LXIV.—*The Chemical Action of Bacillus coli communis and Similar Organisms on Carbohydrates and Allied Compounds.*

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THE following experiments were undertaken with the object of examining quantitatively the mode of action of the organisms of the Colon group as fermentative agents, in the hope of finding some characteristics which might serve as a means of classification or discrimination. The organisms of this group can scarcely be distinguished microscopically, but are found to vary in their action on various sugars and proteid compounds. The *Bacillus coli communis*, at one end of the series, has the following characteristic properties :

- (a) It decomposes glucose with production of (1) acid and (2) gas.
- (b) It curdles milk.
- (c) It produces indole from peptone.

The *Bacillus typhosus*, which may be taken as the other extreme of the series, does not possess properties *b* and *c*, and decomposes glucose with production of acid, but not of gas. Intermediate in the series are a number of organisms, which possess one or more, but not all, of the properties of *B. coli communis* or *B. typhosus*.

The action of *B. coli communis* and of *B. typhosus* on glucose has

therefore been examined, as far as possible quantitatively, in order to obtain a standard with which that of the other organisms might be compared, and this comparison has been effected for three different organisms. The action of *B. coli communis* on *d*-fructose and mannitol has also been examined in detail.

The products of the fermentation of glucose by these organisms comprise lactic acid, succinic acid, acetic acid, ethyl alcohol, formic acid, carbon dioxide, and hydrogen, all of which have been quantitatively estimated.

The *Bacillus coli communis*, and similar organisms, grow well and bring about a vigorous fermentation of glucose in a large number of different nutrient media. Thus they are able to derive their nitrogen from inorganic salts of ammonium and of methylammonium, from simple amino-acids, such as asparaginic acid, from Witte's peptone, and from beef broth. Since the products of the fermentation are acid in character, it is necessary to carry out the process in presence of chalk in order to neutralise the acid as it is produced. When ammonium salts are employed, it is therefore necessary to sterilise the solution of the sugar containing the chalk separately from the solution of the ammonium salt, in order to avoid the loss of ammonia which would otherwise ensue. The solution of the ammonium salt must therefore be sterilised in a flask arranged like an ordinary wash-bottle, so that the liquid, before inoculation, can be driven over into the sugar solution by a current of sterile air. When a culture in such a medium is incubated at 37°, it is found that the fermentation of the sugar commences very slowly, a delay of as much as five days occurring in some cases, although after this initial period the action is as vigorous as in the presence of peptone alone. This delay is no doubt due to the alkalinity of the solution, caused by the liberation of ammonia by the action of the chalk on the ammonium salt, which renders the development of the organism extremely slow until sufficient acid has been produced to render the medium capable of promoting growth at the normal rate.

This is shown by the figures on p. 612, from which it is seen that although in the end nearly the whole of the sugar is decomposed, the evolution of gas commences much more slowly when ammonium or methylammonium salts are present than when peptone alone is employed.

As the action of the free ammonia might seriously affect the mode of growth and action of the organism, this form of nitrogenous nourishment is not well adapted for comparative experiments. Asparaginic acid is equally unsuitable, but from a different and very interesting cause. As will be shown, it undergoes reduction under these circumstances to ammonium succinate, a large proportion of the

Nitrogenous medium.	Gas evolved at the end of days						Total gas evolved c.c.	Total sugar decomposed. Grams.
	1.	2.	3.	4.	5.	6.		
Peptone	500	1560	2270	3400	3500	3600	3700	20
Ammonium sulphate...	—	—	—	100	690	2630	4980	20
Methylammonium sulphate... ..	—	60	960	2210	—	3430	3650	17.87

hydrogen which is evolved when other media are employed being thus absorbed. Moreover, the large amount of succinic acid produced renders the analytical separation of the products more difficult.

It is also difficult to obtain satisfactory analytical results with media containing beef broth or extract of meat, because this contains small quantities of sugar and *d*-lactic acid, and the products derived from these cannot easily be separated from those derived from the sugar undergoing examination. The sum of the products obtained in this medium is invariably greater than the weight of the sugar taken.

The most satisfactory medium, from this point of view, was found to be peptone water, prepared by boiling 10 grams of Witte's peptone with tap water, adding 20 grams of the sugar or other compound to be examined, together with 10 grams of pure calcium carbonate, and making up the whole to 1 litre. In some cases, 2 grams of calcium phosphate were added, but no beneficial effect could be observed. Common salt is purposely omitted from the solution, because in its presence hydrochloric acid invariably passes over both into the volatile acids and the ethereal extract of the non-volatile acids.

The use of Witte's peptone alone has the further advantage that organisms of the Colon group have been found by direct experiment to grow freely in its solutions, without producing volatile acids, acids extractable by ether, or gaseous products in quantities large enough to interfere with the estimation of those produced from the compound under examination.

The action of the organism on the peptone, however, is undoubtedly modified by the presence of sugar, so that complete certainty on this point cannot be readily attained. This is shown by two facts.

1. In the presence of glucose, indole is not produced from peptone, whereas in its absence it is freely formed.

2. In the absence of sugar, practically no gas is evolved from peptone water, whereas in the presence of glucose the gas evolved invariably contains nitrogen, which must have been derived from the peptone. Thus in two experiments in which 250 c.c. of peptone water

were inoculated with this organism, no gas was evolved in one case and only 0.1 c.c. in the other, during a whole week, whilst indole was produced in both cases. On the other hand, a similar solution containing 2 grams of glucose gave about 100 c.c. of gas containing 7.5 per cent. of nitrogen. This result, which is in agreement with that obtained by other observers, is of great importance, since it throws some light on the function of the glucose in the nutrition of the organism.

The fermentations were in every case carried out in an atmosphere of nitrogen; the exact disposition of the apparatus and the methods of collecting and examining the gaseous products have been previously described in detail (*Trans. Jenner Inst.*, 1899, 2, 126).

The method of analysis of the remaining products, which follows the general plan proposed by Nencki (*Centr. Bakt.*, 1891, 9, 305) has, however, undergone considerable modification, and is therefore given in full.

Analytical Methods.

(1) *Residual Carbohydrate*.—Glucose and fructose were estimated by taking 50 c.c. of the clear liquid, adding excess of ammonium oxalate to precipitate the calcium, diluting to 100 c.c., and estimating the sugar present by means of Pavy's copper solution. In some cases, especially when beef broth was employed, the end of the reaction was rendered indistinct by the production of a purple coloration, due to the unaltered peptone and albumoses in the liquid. In such cases, it was found advisable to add four volumes of alcohol to some of the solution, filter an aliquot portion, evaporate off the alcohol, make up to the original bulk and then titrate for the sugar.

Mannitol was determined by Müller's method of measuring the change of rotation produced by the addition of a known weight of borax to the solution. The other substances present were found not to interfere with this measurement.

(2) *Total Acids*.—Fifty c.c. of the liquid were boiled for a short time with pure calcium carbonate, to effectually neutralise any free acid which might be present, and at the same time to remove calcium carbonate held in solution by carbonic acid. The dissolved calcium was estimated in the filtrate by precipitating with ammonium oxalate, and weighing as lime. The peptone, which was usually employed as the nutrient medium, contained calcium and was found to yield 0.106 gram of lime per 10 grams of peptone. This amount was therefore subtracted from the quantity found, in order to obtain the lime equivalent to the acids produced by the fermentation.

(3) *Alcohol*.—About 800 c.c. of the liquid were taken, 10 grams of oxalic acid added, the solution measured, and about 700–800 c.c. filtered off and distilled. The first 250 c.c. of the distillate were

reserved for the estimation of alcohol, and for this purpose were neutralised with normal alkali in presence of phenolphthalein paper, and then redistilled through a fractionating column; 100 c.c. of the distillate were collected and the alcohol calculated from the specific gravity.

(4) *Volatile Acids*.—The distillation of the acidified liquid was continued until only about 100 c.c. remained in the flask, and this was then distilled in steam until 100 c.c. of the distillate only required about 0.2 c.c. of normal alkali for neutralisation. In the presence of lactic acid, it is impossible to attain perfect neutrality, as this acid is itself slightly volatile with steam, the error thus introduced amounting to about 1 c.c. per litre of distillate. The distillate was then neutralised with normal alkali in presence of phenolphthalein, the residue from the alcohol distillation added, and the whole evaporated to dryness on the water-bath, redissolved in water, and diluted to 100 c.c.

In order to ascertain the nature of the acids present, three fractions were obtained by the addition of successive quantities of sulphuric acid and subsequent distillation, and the barium salts prepared from these were analysed. As numerous experiments showed that no higher acid than acetic was produced, this extended examination was not in every case carried out, but the formic acid was directly estimated by oxidation with mercuric chloride, and the acetic acid found by difference. The small amount of lactic acid present was neglected, as it was found that the only satisfactory method of estimating lactic acid—oxidation with alkaline permanganate and estimation of the oxalic acid produced (Ulzer and Seidel)—is inapplicable in the presence of acetic acid.

(5) *Lactic and Succinic Acids*.—The residue from the steam distillation was diluted to 150 c.c., and 25 c.c. were extracted with ether, which removes the lactic and succinic acids, together with some oxalic acid.

Since lactic acid is difficult to extract from aqueous solution, and the nutrient solutions tend to form an emulsion with the ether when shaken up by hand, it is convenient to employ some form of continuous extraction apparatus, and a modification of that described by Foerster (*Chem. Zeit.*, 1898, 22, 421) has been found very suitable.

The extraction was continued for 10 hours, at the close of which time experiment has shown that all the lactic acid is removed; the ether was then evaporated, and the liquid heated on the water-bath with excess of calcium carbonate for several hours. The lactic anhydride which is invariably formed when the ethereal extract is evaporated (Wislicenus) is thus completely converted into calcium lactate. The liquid was then filtered from the calcium oxalate and excess of

carbonate and diluted to 400 c.c.; 150 c.c. of this solution were taken for the estimation of the total acid present by precipitation of the calcium as oxalate.

Separation of Calcium Lactate from Calcium Succinate.—It was found that the principle of the method proposed by Müller for the separation of lactic from succinic acid by means of the difference in the solubility of their barium salts in dilute alcohol could also be applied to the calcium salts. Traces only of calcium succinate dissolve in 90 per cent. alcohol, whereas calcium lactate dissolves to the extent of about 3 grams in 100 c.c. of the liquid. The two salts can therefore be completely separated either by digesting the mixture of the dry salts with 90 per cent. alcohol, or, more conveniently, by dissolving the mixture in a measured volume of water and adding 9 volumes of alcohol. The following experiment, made to test the method, shows that the separation is practically complete.

0.7056 gram of calcium lactate yielded 0.1292 gram of lime.

0.7976 gram of the same salt was mixed with 0.1646 gram of pure calcium succinate, the whole treated with 90 per cent. alcohol, and the calcium estimated in the filtrate. This was found to yield 0.1468 gram of lime, whilst the amount calculated from the foregoing analysis of the lactate is 0.1460 gram.

The following method was therefore adopted for the separation and estimation of the succinic acid. One hundred and fifty c.c. of the solution of the calcium salts were evaporated to dryness on the water-bath in a beaker, the residue was dissolved in 10 c.c. of boiling water, and 90 c.c. of absolute alcohol were added. The liquid was allowed to stand for some time and then filtered, the precipitate washed with 90 per cent. alcohol, and the calcium estimated in the filtrate after the removal of the alcohol by distillation. This gives the equivalent of the lactic acid present, whilst the difference between this and the total acid gives the succinic acid. The precipitated calcium succinate can also be dissolved in water and the calcium in it estimated directly.

(6) *Determination of the nature of the Lactic Acid produced.*—The method usually employed for this purpose is to convert the acid into the zinc salt and estimate the amount of water of crystallisation and the optical rotation of this, the zinc salts of the active acids crystallising with $2\text{H}_2\text{O}$ (12.9 per cent.) and that of the inactive acid with $3\text{H}_2\text{O}$ (18.1 per cent.).

The zinc salts of the inactive and active acids, however, differ in solubility, and it is therefore impossible to prepare the salt from the acids obtained by extracting with ether in a state of purity, without to some extent altering the proportions in which the active and inactive acids are present. The presence of succinic acid, moreover, renders it essen-

tial to remove this substance from the lactic acid before the conversion of the latter into the zinc salt.

Attempts were therefore made to take advantage of the fact that the calcium salts of the active lactic acids have an optical rotation which is the opposite to that of the acids from which they are derived, and to estimate the amount of the active acid present from the change of rotation which occurs when the salt is converted into the acid.

This method suffers from two disadvantages (1) the lactic acids and their calcium salts have a very low specific rotation ; (2) even in an aqueous solution of the acid there is a tendency towards the formation of an anhydride which has a much higher rotation than the acid, and in an opposite sense.

It was, however, found that by working in the manner described below, fairly consistent numbers could be obtained. The results given in the present paper are founded on the numbers obtained by the examination of a sample of calcium *d*-lactate derived from extract of meat, and the rotations were all measured on a Schmitt and Haensch saccharimeter which can be employed with lactic acid for relative determinations of this kind. The observations are being extended to specimens of pure *l*- and *d*-lactates resulting from the fractional crystallisation of zinc ammonium lactate, a supply of which I owe to the kindness of Prof. Purdie, and a full account of the absolute measurements and conditions and limits of accuracy will shortly be published. The numbers given in the present paper are probably accurate to about 5—10 per cent.

This method has the two advantages that a fair estimate of the nature of the whole of the lactic acid is obtained, and that the estimation can be carried out even in the presence of small amounts of optically active substances, such as proteid matter from the nutrient medium, or unfermented sugar, traces of which very frequently pass over into the ether extract in spite of the greatest care, owing to the character of the liquid to be extracted.

To carry out the estimation, the remainder of the 150 c.c. of the liquid left after the removal of the volatile acids by distillation is extracted with ether and the acid thus obtained converted into the calcium salt, which is then freed from calcium succinate in the manner already described, 10 c.c. of water and 90 c.c. of alcohol being used for each 1.5 grams of salt. The alcoholic filtrate is evaporated, decolorised if necessary with animal charcoal, and diluted so as to yield at least 30 c.c. of liquid. Five c.c. of the solution are taken for the estimation of the calcium, and from this the concentration of the solution is found. Two quantities of 12 c.c. are measured out and to one is added 1 c.c. of water, to the other 1 c.c. of aqueous hydrochloric acid containing sufficient acid to liberate the whole of the

lactic acid. Two solutions are thus obtained of the same concentration, one containing the acid and the other the calcium salt. These are then examined in the polarimeter and the difference between their rotations observed. The amount of lime as active acid corresponding to this difference is then found from the table and the relation of this to the total amount of lime present gives the percentage of active acid.

Action of Normal B. coli communis on d-Glucose.

The following table contains the results of five experiments, each carried out with peptone water, as already described, in an atmosphere of nitrogen, 20 grams of pure glucose being used.

Experiment No. 3 was carried out with a solution of ammonium sulphate containing 6.6 grams of this salt per litre, instead of peptone.

The gaseous products are expressed in c.c. per gram of sugar, and the other products in percentages of the weight of sugar fermented.

The organism employed in experiments 1, 2, and 3 was isolated from the stool of an adult, that employed in No. 6 from that of an unweaned child; and those used for Nos. 4 and 5 were isolated from the faeces of a dog and a rabbit respectively. The culture was made in every case into Parietti broth; the resulting growth was plated out, and cultures taken from a single colony. All the organisms gave the usual reactions for the normal bacillus.

Source of organism.	Sugar fermented. Grams.	Lactic acid.	Percentage of excess of <i>l</i> -acid in the lactic acid.	Acetic acid.	Alcohol.	Succinic acid.	Formic acid.	Carbon dioxide, per cent.	C.c. per 1 gram.	
									Carbon dioxide.	Hydrogen.
1. Adult.....	12.99	40.1	{ not determined }	19.95	16.9	5.5	trace	—	—	—
2. „	20	31.9		18.84	12.85	(5.2)	none	18.09	91.8	110
3. „	20	40.1		15.63	9.84	(6.7)	none	16.25	83	86
4. Dog.....	14.68	45.2	„	14.14	8.9	(5.7)	none	12.85	65.2	79.9
5. Rabbit ...	11.63	44.4	75	10.69	10.2	4.6	1.4	12.1	61.6	80.7
6. Child	13.33	46.0	95	12.02	10.9	5.7	2.5	—	—	—

These results illustrate the degree of variation which is found in the action of these organisms. Even parallel experiments, carried out with the same culture under apparently identical conditions, have frequently been found to yield the various products in somewhat different proportions. The weight of lactic acid produced varies from 31.9 to 46 per cent., but never exceeds 50 per cent. of the sugar employed. In every case, this acid consists of a mixture of inactive acid with a preponderating amount of *l*-acid. In spite of this result however, the production of *l*-lactic acid cannot be taken as an essential function of this organism, since the production of the *d*-acid has been frequently observed by various authors, employing this bacillus derived

from various sources. It has also been found by Péré (*Ann. Inst. Past.*, 1898, 12, 63; 1893, 7, 737) that some of these organisms may be made to yield either *d*- or *l*-lactic acid, according to the conditions of nourishment, whilst others yield the same acid under all conditions.

These numbers do not, in every case, account for the whole of the sugar, there being a deficit amounting to from 1/12th to 1/8th of the total carbon, but a careful search has not yet revealed the nature of the change which this remaining portion has undergone. It is possible that a portion of the sugar is reduced either by the hydrogen which is evolved, or by a reaction in which a molecule of water, or perhaps of formic acid, intervenes. Sorbitol, which might be thus produced, is not easily fermented by the organism, and might remain unaltered and escape notice. Further experiments are still in progress with the object of deciding this question.

A clearer view of the nature of the chemical change is afforded by expressing the results in terms of the carbon atoms of the original sugar. This is done in the following table:

Source of organism.	Carbon atoms as					Hydrogen atoms.
	Lactic acid.	Acetic acid.	Alcohol.	Succinic acid.	Carbon dioxide and formic acid.	
1. Adult	2.40	1.2	1.32	0.34	—	—
2. „	1.91	1.13	1.01	0.32	0.74	1.77
3. „	2.40	0.94	0.98	0.41	0.67	1.39
4. Dog	2.71	0.84	0.70	0.35	0.53	1.29
5. Rabbit	2.67	1.18	0.80	0.29	0.50	1.30
6. Child	2.76	0.81	0.85	0.35	—	—

This reveals the fact that whilst the lactic acid accounts for 2—3 of the carbon atoms of the original molecule, the alcohol and acetic acid are formed in nearly equivalent amounts, and each accounts for only one carbon atom of the sugar molecule. The amount of carbon dioxide obtained in different experiments represents less than 1 carbon atom, but never falls below 0.5. The ratio of hydrogen to carbon dioxide is not exactly 1 : 1 by volume, but does not depart widely from this ratio. The production of about half the weight of the sugar as lactic acid, and of acetic acid and alcohol in equivalent amounts, each corresponding to about 1 carbon atom of the sugar molecule, may be represented by a very simple equation, which, however, somewhat over-estimates the amounts of carbon dioxide and hydrogen which are actually found:

It seems probable, however, that this represents the main reaction, but is subject to modification by secondary changes (see p. 626).

In solutions containing beef broth, as already mentioned, the products derived from the sugar cannot be distinguished accurately from those previously present in the broth and formed from it by the action of the organism. The lactic acid obtained in this medium is invariably much less active, and sometimes quite inactive, as has been pointed out in a previous paper (*Trans. Jenner Inst.*, 1899, 2, 126). This is, at all events in part, to be ascribed to the presence of *d*-lactic acid in the broth, since this acid is not affected by the organism (p. 625). Precisely similar relations are found with *lævulose* and *mannitol*.

The Fermentation of Glucose by Bacillus typhosus.

The products of fermentation of glucose by *B. typhosus* are very similar in character to those produced by *B. coli communis*, the only essential difference being that formic acid is produced in large quantity, instead of only in traces. This formic acid doubtless represents the carbon dioxide and hydrogen which are produced by *B. coli communis*, the *B. typhosus* being unable to decompose formates, whilst *B. coli communis*, as will be shown later on, has this power (see also Pakes and Jollyman, this vol., p. 386). The fermentation is not a very vigorous one, and in all cases terminated when about half of the sugar had been decomposed.

The two following experiments were carried out with cultures of virulent typhoid in an atmosphere of nitrogen. The solutions were sterilised at 80° for an hour before being examined :

	I.		II.	
	Per cent.	Carbon atoms.	Per cent.	Carbon atoms.
Lactic acid	31.1	1.87	49.5	2.96
Acetic acid	16.1	0.97	12.7	0.76
Alcohol	10.6	0.83	9.1	0.70
Succinic acid	2.1	0.13	trace	trace
Formic acid	17.2	0.67	17.7	0.69

In both cases, a mixture of inactive with *l*-lactic acid was formed ; that produced in experiment I was found to contain 47.5 per cent. of the active acid, this being a smaller proportion than is produced by the *B. coli communis*.

Action of Abnormal Forms of B. coli communis on Glucose.

Only three of these organisms have so far been employed: 1. Two organisms which differed from the normal only in not curdling milk until after the expiration of 7 or 8 days. One of these (A) was isolated from the faeces of a guinea-pig, and the second (B) from those of a pigeon.

2. An organism (C) which I owe to the kindness of Dr. Gordon, who had isolated it from sewage. This organism gave no gas with sugar solutions, but curdled milk and produced indole in the normal manner.

The results are stated both in percentages and in terms of the carbon atoms of the sugar molecule.

	A.		B.		C.	
	Per cent.	Carbon atoms.	Per cent.	Carbon atoms.	Per cent.	Carbon atoms.
Lactic acid	33·2	1·99	25·4	1·52	76·6	4·6
Succinic acid	3·2	0·2	3·9	0·24	3·3	0·2
Acetic acid	20·95	1·26	24·5	1·47	14·1	0·85
Alcohol	14·33	1·12	20·1	1·57	4·6	0·36
Formic acid	1·4	0·06	3·4	0·13	9·0	0·35
Carbon dioxide ...	14·6	0·60	18·5	0·76	none	none
Per cent. of active l-acid	64·4		80		32	

It appears from this that organisms A and B do not differ essentially from the normal *B. coli communis* in their action on glucose, although they yield rather less lactic acid and more alcohol and acetic acid (see p. 618).

The organism C, on the other hand, differs distinctly in its mode of action, both from *B. coli communis* and *B. typhosus*. The amount of lactic acid is greater, and the proportion of active acid less, whilst the alcohol and acetic acid are produced in different proportions. Like *B. typhosus*, this organism produces formic acid, which it appears to be unable to decompose.

This result shows that an organism which appears by its cultural tests to be closely related to the colon bacillus and to *B. typhosus*, may nevertheless differ from both of these in its action on glucose.

Action of B. coli communis on Lævulose (d-Fructose).

Lævulose is readily fermented by this organism, both in peptone water and in peptone beef broth. The products are identical in character with those produced from glucose. The following results were obtained in peptone water; 20 grams of the sugar containing 0.8 gram of water, and 19.2 grams of lævulose were employed:

	I.		II.	
	Per cent.	Carbon atoms.	Per cent.	Carbon atoms.
Lactic acid	43	2.58	48.2	2.89
Succinic acid	not determined		6.1	0.37
Alcohol	"	"	11.5	0.90
Acetic acid	13	0.78	16.1	0.97
Carbon dioxide ...	15.5	0.63	13.07	0.53
Formic acid	—	—	trace	

The lactic acid produced in both cases was lævorotatory. That derived from experiment I was not further examined. That obtained from experiment II was converted into the zinc salt. As this was found to contain succinic acid, the lactic acid was re-extracted, converted into the calcium salt, and the latter purified and examined as usual. It was found to contain 90 per cent. of the active *l*-lactic acid.

An experiment which was carried out in peptone beef broth yielded a very similar result to experiments with glucose under the same conditions. The lactic acid obtained was greater in quantity (65 per cent.), and contained only 51 per cent. of active acid. As is the case with glucose in this medium, the sum of the products exceeded the weight of the sugar taken.

The difference of constitution between glucose and lævulose, therefore, does not appear to produce any difference in the character of the products of fermentation by this organism. The lactic acid in particular is of the same character, and this shows that the configuration of the lactic acid produced does not depend on that of the carbon atom situated in the α position to the aldehyde group of glucose, since in lævulose this atom is not asymmetric.

Action of B. coli communis on Mannitol.

Although the substitution of lævulose for glucose produces no recognisable difference in the products of fermentation, a very marked

change is observed when the sugar is replaced by a hexatomic alcohol.

Dulcitol is not fermented by this organism. The attempt was made in peptone water, and although the organism developed well, no sign of fermentation (gas evolution, or production of acid) could be observed. A similar power of discriminating between mannitol and dulcitol has previously been observed in other organisms.

Sorbitol appears to undergo only a very slight fermentation when submitted to this organism, and the products have not yet been examined.

Mannitol, on the other hand, is very vigorously and completely acted on, the products being qualitatively the same as those produced from glucose and lævulose.

The following are the complete results of two experiments, I in peptone water, II in beef broth:

	I.		II.	
	Per cent.	Carbon atoms.	Per cent.	Carbon atoms.
Lactic acid	18·6	1·13	24·9	1·51
Succinic acid ¹	8·9	0·55	9·4	0·58
Acetic acid.....	9·5	0·58	8·2	0·50
Alcohol	28·06	2·22	33·5	2·65
Formic acid	3·0	0·12	0·6	0·02
Carbon dioxide ...	28·44	1·16	30·84	1·26
Per cent. of active l-acid	79		29	
Hydrogen (atoms)	2·7		2·7	

The lactic acid from experiment I was first converted into the zinc salt, and this was then treated with pure lime, and the resulting calcium salt purified and examined in the usual manner.

The experiment in beef broth, as usual, yields high results, whilst the lactic acid is less active.

The most noticeable point about this result is the large yield of alcohol, which considerably exceeds 25 per cent. of the weight of the mannitol fermented. It is always produced in this large proportion, two other experiments giving 28·2 per cent. (peptone water) and 26·9 per cent. (sodium asparaginate). When separated by means of potassium carbonate, dried over the same salt and distilled, it boiled constantly at 78°, and was therefore pure ethyl alcohol. The succinic

acid is much larger in amount than that obtained from glucose, whilst the lactic and acetic acids are much smaller. The amount of hydrogen evolved is also greater, the total amount, including that of the undecomposed formate, being nearly three atoms of that contained in the mannitol. The fact that the amount of alcohol corresponds with two of the carbon atoms of the mannitol, whereas glucose only yields one of its carbon atoms as alcohol, seems to be definitely connected with the fact that in mannitol there are two groups of the formula $\text{CH}_2(\text{OH})\cdot\text{CH}(\text{OH})\cdot$, whilst in glucose only one such group occurs.

The view that the alcohol produced originates from this particular group is strikingly confirmed by the action of this organism on glycerol, $\text{CH}_2(\text{OH})\cdot\text{CH}(\text{OH})\cdot\text{CH}_2\cdot\text{OH}$. Quite recently, I have found that the chief products of this action are alcohol and formic acid, or its decomposition products, carbon dioxide and hydrogen, only a very small quantity of other acids being produced.

5.2 grams of glycerol yielded 2.4 grams of alcohol, 1.8 grams of formic acid (partially as carbon dioxide and hydrogen), together with small amounts of two other acids, probably acetic acid and succinic acid.

Reduction of Asparaginic Acid by B. coli communis in presence of Glucose and Mannitol.

When the sole nitrogenous nourishment supplied to this organism consists of sodium asparaginate, glucose and mannitol are fermented in the usual manner, with the single exception that the amount of hydrogen evolved is much less, a large proportion of it serving to reduce the asparaginic acid to ammonium succinate.

This is shown in the following table of results, in which col. I gives (a) the volume of hydrogen actually evolved; (b) the volume of hydrogen taken up by the asparaginic acid, calculated from the ammonia found in the solution, and (c) the volume of hydrogen evolved in another experiment in presence of peptone from the same weight of glucose. Col. II gives the same information for mannitol. The solutions contained 20 grams of sodium asparaginate and 20 grams of glucose and mannitol respectively per litre.

	I. Glucose.		II. Mannitol.
	c.c.		c.c.
a.	663	} 1884	887
b.	1221		2038
c.	1776		3274
			} 2925

The occurrence of this reduction is of some interest, since asparaginic acid is not very easily reduced by ordinary reagents, but requires to be heated with hydriodic acid. Whether the reduction is due to a purely chemical reaction or whether the protoplasm of the organism plays a direct part in the reduction must remain at present undecided.

The reduction is also interesting from another point of view, as showing that an organism like *B. coli communis*, which has very little direct action on proteids, may, nevertheless, under appropriate circumstances, assist in the process of still further decomposing the products formed from them by putrefaction.

Experiments made with aminoacetic and aminopropionic acids to ascertain whether a similar reduction occurred gave negative results, the growth of the organism being very feeble and no ammonia being formed.

Action of B. coli communis on d-Galactose and l-Arabinose.

Both of these sugars are fermented by this organism, with formation of the same products as are derived from glucose. The complete course of the action is still under investigation, but it may be stated that both yield a mixture of *l*-lactic acid with the inactive acid. *l*-Arabinose, moreover, yields alcohol and acetic acid in about the same molecular proportion as glucose, whilst the amount of lactic acid is only half as great.

Action of B. coli communis on Formates and Lactates.

The fact that *B. coli communis* differs in its action on glucose from *B. typhosus*, mainly by the production of carbon dioxide and hydrogen instead of formic acid, led to experiments on the direct action of the former organism on formates. It was found that this organism effects the decomposition of these salts according to the equation

Hoppe-Seyler, in 1876, described a decomposition of this kind by the action of the bacteria of sewage, and quite recently Messrs. Pakes and Jollyman (this vol., p. 386) have drawn attention to this function of *B. coli communis* and other organisms.

The particular organism employed in these experiments exerted comparatively little action on a solution containing 2 per cent. of sodium formate and 1 per cent. of Witte's peptone. In presence of a small amount of sugar, however, a much larger amount of the formate was decomposed, as is shown in the following table, the hydrogen due to the sugar being deducted:

Sodium formate	2 per cent.	2 per cent.	2 per cent.
Glucose	0 „	0.2 „	0.4 „
Hydrogen evolved ...	105 c.c.	1122 c.c.	1481

Increase of pressure appears to favour the formation of formic acid instead of its decomposition products. Thus, under an additional pressure of 0.5 atmosphere, the amount of formic acid produced was increased in one case 3 times, and in a second experiment 10 times.

Pressure.	C.c. of <i>N</i> -formic acid from 1 gram of glucose.
1 atmosphere.	0.15
1.5 „	0.42
1.5 „	1.32

This organism has practically no action on inactive lactic acid in the form of ammonium, calcium, or sodium salt, and even when a small amount of glucose is added, the amount of active lactic acid produced is not greater than is produced from the sugar alone. Thus, a 2 per cent. peptone solution, containing 9 grams of lactic acid as sodium salt, together with 1.8 grams of glucose, only yielded 0.4 gram of active lactic acid, the whole of which is derived from the sugar.

This fact is important as showing that the active lactic acid which results from the action of the organism on so many sugars is almost certainly not formed by the selective decomposition of previously formed inactive acid.

Theoretical Considerations.

The results communicated above seem to show that in the fermentation of *d*-glucose and *d*-fructose, at least two molecules are concerned, and that the chief reaction may be best represented by the equation

which finds a simple diagrammatic expression as follows :

According to this scheme, two groups, $\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot$, are left, which may then be converted into lactic acid or partly break down into other compounds, among which alcohol, acetic acid, hydrogen, and succinic acid are probably to be numbered. This secondary change probably accounts for the fact that in some cases (experiments A and B, p. 620), whilst the lactic acid represents considerably less than 3 atoms of carbon, the alcohol and acetic acid are correspondingly larger in amount.

In the case of mannitol, the fact that the presence of two $\cdot\text{CH}_2(\text{OH})\cdot\text{CH}(\text{OH})\cdot$ groups gives rise to a largely increased yield of alcohol and a decreased yield of acetic acid, must certainly be considered significant. The chief reaction in this case may also be most easily imagined as embracing two molecules:

The two residual groups, $\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot$, then behave in an analogous manner to those derived from the sugars, but yield in this case less lactic acid and more of the other products.

From this point of view the action of the organism on a pentose like *l*-arabinose is of great interest. If the action is of the same type as that on glucose, fructose, and mannitol, the maximum yield of lactic acid should only be 30 per cent. of the weight of the sugar, only three carbon atoms from two molecules being available for the production of the acid, whereas in the case of glucose six atoms are available:

The acetic acid and alcohol, on the other hand, should represent each one carbon atom per molecule of the sugar. The single experiment

so far completed is in good agreement with this scheme, the amounts of alcohol and acetic acid formed bearing nearly the required ratio to the lactic acid, but the experiment must be multiplied before any definite conclusion can be drawn.

The group $\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot\text{CH}(\text{OH})\cdot$, from which it is suggested that the lactic acid is formed, has the same empirical composition as lactic acid, and would yield this acid by the interchange of a hydrogen and oxygen atom between the two terminal groups, or between the terminal groups of a second similar chain derived from another molecule of the sugar.

The unaltered asymmetric group, $\cdot\text{CH}(\text{OH})\cdot$, would then retain its asymmetry in the new molecule of lactic acid thus produced. Such a rearrangement, however, if it could be effected by means of reagents containing only symmetric molecules, would probably result in the production of an inactive acid, the oxidation and reduction taking place to an equal extent at either end of the chain.

The change being, however, brought about in the presence of the asymmetric molecules of the protoplasm of the organism—or possibly of an enzyme—would most probably be influenced so as to proceed entirely in one direction, or more rapidly in one direction than in the other, and thus give rise to an active acid, the activity of which would be independent of the previous configuration of the group and depend only on the nature of the organism. This view is therefore not inconsistent with the fact observed by Péré that the same sugar when submitted to the same organism, but under different conditions of nourishment, may yield lactic acids of opposite signs. Before admitting this explanation, it would, however, be necessary to show that the type of reaction had remained unaltered.

The question of the origin of the lactic acid within the molecule of the sugar may also be attacked by examining the products formed from sugars of different configuration, and the work is being proceeded with in this direction; it is also being extended to embrace a study of the various other types of formation of lactic acid from the carbohydrates, and the action of these organisms on compounds of different constitution.

Summary of Results.

1. In the fermentation of glucose by *B. coli communis*, the lactic acid produced never exceeds in amount one half of the sugar fermented, but may be less. It is optically active, but is probably not derived from previously formed inactive acid. Alcohol and acetic acid are produced in approximately equivalent amounts.

2. The products of fermentation of glucose by *B. typhosus* are similar to those produced by *B. coli communis*, with the exception that formic acid is produced instead of a mixture of carbon dioxide and hydrogen.

3. Certain bacteria which appear by the ordinary tests to be intermediate between *B. coli communis* and *B. typhosus* decompose glucose according to a different type of reaction.

4. *d*-Fructose, when fermented by *B. coli communis*, yields the same products as glucose, and in the same proportion.

5. Mannitol, when fermented by *B. coli communis*, yields about 28 per cent. of alcohol, corresponding to two of its carbon atoms, together with less lactic acid and acetic acid than are formed from glucose. The alcohol appears to be produced from the group $\text{CH}_2(\text{OH})\cdot\text{CH}\cdot\text{OH}$.

6. Asparaginic acid is reduced to ammonium succinate by *B. coli communis* in presence of glucose or mannitol, the volume of hydrogen evolved being correspondingly diminished.

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