed could only be used in small quantities, it was necessary to elaborate a method for the detection of both acetylmethylcarbinol and butylene glycol in small amounts. Attempts were first made to separate these two compounds by cautious evaporation and estimate them individually, but it was found impossible to arrive at any quantitative values in this way.

The method ultimately adopted was as follows:—

The medium in which the organism had been grown was carefully distilled to as small a bulk as possible over a free flame and then to dryness under reduced pressure at 37° C. The distillates were then united and made up to a definite volume and portions tested for reducing power and for Voges and Proskauer's reaction, which, as stated above, is due to the presence of acetylmethylcarbinol. To perform this reaction 3 c.c. of 1 per cent. Witte's peptone water are mixed in a test-tube with an equal quantity of 10 per cent. caustic soda, 2 c.c. of the solution to be tested are then carefully poured on to the surface of the liquid, and the tube is allowed to stand at room temperature. If acetylmethylcarbinol be present, a pink ring forms at the juncture of the two liquids. With very small quantities of carbinol, this may take some hours to develop, but with larger amounts the colour soon appears and quickly spreads through the whole of the solution, a green fluorescence being also produced. It was found that more delicate results could be obtained by the above method than by simply mixing the solutions.

Experiments in connection with other work, shortly to be published, have indicated that the carbinol is alone responsible for the reducing power of the distillates, and hence an estimation of this by Bang's (11) method at once gives the amount of acetylmethylcarbinol present, the reducing power of this substance being known (12).

The estimation of the butylene glycol is not such a simple matter.

A small amount is held in the dry residue after distillation, and must be extracted with dry ether, and the glycol thus obtained, after evaporation of the ether, added to the distillate.

The estimation depends on the fact that the glycol is readily oxidised by bromine under the influence of light to diacetyl, which can be estimated by its reducing power. Any acetylmethylcarbinol present is quantitatively oxidised to diacetyl in this way, but as the quantity of this substance will already have been determined, the amount of diacetyl formed from it is known, and the difference between this and the total diacetyl represents that due to the glycol. The details of the process are as follows:—An aliquot portion of the distillate is treated with a small quantity of bromine (0·1 c.c. for the distillate from the fermentation of 5 grm. of substance), and left exposed for 12 to 15 hours to the light of a 50-candle-power electric lamp. A further addition of bromine is made if the solution becomes completely decolorised, and the exposure continued. Any free bromine is then removed by the cautious addition of sulphurous acid, excess of this being carefully avoided. The solution is next saturated with sodium chloride, and the diacetyl distilled off and estimated by determining the reducing power of the distillate.

The results obtained by the above method are low, as the oxidation of the glycol to diacetyl is not quantitative. Control experiments with known amounts of glycol show that the actual results obtained are two-thirds the correct value, and a correction for this has been made in the tables given below.

In most cases the diacetyl produced by the oxidation was further characterised by the preparation and analysis of the phenylosazone.

Two typical analyses gave the following results:—

1. Substance fermented—galactose. Organism—*B. cloacæ*. Osazone—m.p. 243° C.

0·2109 grm. gave 39·4 c.c. N at 23·5° C. and 765 mm. N = 21·14 per cent.

2. Substance fermented—arabinose. Organism—*B. lactis aërogenes*. Osazone—m.p. 244° C.

0·1349 grm. gave 25 c.c. N at 22° C. and 765 mm. N = 21·13 per cent.

Diacetylphenylosazone, $C_{16}H_{18}N_4$, requires N = 21·05 per cent.

In the sugar experiments the amount unfermented was estimated in the residue left after the extraction of the glycol by dissolving it in water, making up to a known volume, and determining the reducing power after treatment with mercuric nitrate (Patein).

3. *Results Obtained.*

The results obtained with the organisms employed are indicated in the tables given below. The figures given in Columns 8 and 9 are the values calculated from the actual results found for 10 grm. of sugar fermented.

As previously mentioned, the results obtained with *B. coli communis* were in every case negative.

Table I.—Action of *B. lactis aërogenes* (Escherich) and *B. cloacæ* (Jordan) on various Sugars.

Experiment.	Organism.	Sugar.	Weight fermented.	Time of growth.	Carbinol per 10 grm. sugar fermented.	Glycol per 10 grm. sugar fermented.
			grm.		grm.	grm.
1	*B. lactis aërogenes*	Glucose	4·66	3 weeks	0·11	1·42
2	,, ,,	,,	4·85	,,	0·12	1·39
3	,, ,,	,,	5·00	,,	0·11	1·36
4	,, ,,	,,	2·47	,,	0·11	1·44
5	*B. cloacæ*	Fructose	4·67	,,	0·11	1·55
6	,,	,,	4·15	,,	0·11	1·51
7	,,	Mannose	2·32	,,	0·11	1·36
8	*B. lactis aërogenes*	,,	2·45	,,	0·10	1·42
9	,, ,,	Galactose	4·03	,,	0·06	0·86
10	,, ,,	,,	2·40	,,	0·07	0·85
11	*B. cloacæ*	,,	4·74	,,	0·08	1·41
12	,,	,,	4·38	,,	0·08	1·36
13	*B. lactis aërogenes*	Arabinose	3·92	,,	0·07	1·18
14	,, ,,	,,	2·39	,,	0·08	1·19
15	*B. cloacæ*	,,	5·00	,,	0·06	1·19
16	,,	,,	1·83	,,	0·08	1·18
17	*B. lactis aërogenes*	Isodulcite	2·03	32 days	0·43	1·45
18	,, ,,	,,	1·77	3 weeks	0·67	1·54
19	*B. cloacæ*	,,	2·03	,,	0·04	0·81

It will be seen from the above table that glucose, fructose, and mannose have given practically the same quantities of carbinol and glycol respectively, whilst with galactose the carbinol is slightly less in amount with both organisms used, and the yield of glycol varies with the organism employed. The results obtained in the case of arabinose show close agreement for both organisms, although in all four cases lower figures are obtained than with the hexoses. The question of isodulcite is interesting, as the amount of carbinol obtained by means of *B. lactis aërogenes* is abnormally high compared with that obtained from the other sugars or by using *B. cloacæ*.

The galactose and arabinose used in the above experiments were previously purified from traces of glucose by fermentation with yeast.

The amount of glycol produced from glucose is decidedly lower than that obtained by Harden and Walpole, a result which is possibly due to the employment of a different strain of the organism.

Table II.—Action of *B. lactis aërogenes* (Escherich) and *B. cloacæ* (Jordan) on various Alcohols.

Experiment.	Organism.	Alcohol.	Weight taken.	Carbinol per 10 grm. taken.	Glycol per 10 grm. taken.
			grm.	grm.	grm.
20	*B. lactis aërogenes*	Glycerol	5	Nil	0·05
21	„ „	„	50	„	0·05
22	„ „	„	10	„	0·05
23	„ „	„	10	„	0·04
24	*B. cloacæ*	„	10	„	Nil
25	*B. lactis aërogenes*	Ethylene glycol	5	„	0·08
26	„ „	„	5	„	0·08
27	„ „	„	5	„	0·09
28	„ „	Adonitol	2·50	0·06	0·22
29	„ „	„	2·50	0·06	0·24
30	„ „	Mannitol	5	0·03	0·23
31	„ „	„	5	0·03	0·04

The growth was continued for three weeks, except in the case of Experiment 31, which was for two weeks. The residual alcohols were not estimated, so that the results stated above are not corrected for amount of substance unfermented. Only in the case of mannitol and adonitol was any carbinol detected. All these alcohols, however, yielded glycol. Citric and malic acids gave negative results with both organisms.

The action of *B. lactis aërogenes* on dihydroxyacetone, $CH_2(OH)\cdot CO\cdot CH_2OH$, was also tried, but here again without positive result.

4. *Experiments on the Synthesis of* 2.3-*Butylene Glycol from Acetaldehyde by means of* B. lactis aërogenes.

For each experiment a litre of Witte's peptone water was made up and sufficient chalk added to neutralise any acids which might be formed during fermentation. The medium was then inoculated with *B. lactis aërogenes* and incubated at 37° C. for 24 hours before any addition of acetaldehyde was made, in order to establish a good growth of the organism.

In some experiments calcium formate (10 grm. formic acid per 100 c.c. water neutralised with $CaCO_3$) was added with the acetaldehyde (10 grm. per 100 c.c.); in others acetaldehyde was used alone.

Two cubic centimetres of the above solutions were added to the culture medium each day under sterile conditions, so that each flask received a daily addition of 0·2 grm. of acetaldehyde and the same quantity of formic acid (as formate) in the cases where this was used.

This treatment was continued until in two experiments 60 c.c. of the above solutions had been added, and in two others until 80 c.c. had been

added. At the end of this time and occasionally during the progress of the experiment sub-cultures were made to show that the organism in question was still alive and uncontaminated. In two experiments the whole of the acetaldehyde had not been used up, so that the liquid obtained by distillation of the culture medium was strongly reducing to Fehling's solution. In two other cases sufficient time was allowed to elapse between the last addition of aldehyde and the examination of the products to ensure the complete removal of this factor.

In these two cases the distillates obtained had no reducing power. In every experiment the distillate was tested by means of the Voges and Proskauer reaction for acetylmethylcarbinol, and in no case could any trace of this be detected.

The liquid was further examined for butylene glycol, which was in every case found to be present, though in extremely small quantities. It was detected as described above by the formation of diacetyl, this substance being proved to be present in every case by the positive results given by the Voges and Proskauer reaction after oxidation of the culture distillate.

In one case sufficient osazone was prepared from the diacetyl for a determination of the melting point, which was found to be 244° C. Among the products of the reaction were also found ethyl alcohol, acetic acid, and some succinic acid. Lactic acid was not present. These products were estimated in the manner previously described by Harden (13) with a few slight modifications. In the estimation of the alcohol any unchanged acetaldehyde present was removed by oxidation with moist silver oxide. The acetic acid was determined by the method of Macnair (14).

The table below shows the results obtained in three typical experiments:—

Table III.

	1.	2.	3.
Total acetaldehyde added	6·07 grm.	8·0 grm.	7·6 grm.
Total formic acid added (as Ca formate)	6·07 „	Nil	7·6 „
	grm.	grm.	grm.
Alcohol	6·5	4·02	4·5
Acetic acid	2·5	1·38	1·3
Succinic acid	Not estimated	1·25	0·12
Butylene glycol	+	0·677	0·109

From the point of view of the production of butylene glycol the presence of calcium formate appears to be detrimental, and it is also interesting to note the somewhat large quantity of succinic acid produced in Experiment 2, which also gave the largest yield of glycol.

Summary of Results.

1. *B. lactis aërogenes* and *B. cloacæ*, when grown in a peptone solution containing either glucose, fructose, mannose, galactose, arabinose, isodulcite, mannitol or adonitol, produce both acetylmethylcarbinol and 2.3-butylene glycol.

2. Glycerol, ethylene glycol and acetaldehyde, under similar conditions, also give rise to 2.3-butylene glycol in presence of *B. lactis aërogenes*, but no acetylmethylcarbinol is produced. In these three cases a carbon synthesis is involved, analogous to that which occurs in the butyric fermentation of glycerol and lactic acid.

3. The fermentation of citric and malic acids, of dihydroxyacetone, and of peptone water, gives rise to neither carbinol nor glycol.

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