CLXVI. STUDIES IN THE BIOCHEMISTRY OF
MICRO-ORGANISMS.
XXI. QUANTITATIVE EXAMINATION BY THE CARBON
BALANCE SHEET METHOD OF THE TYPES OF
PRODUCTS FORMED FROM GLUCOSE BY
SPECIES OF BACTERIA.

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(Received July 29th, 1931.)

IN an earlier paper of this series [Birkinshaw and Raistrick, 1931], a full description was given of a method for the determination of the products formed by micro-organisms by the carbon balance sheet method, with particular reference to moulds. A number of balance sheets in later papers [Raistrick *et al.*, 1931] show that by this means a useful general survey of the types of mould metabolism products can be obtained and may be used as a basis for the biochemical classification of these organisms. The method as originally evolved, however, was intended to cover the whole field of micro-organisms, and the present work was undertaken largely with a view to determining how far the original method would apply, and what modification would be necessary, in order to study the metabolic products of bacteria. In addition it was hoped to confirm and supplement in a quantitative manner the results obtained by Harden, Neuberg, Kluyver and other workers in this field. It was further anticipated that some of the organisms examined would show hitherto unsuspected amounts of some types of products, or of "carbon unaccounted for," and would thus open up interesting possibilities in the way of detailed investigation of the metabolism of the organism so selected.

Medium.

The Czapek-Dox-glucose medium used in the work on moulds was purposely chosen on account of its simplicity and its lack of any source of carbon other than glucose. Moulds are remarkably tolerant of acid, consequently the low p_H obtained with this medium when organic acids are produced does not markedly affect the growth or activity of these organisms. With bacteria, the

case is otherwise. They are known to be much more sensitive to acidity, and it was necessary, therefore, to adopt some method of neutralising the acid as formed. Both phosphate buffering and addition of calcium carbonate were tried, the latter proving more useful. Growth of *B. coli* (used as a test organism) on Czapek-Dox medium modified by addition of carbonate was unsatisfactory. It was considered that the form of nitrogen (NaNO_3) of the medium was probably unsuitable and a series of the commoner bacteria was therefore sown on to a Czapek-Dox medium containing the nitrogen in various forms, but in each case equivalent in nitrogen to the 0.2 % sodium nitrate of the original medium. Only 1 % of glucose was used so that an accumulation of acid was impossible, and calcium carbonate could thus be omitted without detriment to growth. The growth of the organisms after 5 days on the modified medium is indicated in Table I.

Table I. *Growth of stock strains of bacteria on modified Czapek-Dox solution containing various sources of nitrogen. Temp. 34°.*

	Time of incubation								
	5 days								48 hours Micro- cosmic salt + CaCO ₃
	Sod. nitrate	Sod. nitrite	Micro- cosmic salt	Amm. nitrate	Amm. sulphate	Amm. chloride	Calc. nitrate	Pot. nitrate	
C 1. <i>B. mesentericus</i>	+++	—	+++	—	+++	+++	—	—	+++
C 3. <i>B. coli</i> (<i>Escherichia coli</i>)	—	—	+++	++	++	++	—	—	+++
C 4. <i>B. proteus-vulgaris</i>	—	—	—	—	—	—	—	—	.
C 6. <i>Bact. acidilactici</i> (<i>Escherichia acidilactici</i>)	—	—	+++	++	—	++	—	—	+++
C 12. <i>Aerobacter cloacae</i>	—	—	+++	+	+++	+++	—	—	+++
C 13. <i>B. figurans</i>	—	—	—	—	—	—	—	—	.
C 17. <i>Serratia marcescens</i>	—	—	—	—	—	—	—	—	.
C 24. <i>B. lactis-aerogenes</i> (<i>Aerobacter aerogenes</i>)	+++	—	+++	++	+++	.	—	—	++++
C 25. Leather bac. Houston	++	—	+++	+	—	++	—	+++	++++
C 26. Lactose acidifier (<i>Aerobacter</i>)	—	—	++	—	—	—	—	—	+
C 27. Lactose fermenter (<i>Aerobacter</i>)	++	—	+++	+	+++	+++	—	+++	+++
C 28. <i>B. mycoides</i>	—	—	+++	+++	+++	+++	—	+++	++
C 30. Sporing rod bacillus	++	—	+++	+++	+	++	—	+++	++
C 33. <i>Sarcina lutea</i>	—	—	—	—	—	—	—	—	.
C 34. <i>B. coli</i> (<i>Escherichia coli</i>)	—	—	+++	+	+	++	—	—	+++

The medium contained only 1 % of glucose.

The microcosmic salt ($\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$) medium showed definitely the best growth for the largest number of bacteria and was employed in a further trial. This time 1 % of calcium carbonate was added to the medium to determine whether this inhibited growth, since it would be necessary or at least desirable to use 5 % of glucose in the metabolism experiments with a correspondingly greater acid production. No inhibition was observed. The growth is shown in the last column of Table I.

It was thus evident that a number of organisms could be selected which grew rapidly on a medium containing merely inorganic salts apart from the glucose. A further batch of cultures shown in Table II yielded in most cases disappointing results, in respect of their growth on this medium.

Table II. *Growth of various stock strains of bacteria on modified Czapek-Dox solution containing various sources of nitrogen.*

Time of incubation 3 days at 34°.								
	Sod. nitrate	Sod. nitrite	Amm. chloride	Amm. sulphate	Pot. nitrate	Calc. nitrate	Micro- cosmic salt	Amm. nitrate
C 38. <i>Streptococcus lacticus</i>	—	+	—	+	—	—	—	—
C 39. <i>B. guntheri</i> (<i>Streptococcus lactis</i>)	—	?	—	++	—	—	+	—
C 40. <i>B. bulgaricus</i> (<i>Lacto-bacillus bulgaricus</i>)	+	+	—	—	+	—	—	+
C 41. <i>B. acidophilus</i> (<i>Lacto-bacillus acidophilus</i>)	?	—	—	—	—	—	—	—
C 44. <i>B. pyocyaneus</i> (<i>Pseudomonas aeruginosa</i>)	++	—	++	+++	+++	—	+++	+
C 46. <i>Pseudomonas</i> (denitrifying)	+	—	—	—	—	—	+++	—
C 54. <i>B. acidilactici</i>	—	—	+	+	—	—	++	+
C 60. <i>B. faecalis-alkaligenes</i>	—	—	—	+	—	—	—	—
C 62. <i>B. acidilactici</i> (St Georges)	—	—	+	+	—	—	++	+
C 66. <i>B. butyricus</i> (Hueppe)	—	—	—	—	—	—	—	—
C 67. <i>B. mesentericus</i>	—	—	—	—	—	—	—	—
C 70. <i>B. asiaticus-mobilis</i>	++	—	+++	++	+	+	++++	+
C 73. <i>B. alkalescens</i>	—	—	+	—	.	—	++	—
C 80. <i>Mycobact. phlei</i>	—	—	—	—	.	—	—	—
C 81. Butter bac. (Rabinowitsch)	—	—	+	—	.	—	+	—
C 82. Moeller's "Mist" bacillus	—	—	++	+	.	—	++	+

At this stage it became evident that the number of species which would grow on a purely synthetic medium, containing only inorganic salts and glucose, was disappointingly small, and it appeared possible that the addition of amounts of organic nitrogen which would introduce carbon in quantities representing only a small percentage of that present as glucose would render a much larger number of bacteria available, whilst not appreciably affecting the carbon balance. Large amounts of organic nitrogen would be out of the question, since they would vitiate the carbon balance or, at least, necessitate a cumbersome technique. Growth on media obtained by addition of 2 drops of 10 % peptone, 10 % asparagine, caseinogen digest or double strength yeast water to 10 cc. of the Czapek-Dox microcosmic salt medium was compared for a series of organisms, which failed to grow in the absence of organic nitrogen (see Table III).

Table III. *Effect of additions of small amounts of organic nitrogen to modified Czapek-Dox medium.*

Incubated 4 days at 34°.				
	Peptone	Asparagine	Caseinogen digest	Yeast water
C 38. <i>Streptococcus lacticus</i>	+	—	+	—
C 39. <i>B. guntheri</i> (<i>Streptococcus lactis</i>)	++	—	—	+
C 40. <i>B. bulgaricus</i> (<i>Lacto-bacillus bulgaricus</i>)	+	—	—	—
C 41. <i>B. acidophilus</i> (<i>Lacto-bacillus acidophilus</i>)	—	—	—	—
C 60. <i>B. faecalis-alkaligenes</i> (<i>Alkaligenes faecalis</i>)	+	—	++	—
C 66. <i>B. butyricus</i> (Hueppe)	++	—	++	—
C 67. <i>B. mesentericus</i> , No. 4	+	—	+++	++
C 80. <i>M. phlei</i>	+	—	+	+++
C 81. Butter bac.	++	++	++	++

As the addition of peptone appeared to give the best results it was adopted as a standard constituent to be added to the medium. The amount was fixed at 0.2 % corresponding to only 4 % by weight of the sugar, since this produced a considerable increase in growth whilst not interfering appreciably with the carbon balance sheets.

With the series of bacteria growing best at 25–30° the inorganic nitrogen proved relatively inefficient though here again the microcosmic salt was most favourable. The addition of peptone again rendered the medium much more suitable for bacterial growth.

The Czapek-Dox medium had been rather arbitrarily selected as the synthetic medium owing to its previous use for mould metabolism experiments. In order to justify its retention it was compared with other synthetic media, notably those of Prazmovski and Raulin (the latter *minus* tartrate). No advantage was obtained by the use of these two media, in fact the Czapek-Dox microcosmic salt and peptone medium was definitely superior.

The modified Czapek-Dox media employed were then as follows.

Medium A.

Microcosmic salt ($\text{NaNH}_4\text{HPO}_4, 4\text{H}_2\text{O}$)	...	...	4.921 g.
K_2HPO_4 ...	...	...	1.00 g.
KCl ...	...	...	0.50 g.
$\text{MgSO}_4, 7\text{H}_2\text{O}$...	...	...	0.50 g.
$\text{FeSO}_4, 7\text{H}_2\text{O}$...	...	...	0.01 g.
Glucose ...	...	...	50.00 g.
CaCO_3 ...	...	...	28.00 g.
Distilled water ...	...	...	1000 cc.

250 cc. of medium, in a 750 cc. conical flask, were used in each experiment.

Medium B. To the above medium was added 0.2 % of peptone for certain species in order to increase the range of species which could be employed.

Medium C. This medium represented an attempt to eliminate the use of calcium carbonate by the employment of a phosphate solution of high buffering power. Medium C contains 8.70 g. of dipotassium phosphate per litre in place of 1 g. as in Medium A, the calcium carbonate being omitted. Thus glucose represents the only source of carbon, resulting in simplification of the balance sheet. Only two examples of its use are given.

Modifications in technique.

Certain departures from the standard technique as evolved for the study of moulds were necessitated by the different habits of growth of bacteria and by the difference in the medium used.

When calcium carbonate was employed, a known amount was sterilised dry in a plugged tube which was weighed, and the contents were added to the metabolism flask under aseptic conditions. The empty tube and plug were next weighed and the exact weight of carbonate obtained by difference. The flask was then sown with the organism to be examined and incubated in an apparatus similar to that used in the mould work.

In order to allow for a considerable evolution of carbon dioxide from the action of organic acids on the calcium carbonate present, a new type of potassium hydroxide absorption bulb was fitted immediately after the first

sulphuric acid bubbler. In this the carbon dioxide entering was allowed to impinge on the surface of the potassium hydroxide solution, the delivery tube being cut off about half an inch above the surface of the absorbent liquid. Much of the carbon dioxide was removed in this manner without any danger of blocking the tube with solid potassium bicarbonate, as occasionally happened with the moulds.

The air supplied to aerobic bacteria was the same as for moulds, *viz.* about 500 cc. daily. The air inlet tube in most cases dipped below the surface of the medium, so that the air on entering bubbled through the liquid and to some extent agitated the calcium carbonate. If the bacteria under examination were known to be film formers, however, the standard mould procedure was adopted, the air being admitted above the liquid surface. In the case of anaerobic bacteria the air was swept out of the apparatus with a current of nitrogen, and no further attention was given until the end of the experiment when the gaseous metabolism products were again removed by nitrogen.

After the incubation had proceeded for the length of time necessary to metabolise the greater part of the sugar as determined from a control experiment the apparatus was swept out with a current of air (or nitrogen) and then the several portions of the apparatus treated substantially as in the mould experiments. Only in the case of the flask contents was a modification necessary.

No preliminary titration of the flask contents was undertaken owing to the presence of calcium carbonate. An idea of the amount of acid formed is obtained from the amount of carbonate which disappears during the experiment. This approximate figure is inserted in the balance sheet in place of the figure obtained from the titration of the metabolism solution.

The contents of the flask are filtered through a filter-paper on which is distributed a weighed amount (about 0.5 g.) of "Supercel Hyflo" in place of kieselguhr. This was found to give easier filtration and to be practically carbon-free. A further weighed amount (1.5 g.) was added to the metabolism solution which was well shaken before filtration. The dried bacterial or other insoluble residue, together with the unused calcium carbonate and the supercel, remains on the paper. After drying it peels off readily and is weighed and ground up in a mortar. The residual carbonate present is determined by warming a portion of the mixture with dilute acid in the wet combustion apparatus, the carbon dioxide being determined in the usual way. On another portion a total carbon estimation is performed. In this way the value for "carbon in bacteria" is obtained.

The main filtered solution is made up to a known volume (500 cc.) and the determinations necessary to construct a carbon balance sheet are made.

It was found that lactic acid and butylene glycol, which would normally be included in the "carbon unaccounted for" are such frequent products of bacterial metabolism that the separate determination of them was necessary, in order to decide whether other products were present in this fraction.

Procedure.

The residue from the determination of the "volatile neutral carbon" (for which 100 cc. is taken in the ordinary method) is made up to 100 cc. and 25 cc. removed. This neutral solution is extracted with ether for some 12 hours in a continuous apparatus. This has the effect of removing all the butylene glycol (along with any compounds of similar nature, if present). The extract is set aside for glycol determination and the residue is acidified with phosphoric acid and the extraction continued for another 12 hours. The resulting extract is used for the estimation of lactic acid.

Determination of butylene glycol.

Preliminary tests of the method suggested by Malaprade [1928] for polyhydric alcohols showed that butylene glycol when oxidised by periodic acid behaves in a similar manner to ethylene glycol. One molecule of butylene glycol requires 1 atom of periodate oxygen according to the equation:

Although this method would not in itself distinguish between butylene glycol and other polyhydric alcohols, it is a valuable aid to the construction of the carbon balance sheets when applied with discrimination and supplemented in doubtful cases by specific tests for, or by the isolation of, butylene glycol.

A standard solution of potassium periodate was made up such that on acidification and addition of excess potassium iodide it yielded a *N*/10 solution of iodine (calculated on the original volume). A known amount of the material extracted by ether from the neutral metabolism solution containing not more than 0.05 g. of butylene glycol was treated with 100 cc. of the periodate solution and 10 cc. of 10 % sulphuric acid. The mixture was allowed to stand overnight and then an excess of potassium iodide added and the free iodine titrated with *N*/10 thiosulphate. This titration figure must be more than 75 cc. which would correspond with the total conversion of the reagent.

Determination of lactic acid.

The method involving distillation with dilute permanganate and titration of the acetaldehyde evolved has been frequently modified, though the original form of the method is associated with the names of von Fürth and Charnass [1910].

The actual procedure is as follows.

A known amount of the solution containing 0.5–5.0 mg. of lactic acid is introduced into a Claisen flask together with 10 cc. of 10 % sulphuric acid. The flask is attached to an efficient Liebig condenser and a receiver consisting of a Volhard flask containing 20 cc. of *N*/20 sodium bisulphite. The Claisen flask is heated in a boiling water-bath and a current of steam passed in through

one neck. Through the other neck *N*/200 potassium permanganate solution is slowly dropped in until the liquid in the flask attains a permanent pink colour.

After a further 5 minutes the steam is shut off, the condenser washed down into the bisulphite and the contents of the Volhard flask washed out into a conical flask. Starch is added and the excess bisulphite is titrated with *N*/20 and finally *N*/1000 iodine until a pale blue colour is obtained. Excess of saturated sodium bicarbonate solution is then added to decompose the aldehyde-bisulphite compound and the solution is titrated with *N*/1000 iodine to re-establish the colour. With 1 cc. of 0.0997 % lithium lactate duplicate titrations obtained were 20.2 and 20.9 cc. of *N*/1000 iodine. The mean 20.55 cc. corresponds to 0.986 mg. lithium lactate, giving an error of 1 %.

The one difficulty which remains is due to the slight volatility of lactic acid. During the distillation of the volatile acids a certain amount of the lactic acid present distils over and is recorded as volatile acids. Since a separate lactic acid estimation is performed on another portion of the metabolism solution the carbon accounted for is slightly higher than it should be. This is borne out by the fact that in some cases the total carbon accounted for is higher than that present in solution. The error introduced in this manner is however fairly small. In distillation tests carried out with lactic acid present in about double the amount that was present in any metabolism experiment, lactic acid distilling over under the conditions of the experiment corresponded to 0.4 g. of carbon. This would, of course, represent a maximum error; it will be seen from the balance sheets that the actual error is usually considerably less, since the total volatile acid is frequently less than this figure even when lactic acid is present.

An attempt was made to calculate the respiration coefficient as for the moulds but when calcium carbonate is present there is difficulty in distinguishing between carbon dioxide arising from the carbonate and that due to respiration. It was assumed, however, for this purpose that all the carbonate disappearing had given rise to the theoretical amount of carbon dioxide, which was therefore deducted from the total carbon dioxide recovered.

Since the weight of bacteria was only determinable indirectly and was in any case small, this weight could not be accurately obtained. Hence the figure for the % carbon in bacteria was unreliable and was not determined although, of course, the total carbon in the bacteria was estimated.

It was found that a number of the organisms which grow quite well on the medium selected are unable to metabolise more than a small portion of the sugar supplied. Consequently, the standard procedure adopted was to carry out a control experiment with each organism giving good growth on the synthetic medium.

In these control experiments (Table IV) the organism was sown into a flask, fitted with tubes for aeration, and containing the medium as used in the metabolism experiments. It was incubated at a suitable temperature,

Table IV.

Cat. No.	Name	Source	Medium	Temp. °C.	Days	Sugar %	
						Start	Finish
C 39.	<i>B. glntheri</i> (<i>Streptococcus lactis</i>)	N.C.T.C. No. 661	B	34	26	5-02	3-16
C 38.	<i>Streptococcus lactis</i>	N.C.T.C. No. 1551	B	34	21	5-17	3-74
C 87.	<i>Leuconostoc</i> species	Isolated from molasses at Ardeer	B	34	16	5-02	1-83
C 79.	<i>M. flarus</i> (Flügge)	N.C.T.C. No. 1629	B	28	14	5-39	5-25
C 78.	<i>M. roseus</i> (Bumm)	N.C.T.C. No. 195	B	28	28	4-91	4-63
C 44.	<i>B. pyocyaneus</i> (<i>Pseudomonas aeruginosa</i>)	N.C.T.C. No. 1999	A	34	8	5-14	4-68
C 44.	<i>B. pyocyaneus</i> (<i>Pseudomonas aeruginosa</i>)	N.C.T.C. No. 1999	B	34	31	5-02	2-44
C 46.	<i>Pseudomonas</i> (dentrifying)	N.C.T.C. No. 933	A	34	6	5-14	4-56
C 35.	<i>B. rossicus</i> Kell. (<i>Cellulomonas rossica</i>)	N.C.T.C. No. 1655	A	34	11	5-14	4-83
C 4.	<i>B. proteus-vulgaris</i> (<i>Proteus vulgaris</i>)	N.C.T.C. No. 67	A	34	6	5-21	3-66
C 47.	<i>B. phytophthorus</i> Appel	N.C.T.C. No. 1985	B	28	20	5-50	3-12
C 45.	<i>B. aroidae</i> Townsend (<i>Erwinia aroidae</i>)	N.C.T.C. No. 1987	B	28	31	5-35	0-10
C 51.	<i>B. carotovorus</i> Jones (<i>Erwinia carotovora</i>)	N.C.T.C. No. 1975	B	28	27	4-56	1-90
C 43.	<i>B. marginale</i> N. A. Brown (<i>Phytomonas marginale</i>)	N.C.T.C. No. 1981	B	28	15	5-12	4-70
C 50.	<i>B. campestre</i> Pammel (<i>Phytomonas campestre</i>)	N.C.T.C. No. 1958	B	28	17	5-45	4-60
C 63.	<i>B. hyacinthi</i> Walker	N.C.T.C. No. 387	B	28	26	5-54	4-68
C 64.	<i>B. aptatum</i> Brown and Jamieson (<i>Phytomonas aptata</i>)	N.C.T.C. No. 1977	B	28	28	4-89	2-41
C 65.	<i>B. mabuccarum</i> E. F. Smith (<i>Phytomonas mabuccera</i>)	N.C.T.C. No. 1960	B	28	11	5-20	5-20
C 75.	<i>B. cannae</i> Bryan (<i>Phytomonas cannae</i>)	N.C.T.C. No. 1956	B	28	15	5-49	5-48
C 54.	<i>B. acidi-lactici</i>	N.C.T.C. No. 87	B	34	6	5-02	2-24
C 6.	<i>B. acidi-lactici</i> Grotenfelt, Hueppe (<i>Escherichia acidi-lactici</i>)	N.C.T.C. No. 413	A	34	6*	2-19	2-24
C 62.	<i>B. acidi-lactici</i> (St Georges)	N.C.T.C. No. 123	B	34	10	5-02	0-36
C 34.	<i>B. coli</i> Esch. (<i>Escherichia coli</i>)	N.C.T.C. No. 86	A	34	6*	4-91	2-51
C 37.	<i>Esch.</i> species	Isolated from milk at Ardeer	A	34	6*	4-91	1-09
C 26.	<i>Esch.</i> species	Isolated from milk at Ardeer	A	34	6*	4-91	3-12
C 24.	<i>B. lactis-aerogenes</i> Esch. (<i>Aerobacter aerogenes</i>)	N.C.T.C. No. 418	A	34	9*	4-91	0-02
C 24.	<i>B. lactis-aerogenes</i> Esch. (<i>Aerobacter aerogenes</i>)	N.C.T.C. No. 418	C	34	7*	4-93	1-37
C 12.	<i>Aerobacter cloacae</i>	Isolated at Ardeer	A	34	6*	4-91	0-86
C 12.	<i>Aerobacter cloacae</i>	Isolated at Ardeer	C	34	6*	4-93	2-77
C 25.	Leather bacillus (Houston)	N.C.T.C. No. 672	A	34	6*	4-91	0-08
C 27.	<i>Aerobacter</i> species	Isolated at Ardeer	A	34	6*	4-91	0-08
C 70.	<i>B. asiaticus-mobis</i> Castellani	N.C.T.C. No. 1747	A	34	2	5-14	0-0
C 73.	<i>B. alkaliscens</i> Andrews	N.C.T.C. No. 1601	B	34	17	5-02	1-95
C 60.	<i>B. fecalis-alcigenes</i> Petruschky (<i>Alcaligenes fecalis</i>)	N.C.T.C. No. 415	B	34	8	5-02	4-64
C 72.	<i>B. ureae</i> Leube	N.C.T.C. No. 1654	B	28	26	5-06	2-13
C 98.	<i>B. albolactis</i> (Löffler) Migula (298)	American Type Culture Collection, No. 3	B	28	17	5-22	2-29
C 67.	<i>B. mesentericus</i> No. 4	N.C.T.C. No. 2589	B	34	17	5-02	3-35
C 1.	<i>B. mesentericus</i>	Isolated at Ardeer	B	34	8	5-02	4-31
C 90.	<i>B. megatherium</i> De Bary	N.C.T.C. No. 654	B	34	10	4-77	3-92
C 80.	Rod bacillus, oval spores	Isolated at Ardeer	B	34	21	5-17	3-39
C 28.	<i>B. mycoides</i>	Isolated at Ardeer	B	34	21	5-17	2-86
C 69.	<i>B. bifermantans</i> Tissier and Martelly (<i>Clostridium bifermantans</i>)	N.C.T.C. No. 1340	B	34	15	4-77	3-38
C 96.	<i>Clostr. saccharobutyricum</i> Beijerinck	American Type Culture Collection, No. 860	B	34	7	5-17	1-47
C 94.	<i>Clostr. pasteurianum</i> Winogradsky	American Type Culture Collection, No. 861	B	34	7	5-17	1-39
C 80.	<i>Myc. phlei</i> Sohn.	N.C.T.C. No. 1471	B	34	21	5-17	4-23
C 82.	Moeller's "Mist" Bacillus (<i>Myc. stercoris</i>)	N.C.T.C. No. 526	B	34	17	5-02	3-97
C 81.	Bacillus (acid fast) from butter (butter bacillus of Rabinowitsch)	N.C.T.C. No. 524	B	34	8	5-02	4-64

N.C.T.C. refers to the British National Collection of Type Cultures, Lister Institute.

samples being withdrawn aseptically from time to time for sugar determination. Only if the sugar disappeared at a reasonable rate was it considered worth while to perform a complete metabolism experiment. In the event of the metabolism experiment being carried out, the control gave a useful indication as to the rate of progress of the fermentation, so that the correct time could be allotted for the incubation period.

Preliminary controls are lacking for some of the organisms used for the earlier balance sheets. In these cases the balance sheet figures are inserted in the table for the sake of completeness. Where this is done it is indicated by an asterisk. The results of the carbon balance sheet determinations are summarised in the Tables V-IX.

Table V.

	<i>Leuconostoc</i> species	<i>B. aroideae</i>	<i>B. caroto-</i> <i>vorus</i>	<i>B. proteus-</i> <i>vulgaris</i>	<i>B. proteus-</i> <i>vulgaris</i>
Medium used ...	B	B	B	B	B
Catalogue number ...	C 87	C 45	C 51	C 4	C 4
Temperature ...	34°	28°	28°	34°	34°
Incubation period in days ...	13	26	26	6	27
Carbon balance sheet (g.)					
Carbon in solution (start) ...	5.109	5.189	5.189	5.214	5.189
„ added as CaCO ₃ ...	0.840	0.837	0.839	0.838	0.835
Total carbon (start) ...	5.949	6.026	6.028	6.052	6.024
Carbon in H ₂ SO ₄ ...	0.002	0.007	0.003	0.002	0.007
„ in CO ₂ ...	0.651	1.324	0.576	0.383	0.435
„ in bacteria ...	0.100	0.233	0.059	0.081	0.167
„ in residual CaCO ₃ ...	0.450	0.579	0.450	0.625	0.527
„ in solution ...	4.679	3.786	4.848	4.941	4.840
„ accounted for ...	5.882	5.929	5.936	6.032	5.976
„ accounted for (%) ...	98.9	98.4	98.5	99.7	99.2
Analysis of solution (g.)					
Carbon in residual glucose ...	2.266	0.042	2.416	3.662	3.328
„ in CO ₂ in solution ...	0.006	0.006	0.005	0.006	0.005
„ in volatile acids ...	0.457	0.288	0.475	0.344	0.313
„ in non-volatile acids (Ca ppt.) ...	0.146	0.336	0.323	0.084	0.117
„ in volatile neutral compounds ...	0.194	0.269	0.225	0.237	0.420
„ in synthetic compounds ...	0.063	0.119	0.101	0.057	0.053
„ as butylene glycol ...	0.311	2.136	Nil	—	0.168
„ as lactic acid ...	1.008	0.288	1.152	—	0.360
Total carbon accounted for ...	4.451	3.484	4.697	4.390	4.764
Total carbon in solution ...	4.679	3.786	4.848	4.941	4.840
Carbon unaccounted for (by difference) ...	0.228	0.302	0.151	0.550	0.076
Residual glucose (%)					
Glucose (by polarimeter) ...	0.828	Nil	1.187	1.774	1.653
„ (by Shaffer-Hartmann) ...	1.133	0.021	1.208	1.831	1.664
„ (by Wood-Ost) ...	1.104	0.021	1.182	1.805	1.692
Acids					
Titration (calculated) (cc. N/1) ...	65.0	43.0	64.8	35.5	51.3
Volatile acids (cc. N/1) ...	12.19	6.75	15.98	14.4	13.4
Barium salts (weight in g.) ...	2.050	1.083	2.584	1.996	1.692
Calcium salts (weight in g.) ...	0.451	1.020	0.918	0.104	0.098
Volume of oxygen absorbed (cc.) ...	395	590	350	93	210
Respiration coefficient ...	1.23	3.37	10.0	3.41	1.13
Volume of hydrogen evolved (cc.) ...	Nil	Nil	Nil	Trace	Nil

The bacterial strains tested in the control experiments (Table IV), and those employed in the complete metabolism experiments (Tables V to IX)

Table VI.

	<i>B. acidi-lactici</i>	<i>B. acidi-lactici</i> Grotten-felt Hueppe	<i>B. acidi-lactici</i> (St Georges)	<i>B. coli</i> (<i>E. coli</i>)	<i>Esche-richia</i> species from milk	<i>Esche-richia</i> species from milk
Medium used	B	A	B	A	A	A
Catalogue number	C 54	C 6	C 62	C 34	C 37	C 26
Temperature	34°	34°	34°	34°	34°	34°
Incubation period in days ...	6	6	6	6	6	6
Carbon balance sheet (g.)						
Carbon in solution (start)	5.214	4.912	5.214	4.912	4.912	4.912
„ added as CaCO ₃	0.840	0.833	0.837	0.839	0.835	0.834
Total carbon (start)	6.054	5.745	6.051	5.751	5.747	5.746
Carbon in H ₂ SO ₄	0.005	0.003	0.003	0.004	0.004	0.002
„ in CO ₂	0.920	0.566	0.791	0.590	0.838	0.411
„ in bacteria	0.202	0.091	0.205	0.262	0.156	0.054
„ in residual CaCO ₃	0.200	0.388	0.331	0.288	0.207	0.549
„ in solution	4.568	4.593	4.636	4.617	4.443	4.653
„ accounted for	5.895	5.641	5.966	5.761	5.648	5.669
„ accounted for (%)	97.4	98.2	98.6	100.2	98.3	98.6
Analysis of solution (g.)						
Carbon in residual glucose	1.101	2.190	2.030	2.508	1.092	3.121
„ in CO ₂ in solution	0.008	0.006	0.003	0.004	0.005	0.004
„ in volatile acids... ..	0.524	0.253	0.719	0.434	0.659	0.381
„ in non-volatile acids (Ca ppt.)	0.311	0.263	0.097	0.105	0.115	0.045
„ in volatile neutral compounds	0.539	0.269	0.436	0.287	0.376	0.186
„ in synthetic compounds	0.074	0.052	0.032	0.040	0.047	0.038
„ as butylene glycol	Nil	Nil	Nil	0.022	0.042	0.072
„ as lactic acid	1.395	1.260	0.990	0.882	1.518	0.900
Total carbon accounted for	3.952	4.293	4.307	4.282	3.854	4.747
Total carbon in solution	4.568	4.593	4.636	4.617	4.443	4.653
Carbon unaccounted for (by difference)	0.616	0.300	0.329	0.335	0.589	—
Residual glucose (%)						
Glucose (by polarimeter)	0.600	1.280	0.988	1.345	0.662	1.539
„ (by Shaffer-Hartmann)	0.551	1.095	1.015	1.254	0.546	1.560
„ (by Wood-Ost)	0.585	1.128	1.019	1.290	0.598	1.525
Acids						
Titration (calculated) (cc. N/1) ...	106.7	74.2	84.4	91.9	104.7	47.5
Volatile acids (cc. N/1)	20.2	7.50	26.1	16.6	23.5	14.9
Barium salts (weight in g.)	2.882	1.331	4.019	2.400	3.457	2.182
Calcium salts (weight in g.)	0.812	0.772	0.055	0.034	0.146	—
Volume of oxygen absorbed (cc.) ...	119	13	131	125	66	77
Respiration coefficient	4.40	17.3	4.06	0.57	6.05	3.07
Volume of hydrogen evolved (cc.) ...	285	82.5	388	Nil	165	Nil

are listed by the names under which they were received, the source from which they were obtained being indicated in each case. No attempt has been made to study these strains from the systematic point of view; nor, in the present confused state of bacterial nomenclature, has it seemed desirable to insert alternative names, in accordance with any one of the systems in current use.

The strains brought together in Tables VI and VII are certainly closely related. Those in Tables V and IX include widely separated species. Our

Table VII.

		<i>B.</i> <i>lactis-</i> <i>aero-</i> <i>genes</i>	<i>B.</i> <i>lactis-</i> <i>aero-</i> <i>genes</i>	<i>A.</i> <i>cloacae</i>	<i>A.</i> <i>cloacae</i>	Leather bacillus Houston	<i>Aero-</i> <i>bacter</i> species from milk
Medium used	...	A	C	A	C	A	A
Catalogue number	...	C 24	C 24	C 12	C 12	C 25	C 27
Temperature	...	34°	34°	34°	34°	34°	34°
Incubation period in days	...	9	7	6	6	6	6
Carbon balance sheet (g.)							
Carbon in solution (start)	...	4.912	4.928	4.912	4.928	4.912	4.912
„ added as CaCO ₃	...	0.835	—	0.831	—	0.836	0.839
Total carbon (start)	...	5.747	4.928	5.743	4.928	5.748	5.751
Carbon in H ₂ SO ₄	...	0.019	0.004	0.010	0.005	0.004	0.006
„ in CO ₂	...	1.677	0.864	1.131	0.478	1.251	1.254
„ in bacteria	...	0.256	0.090	0.183	0.003	0.168	0.138
„ in residual CaCO ₃	...	0.693	—	0.504	—	0.306	0.371
„ in solution	...	2.750	3.784	3.842	4.494	3.948	3.885
„ accounted for	...	5.395	4.742	5.670	4.980	5.677	5.654
„ accounted for (%)	...	93.9	96.2	98.7	101.0	98.8	98.3
Analysis of solution (g.)							
Carbon in residual glucose	...	0.017	1.366	0.863	2.768	0.081	0.083
„ in CO ₂ in solution	...	0.008	0.006	0.006	0.006	0.006	0.006
„ in volatile acids...	...	0.155	0.106	0.326	0.198	0.648	0.618
„ in non-volatile acids (Ca ppt.)	...	0.062	0.341	0.104	0.483	0.072	0.030
„ in volatile neutral compounds	...	0.687	0.518	0.684	0.362	0.604	0.527
„ in synthetic compounds	...	0.020	0.008	0.016	0.003	0.057	0.048
„ as butylene glycol	...	1.527	0.912	1.603	0.465	0.661	0.968
„ as lactic acid	...	0.157	0.504	0.128	0.072	1.710	1.710
Total carbon accounted for	...	2.633	3.761	3.730	4.357	3.839	3.990
Total carbon in solution	...	2.750	3.784	3.842	4.494	3.948	3.885
Carbon unaccounted for (by difference)	...	0.117	0.023	0.112	0.137	0.109	—
Residual glucose (%)							
Glucose (by polarimeter)	...	0.113	0.773	0.462	1.418	0.275	0.243
„ (by Shaffer-Hartmann)	...	0.008	0.683	0.432	1.384	0.203	0.042
„ (by Wood-Ost)	...	—	0.686	0.435	1.378	0.192	0.038
Acids							
Titration (calculated) (cc. N/1)	...	23.6	15.0	54.6	8.0	88.3	77.9
Volatile acids (cc. N/1)	...	—	3.37	20.3	6.88	18.4	12.8
Barium salts (weight in g.)	...	0.085	0.450	2.579	1.195	2.902	2.367
Calcium salts (weight in g.)	...	0.114	4.108	0.155	4.539	—	—
Volume of oxygen absorbed (cc.)	...	652	104	117	58	217	151
Respiration coefficient	...	4.4	15.4	12.8	15.4	6.2	9.7
Volume of hydrogen evolved (cc.)	...	392	Trace	Nil	Nil	262	196

purpose, in these preliminary experiments, has been to demonstrate the applicability of this method to the study of bacterial metabolism, and to cover a wide range of species or genera before selecting some of them for more detailed systematic study.

Table VIII.

	<i>B. asiaticus-mobilis</i> (Cast)	<i>B. asiaticus-mobilis</i> (Cast)	<i>B. alkalescens</i> (Andrewes)
Medium used	A	A	B
Catalogue number	C 70	C 70	C 73
Temperature	34°	34°	34°
Incubation period in days	2	2	41
Carbon balance sheet (g.)			
Carbon in solution (start)	4.912	4.912	5.191
„ added as CaCO ₃	0.840	0.838	0.840
Total carbon (start)	5.752	5.750	6.031
Carbon in H ₂ SO ₄	0.010	0.012	0.003
„ in CO ₂	1.140	1.451	0.540
„ in bacteria	0.233	0.248	0.190
„ in residual CaCO ₃	0.613	0.585	0.236
„ in solution	3.217	3.407	4.870
„ accounted for	5.213	5.703	5.839
„ accounted for (%)	90.6	99.2	96.8
Analysis of solution (g.)			
Carbon in residual glucose	0.013	0.000	1.960
„ in CO ₂ in solution	0.006	0.006	—
„ in volatile acids	0.588	0.326	0.515
„ in non-volatile acids (Ca ppt.)	0.142	0.097	0.221
„ in volatile neutral compounds	0.941	0.984	0.122
„ in synthetic compounds	0.065	0.185	0.102
„ as butylene glycol	1.339	1.551	0.380
„ as lactic acid	Nil	Nil	1.300
Total carbon accounted for	3.094	3.149	4.600
Total carbon in solution	3.217	3.407	4.870
Carbon unaccounted for (by difference)	0.123	0.258	0.270
Residual glucose (%)			
Glucose (by polarimeter)	0.113	0.162	1.191
„ (by Shaffer-Hartmann)	0.007	Nil	0.980
„ (by Wood-Ost)	—	Nil	0.860
Acids			
Titration (calculated) (cc. N/1)	37.9	42.2	100.8
Volatile acids (cc. N/1)	8.54	6.49	17.4
Barium salts (weight in g.)	2.064	1.203	2.614
Calcium salts (weight in g.)	0.436	0.215	0.626
Volume of oxygen absorbed (cc.)	79	89	580
Respiration coefficient	21.6	25.1	—
Volume of hydrogen evolved (cc.)	308	430	Nil

Review of balance sheets.

The figures given in parentheses below are taken direct from the balance sheets and denote the weight of carbon, in the class of products mentioned, which is obtained from 5 g. of carbon as glucose. Hence the approximate yield % is obtained by multiplying the quoted figure by 20.

Leuconostoc. The species employed gives a high yield of lactic acid (1.008) and moderate yields of volatile acids (0.457) and of butylene glycol (0.311), considering that nearly half the original sugar remains unfermented.

B. aroideae and *B. carotovorus*. The balance sheets for these two species

Table IX.

Medium used	...	...	<i>B. ureae</i> Leube (<i>Urobacillus</i>) B	<i>B. albo-</i> <i>lactis</i> B	<i>Clostridium</i> <i>saccharo-</i> <i>butyricum</i> B (anaerobic)	<i>Clostridium</i> <i>pasteurianum</i> B (anaerobic)
Catalogue number	...	...	C 72	C 98	C 96	C 94
Temperature	...	...	28°	28°	34°	34°
Incubation period in days	...	...	27	27	7	9
Carbon balance sheet (g.)						
Carbon in solution (start)	...	...	5.189	5.189	5.477	5.315
„ added as CaCO ₃ ...	...	...	0.839	0.839	1.681	1.666
Total carbon (start)	...	...	6.028	6.028	7.158	6.981
Carbon in H ₂ SO ₄	...	...	0.001	0.001	0.001	0.003
„ in CO ₂	...	...	0.650	0.633	1.211	1.198
„ in bacteria	...	...	0.157	0.155	0.506	0.509
„ in residual CaCO ₃	...	...	0.555	0.336	1.123	1.080
„ in solution	...	...	4.641	4.781	4.000	3.903
„ accounted for	...	...	6.004	5.906	6.841	6.693
„ accounted for (%)	...	...	99.6	98.0	95.6	95.9
Analysis of solution (g.)						
Carbon in residual glucose	...	...	2.486	1.840	1.346	1.538
„ in CO ₂ in solution	...	...	0.005	0.006	0.006	0.006
„ in volatile acids...	...	...	0.287	0.532	1.892	1.744
„ in non-volatile acids (Ca ppt.)	...	...	0.184	0.081	0.104	0.121
„ in volatile neutral compounds	...	...	0.136	0.066	0.145	0.291
„ in synthetic compounds	...	...	0.061	0.075	0.109	0.126
„ as butylene glycol	...	...	0.841	0.062	—	—
„ as lactic acid	...	...	0.756	1.530	—	—
Total carbon accounted for	...	...	4.756	4.202	3.602	3.826
Total carbon in solution	...	...	4.641	4.781	4.000	3.903
Carbon unaccounted for (by difference)	...	...	0.115	0.579	0.398	0.077
Residual glucose (%)						
Glucose (by polarimeter)	...	...	0.911	0.563	0.583	0.664
„ (by Shaffer-Hartmann)	...	...	1.243	0.920	0.673	0.769
„ (by Wood-Ost)	...	...	1.206	0.900	0.664	0.710
Acids						
Titration (calculated) (cc. N/1)	...	...	47.3	83.8	93.0	97.7
Volatile acids (cc. N/1)	...	...	7.80	16.0	49.3	45.7
Barium salts (weight in g.)	...	...	1.070	2.590	12.51	10.65
Calcium salts (weight in g.)	...	...	0.537	Nil	0.162	0.216
Volume of oxygen absorbed (cc.)	...	...	430	470	Nil (anaerobic)	Nil (anaerobic)
Respiration coefficient	...	...	1.59	0.52	—	—
Volume of hydrogen evolved (cc.)	...	...	Nil	Nil	1263	1299

show considerable differences. *B. aroideae*, C 45, gives a very high yield of butylene glycol (2.136) equivalent to over 40 % of the original sugar, and very little lactic acid (0.288), whereas *B. carotovorus*, C 51, gives no butylene glycol and a high yield of lactic acid (1.152), corresponding to about 40 % on the sugar consumed.

B. proteus-vulgaris. In the first balance sheet the fermentation had not proceeded far although the incubation period was 6 days; therefore butylene

glycol and lactic acid were not determined. A second balance sheet experiment was performed in which the culture was incubated for 27 days. It was found that very little further fermentation had taken place, although the culture was still viable. With this organism, up to 30 % of the original sugar disappears in the early stages, while very little subsequent change is obvious. The early cessation of fermentation may be due to the exhaustion of one of the nutrients of the medium or to inhibition by products of bacterial metabolism.

B. acidi-lactici and *B. coli* group. This group is notable for the fact that it produces large amounts of lactic acid (up to 1.52) but negligible amounts of butylene glycol. A fair amount of volatile acid is produced (0.25–0.72), and a smaller amount of volatile neutral compounds (0.19–0.54). Too much importance should not be attached to the volatile acid determination, owing to the presence of large amounts of lactic acid. For the same reason, the "carbon unaccounted for" will tend to be low. The most interesting point is the fact that the "carbon unaccounted for" is not negligible; in two cases, it reaches the figures of 0.62 and 0.59 respectively, although 20 % of the original sugar still remains, and in spite of the fact that the "carbon unaccounted for" figure is probably too low, for the reason given above. These two species C 54 and C 37 are marked out for further investigation.

Aerobacter. This group produces lactic acid and butylene glycol. All grades are shown from a high yield of lactic acid (1.71) and a moderately low yield of butylene glycol to a low yield of lactic acid and a high yield of butylene glycol (1.60). In all cases, a fair amount of volatile neutral substances (probably ethyl alcohol) is formed (0.36–0.68). In no case is there any appreciable amount of "carbon unaccounted for". The balance sheets for *B. lactis-aerogenes* and *A. cloacae* bear out the results obtained by Harden *et al.* [1906; 1912, 1, 2].

The *B. asiaticus-mobilis* Castellani shows a remarkably rapid fermentation, the sugar in a 5 % solution being completely metabolised in less than 48 hours. The chief characteristic is the high yield of butylene glycol (1.34 and 1.55) which would make the organism specially suitable for the preparation of this glycol. A considerable amount of alcohol (0.94, 0.98) is formed, and a fair amount of volatile acid (0.59, 0.33). No lactic acid is produced, and the carbon unaccounted for is negligible.

A quantity of butylene glycol was prepared in the laboratory from the fermentation of glucose by *B. asiaticus-mobilis*. The fermentation was carried out in 750 cc. and 1 litre flasks containing 250 cc. and 350 cc. of medium respectively, also in 5 litre flasks containing 4 litres of medium. There was little difference in the yield obtained in the two cases, but the fermentation period was longer in the large flasks, due probably to the settling of the calcium carbonate with less rapid neutralisation of the acid produced. This effect was naturally less pronounced when the liquid was distributed in a shallow layer. Aeration had little or no effect on the fermentation, but as it provided a means of agitation of the carbonate it was advantageous from this point of view.

The best yields were obtained from a comparatively slow fermentation (7 days) occurring in presence of heavy carbonate and representing a 28–29 % recovery. No explanation is offered for this somewhat curious result.

The fermentation of 5 % sugar solution in small flasks occupied 2 days, but with higher concentrations of sugar longer periods were required—thus 12½ % sugar with increased amount of carbonate present needed 14 days for complete fermentation.

The method of recovery of the butylene glycol was very simple. The solution was filtered from undissolved calcium carbonate, evaporated to small bulk *in vacuo* and finally distilled *in vacuo*. Usually the solid residue remaining after distillation was washed with alcohol and a further small amount of the glycol recovered in this way. The total amount of butylene glycol recovered was 2435 g. representing an average yield of 22·3 % on the sugar fermented. The results of the experiments are summarised in Table X.

Table X. *Production of butylene glycol.*

Time in days	Initial sugar		Final sugar		Sugar fermented g.	Glycol recovered g.	% yield	
	%	Weight in g.	%	Weight in g.				
5	5	25	0·21	1·0	24	20	20·2	—
25	10	50	Nil	Nil	50			
20	5	25	Nil	Nil	25			
4	5	1750	Nil	Nil	1750	314	15·5	—
7	5	200	0·22	8·8	191			
4	5	87·5	Nil	Nil	88			
6	5	1663·5	0·28	93·2	1570	450	28·6	Heavy CaCO ₃
2	5	1750	0·45	157·5	1592	287	18·0	—
8	5	1200	0·40	96·0	1104	261	23·7	—
3	5	1750	Nil	Nil	1750	496	25·6	—
7	5	200	0·32	12·8	187			
7	5	440	Nil	Nil	440	126	28·6	Heavy CaCO ₃
2	5	600	Nil	Nil	600	125	20·8	Aerated
4	5	400	0·22	17·6	382	87	22·8	Non-aerated
2	7·5	300	0·42	16·8	283	68	24·0	Aerated
6	10	400	Nil	Nil	400	89	22·3	Aerated
14	12·5	500	0·2	8	492	112	22·8	Aerated
Total					10,928	2435	22·3	

B. alkalescens Andrewes, C 73. This organism produces a large amount of lactic acid (1·30), considerably smaller amounts of butylene glycol (0·38) and volatile acids (0·515). The carbon unaccounted for is small in amount.

B. ureae and *B. albolactis*. These two organisms show several points of interest. *B. ureae* (C 72) shows a small surplus in the balance sheet which is most unusual though possibly accounted for by the volatility of lactic acid. *B. albolactis* (C 98) on the other hand shows a “carbon unaccounted for” figure of 0·58, which is considerable in view of the amount of unfermented glucose remaining. The amount of “non-volatile acids” is low in both cases, but a fair amount of “volatile acids” is formed, particularly in the case of C 98. There is a curious discrepancy in both cases between the glucose determinations, the polarimeter figure being considerably lower than that obtained

by copper reduction. This appears to indicate the formation of some laevo-rotatory compound. *B. albolactis* thus merits further investigation.

Clostridium. These anaerobic species are remarkably similar in behaviour. The characteristic is the production of a large amount of volatile acids (1.89 and 1.74). Butylene glycol and lactic acid were not determined, but could only be present in small amount, since the "carbon unaccounted for" is low.

DISCUSSION.

In performing these balance sheet experiments the time allotted for each fermentation varies since the time was chosen according to the rate of fermentation. An endeavour was made in each case to interrupt the experiment at such a point that most, but not all, of the sugar had disappeared, so that the amount of intermediate metabolic products should be as large as possible. This involves wide differences in the incubation periods of the various organisms. In cases where the fermentation is slow, there is a greater likelihood of bacterial autolysis. This introduces the possibility of carbohydrate breakdown or attack on the intermediate products by the enzymes liberated by cell autolysis, in addition to the coordinated fermentative action of these enzymes when under the control of the living cell. This difficulty cannot be entirely obviated in work of this type, but it would be advisable in future experiments to determine growth curves for each organism studied, in order to correlate the fermentation during the rapid growth period with that occurring during the autolysis period, as indicated by a decline in the viable count.

The possibility of autolysis raises an interesting point in the interpretation of the balance sheets. The "carbon in bacteria" will not represent the total amount of carbon utilised to form cell-matter if a portion of the cells autolyses in the later stages of fermentation. However, it appears likely that a large proportion of the autolysis products will, in such cases, be found in the "carbon in synthetic products", *i.e.* in the carbon compounds precipitated by "colloidal iron."

It has been shown that the quantitative method for the classification of the types of metabolic products of micro-organisms although hitherto used only for moulds, applies equally well to bacteria provided certain minor modifications are made. The chief difficulty consists in finding a suitable synthetic medium containing no carbon apart from the glucose supplied for fermentation. Although some of the organisms were able to grow and ferment on such a medium, the number was found to be very limited, and a much wider field became available by the introduction of small amounts of organic nitrogen in the form of peptone. This is justifiable so long as the amount of carbon thus introduced is small in relation to the carbon present as glucose.

Reference has already been made to the fact that addition of calcium carbonate to the medium was found more generally useful as a method of neutralising the acidity formed than the use of large amounts of phosphate.

In the case of two organisms, *Aerobacter aerogenes* and *cloacae* (C 24 and C 12), comparative balance sheets were obtained using the carbonate and the phosphate buffer media. A study of these reveals the fact that although fermentation was retarded on the phosphate medium, the products were not widely different in nature, the chief difference being a considerable increase of the non-volatile acid fraction with excess of phosphate. This may be due to the formation of hexosephosphate. In a third case—that of *B. acidi-lactici* (Hueppe), C 6, on the phosphate medium practically no gas was liberated during the 6 days of the experiment, only about half the sugar had disappeared and at the end of the period the organism was apparently dead. The balance sheet is not given. On the carbonate medium, the fermentation was definitely faster and was still in progress at the end of 6 days. The phosphate buffering appears inadequate when the organism produces a large amount of acid, as in the case of C 6.

Many of the bacteria appear to utilise glucose merely for growth purposes. This is shown by the large number of control experiments in which only a small proportion of the sugar disappeared. These organisms cannot therefore be said to ferment the sugar in the usual sense of the term.

We may now compare briefly the types of products obtained from bacteria with those derived from moulds. The mould products (apart from mycelium and carbon dioxide) group themselves chiefly in the classes "volatile neutral substances" (ethyl alcohol), "non-volatile acids" and "carbon unaccounted for", the last named, when large in amount, often consisting of mannitol or glycerol. Only traces of volatile acids are produced. Lactic acid and butylene glycol have never been reported as mould metabolic products. With moulds, on the other hand, various synthetic substances such as polysaccharides, sterols, pyrones, quinonoid and phenolic substances, complex fatty acids, etc., are formed.

The main products of bacterial metabolism are "volatile neutral substances", "volatile acids" and much smaller amounts of "non-volatile acids". Lactic acid and butylene glycol are of frequent occurrence and are often produced in considerable quantity. Thus species of *Aerobacter* give varying amounts of both products, of *Escherichia* give considerable amounts of lactic acid but little butylene glycol, and of *B. asiaticus mobilis* (Cast.) large amounts of butylene glycol but no lactic acid.

Hydrogen is a frequent product of bacterial action, particularly in the case of anaerobes, but has never been obtained from moulds.

Although polysaccharides are known to be formed [Grey, 1924], no evidence has yet been obtained of any phenolic substances such as are elaborated by moulds.

The products of bacterial metabolism have been shown to be, in general, more volatile than those of moulds. Thus, volatile acids replace the non-volatile acids, butylene glycol is obtained instead of mannitol or glycerol, and so on. This may be due to the fact that moulds are obligate aerobes, whilst many bacteria are facultative anaerobes, and consequently mould

metabolic products are more highly oxidised. It is well known that an increase in the oxygen content of organic substances (as hydroxyl or carboxyl groups) lowers their volatility. In this connection it is interesting to note [Hermann and Neuschul, 1931] that eight strains of acetic acid bacteria, the reactions of which are almost purely oxidative and only feebly hydrolytic, give products such as gluconic acid, substances which are more akin to those of mould metabolism.

The oxidative nature and greater synthetic activity of mould fermentation may be said to be the chief characteristics which differentiate it from bacterial fermentation.

SUMMARY.

The Czapek-Dox medium as used for the determination of mould metabolism products by the balance sheet method has been modified so as to render it suitable for the cultivation of bacteria. This involves the substitution of the sodium nitrate of the original medium by microcosmic salt of equivalent nitrogen content. The number of organisms showing growth and fermentation on this medium was small but was greatly increased by addition also of 0.2 % peptone, an amount insufficient to interfere appreciably with the carbon balance sheet estimations. Calcium carbonate proved to be the most useful buffering agent. Certain modifications necessary when applying the standard balance sheet technique to bacteria are detailed, and methods employed for the determination of 2 : 3-butylene glycol and lactic acid, which are of frequent occurrence, are described.

Fifty species of bacteria were found to grow readily on the selected medium and of these, 20 species were chosen for examination by the balance sheet method at 28° and 34°. The balance sheets show that the chief classified products are volatile acids, volatile neutral substances, lactic acid and 2 : 3-butylene glycol.

Of these organisms, *B. asiaticus-mobilis* proved interesting in that it gave about a 30 % yield of butylene glycol and the rate of fermentation was faster than for any of the other organisms examined, whilst the balance sheets of three other organisms (*B. acidilactici*, C 54, *Esch.* species from milk, C 37, and *B. albolactis*, C 98) indicate that these species merit further investigation.

We greatly appreciate the interest taken in this work by Professors H. Raistrick and W. W. C. Topley, and our best thanks are due to them for their advice and helpful criticism. We are also indebted to Mr W. Rintoul and the Directorate of Imperial Chemical Industries for permission to publish this work.

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