

XXIX.—*Fermentations induced by the Pneumococcus of Friedländer.*

By PERCY F. FRANKLAND, Ph.D., B.Sc. (Lond.), A.R.S.M., F.I.C.,
ARTHUR STANLEY, F.C.S., and WILLIAM FREW, F.C.S.

AMONGST the numerous contributions which have been made by Brieger to our knowledge of the chemical products resulting from the vital processes of micro-organisms, there are two (*Zeit. physiol. Chem.*, 8, 306—31, and 9, 1—7) on the substances formed in the cultivation of the well known *Pneumococcus* of Friedländer in certain media.

By growing this specific micro-organism in suitable solutions of grape or cane sugar, Brieger obtained principally acetic acid, together with some formic acid and ethyl alcohol. The same products were also obtained by the growth of this organism in solutions of calcium lactate and creatine. As these transformations are only incidentally described by Brieger, without the precise relative proportions of the products having been determined, we have endeavoured to place these fermentations on a more quantitative basis, and to further investigate the behaviour of this micro-organism towards other carbohydrates.

In our experiments, the *Pneumococcus* employed was originally obtained by one of us from the Hygienic Institute of Berlin, in January, 1886, and continuously further cultivated for a period of nearly three years on gelatin-peptone before being used for fermentation purposes.

In order to ensure the purity of the organism used in the following experiments, we submitted one of the growths to plate-cultivation, and thus made a single colony the basis of further operations.

It is unnecessary to enter into a description of the morphological characters of the *Pneumococcus* (Friedländer) which is to be found in any of the modern text-books of bacteriology.

Fermentation of Glucose.

The following solution was prepared for the purpose:—

Glucose, 60 grams	} the whole made up with distilled water to 2000 c.c.
Peptone, 6 grams	
Liebig's <i>extract. carnis</i> , 2 grams.	
Calcium carbonate, 20 grams.	

This solution was placed in a capacious flask, plugged with sterile cotton-wool, and the whole was then finally sterilised by steaming for two hours on each of three successive days.

The sterile solution was inoculated with a single loop of a platinum needle, which had been dipped into a culture derived from one of the colonies referred to above, the flask being then placed in the incubator at 39° C. On the following day the liquid was already in active fermentation, which, however, only continued for a period of two days, and as by the sixth day all visible action had ceased, the liquid was forthwith submitted to analysis.

Two-thirds of the liquid were, in the first place, distilled off, and the distillate worked up for alcohols. To this end it was redistilled several times, two-thirds of the liquid only being taken over on each occasion. When the liquid had in this manner been reduced to about 1000 grams, its density was carefully taken by means of the specific gravity bottle. In this way 64·6876 grams of liquid, sp. gr. 0·9983 (at 15·5°), were obtained, corresponding to 0·91 per cent., or 0·5897 gram ethyl alcohol.

As a check on this determination, the liquid was again repeatedly distilled, until only about one-half of the former volume remained. On then again taking the density, the 33·4826 grams of liquid were found to have a sp. gr. of 0·99757, corresponding to 1·34 per cent., or 0·4487 gram ethyl alcohol.

The residue from which the alcohol had been distilled in the first instance, and which would contain any acids in the condition of calcium salts, was treated with 340 c.c. of normal hydrochloric acid (the quantity required to neutralise the 20 grams of calcium carbonate used was 348·3 c.c.), an insufficient quantity of acid being added in order to only partially liberate the organic acids, and thus obtain a purer product on distillation.

The liquid thus partially neutralised with hydrochloric acid was distilled down to a small bulk, and the distillate boiled up with excess of barium carbonate (see Frankland and Fox, "On a Pure Fermentation of Mannite and Glycerin," *Proc. Roy. Soc.*, **46**, 345—357). In this manner, 1·5422 gram barium salt, dried at 130°, was obtained, yield-

ing on ignition with sulphuric acid, 90·73 per cent. barium sulphate. Barium acetate yields 91·37 per cent. barium sulphate.

The above salt must, therefore, have been almost pure barium acetate, possibly mixed with a trace of a salt of a fatty acid of higher molecular weight.

To the residue in the distilling flask, water was added, and the distillation again carried to small bulk; from the distillate, 1·4434 gram of barium salt was similarly obtained, yielding 94·72 per cent. of barium sulphate. This second salt must, therefore, have been a mixture of barium acetate and formate, thus:—

Barium formate yields 102·64 per cent. barium sulphate.

In fact, the percentage 94·72 barium sulphate corresponds to—

1·0144 gram of barium acetate.

0·4290 „ „ formate.

The full quantity of hydrochloric acid was now added to the residue in the distilling flask, and the two further distillates obtained were united and converted into 0·3752 gram of barium salt, yielding 92·06 per cent. of barium sulphate, thus corresponding to—

0·3522 gram of barium acetate.

0·0230 „ „ formate.

Six more distillates were obtained giving together only 0·1620 gram of barium salt, yielding 90·99 per cent. of barium sulphate, and thus consisting of almost pure barium acetate.

The residue left after distilling off the volatile acids as above was repeatedly shaken out with ether, and the united ethereal extract, after driving off the ether, was treated with excess of carbonate of soda. The sodium salt thus obtained was extracted with absolute alcohol, and the insoluble residue, after being dissolved in water, was acidified with hydrochloric acid, and repeatedly shaken out with ether. The ethereal extract yielded, on evaporation, a residue weighing 0·0280 gram, which was presumably succinic acid, as it gave the characteristic irritating fumes of the latter on ignition.

The products of the fermentation in question may be thus summarised:—

Ethyl alcohol. . . 0·5897 gram.

Acetic acid. . . . 1·4451 „ (Barium acetate = 3·0708 gram).

Formic acid .. 0·1832 „ (Barium formate = 0·4520 „).

Succinic acid.. 0·0280 „

Fermentation of Mannitol. I.

The following solution of mannitol

Mannitol	60	grams	} the whole made up with distilled water to 2000 c.c.
Peptone	2	„	
Carbonate of lime	15	„	
Salt solution	200	c.c.	

was prepared, and, after due sterilisation, was inoculated with a single loopfull of a pure cultivation of the pneumococcus in a similar mannitol solution. After inoculation the mannitol solution was placed in the incubator at 39°, and remained there 40 days, until the slow fermentation which set in had entirely ceased. The liquid was then submitted to a perfectly similar process of analysis to that above described for the glucose fermentation. The volatile acids yielded the following barium salts:—

- I. 5·0513 grams of barium salt (dried at 130°), yielding 4·6161 grams BaSO_4 = 91·38 per cent. BaSO_4 (barium acetate yields 91·37 per cent. BaSO_4).
- II. 1·2320 gram barium salt, yielding 1·0976 gram BaSO_4 = 89·09 per cent. BaSO_4 .
- III. 0·0750 gram barium salt, yielding 0·0628 gram BaSO_4 = 83·74 per cent. BaSO_4 .

Thus, as in the case of the glucose fermentation, the volatile acids consisted chiefly of acetic acid, but traces of formic acid were also detected qualitatively, and the salts II and III, from the percentage of barium sulphate which they yielded, appeared to contain some higher fatty acid, but owing to the small total quantity of salt, the nature of this acid could not be further investigated.

The distillate from the fermented liquid, after repeated rectification, yielded 64·0874 grams of liquid of sp. gr. (at 15·5°) 0·9890, corresponding to 6·42 per cent. of ethyl alcohol by weight or 4·11 grams of ethyl alcohol. On further concentrating the liquid by repeated rectification, 31·7790 grams of liquid of 0·9809 sp. gr. were obtained, corresponding to 12·46 per cent. or 3·96 grams of ethyl alcohol. The liquid was dried with anhydrous potassium carbonate, after which its boiling point was found to be 79°.

From the residue, after distilling off the volatile acids as described above, a minute quantity (0·0108 gram) of succinic acid was obtained.

Fermentation of Mannitol. II.

A second experiment on mannitol was made in order to ascertain whether the fermentation would be more complete if a larger quan-

tity of nitrogenous nutriment were provided for the organism. For this purpose the following solution was prepared:—

Mannitol	60	grams	} the whole made up with distilled water to 2000 c.c.
Liebig's <i>extract. carnis</i> ...	2	„	
Peptone	6	„	
Carbonate of lime	20	„	

This solution, after sterilisation, was inoculated as before from a mannitol cultivation of the pneumococcus, and submitted to analysis after remaining 36 days in the incubator at 39°.

The following products were obtained:—

Volatile Acids.

- I. 4.1798 grams barium salt (dried at 130°), yielding 3.8154 grams BaSO_4 = 91.28 per cent. BaSO_4 (barium acetate yields 91.37 per cent. BaSO_4).
- II. 1.3784 gram barium salt, yielding 1.2498 gram BaSO_4 = 90.67 per cent. BaSO_4 .
- III. 1.1604 gram barium salt, yielding 0.9808 gram BaSO_4 = 84.52 per cent. BaSO_4 (barium propionate yields 82.32 per cent. BaSO_4).

Thus again whilst the principal volatile acid is acetic there is evidence of a considerable proportion of a higher fatty acid, probably propionic. The last fractions of salts obtained as above are, however, always gummy in appearance, and the low percentage of barium may, therefore, not impossibly be due to some other impurity and not to a higher fatty acid; this applies also to the last fractions of barium salt obtained in the previous fermentation (see p. 255).

Alcohols.—After repeated rectification, 63.9424 grams of liquid of sp. gr. 0.9868, corresponding to 7.92 per cent. of alcohol, or 5.06 grams, were obtained. This liquid, after further repeated rectification, yielded 31.6842 grams of sp. gr. 0.9779, corresponding to 14.83 per cent. of alcohol, or 4.70 grams. On drying the alcohol with anhydrous potassium carbonate, its boiling point was found to be 79°, or that of ethyl alcohol.

Fixed Acid.—A trace of what was apparently succinic acid was extracted from the residue after distilling off the volatile acids.

The products of the three fermentations examined may be thus summarised:—

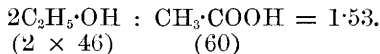
	I.	II.	III.
	Glucose, with peptone and extract of meat.	Mannitol, with peptone only.	Mannitol, with peptone and extract of meat.
Ethyl alcohol	0.5897 grm.	4.11 grms.	4.06 grms.
Volatile acids calculated as acetic acid }	1.6578 „	2.9921 „	3.1617 „

The above results show that in each case, and especially in that of the glucose, only a very partial decomposition had taken place, moreover neither the extent nor the nature of this fermentative decomposition was materially altered in the more nutritious medium produced by the addition of extract of meat and a larger quantity of peptone.

The products of the fermentation, essentially ethyl alcohol and acetic acid, are similar to those obtained by one of us (*loc. cit.*) in the fermentation of mannitol by a totally different organism, the *Bacillus ethaceticus*. Indeed, not only are the products of fermentation similar in their nature, but the proportion in which they are respectively formed is almost identical in the two cases; thus—

Proportion of alcohol to volatile acids, calculated as acetic acid.	Mannitol fermented by <i>Pneumococcus</i> .	
	I.	II.
	1·37	1·60
	Mannitol fermented by <i>Bacillus ethaceticus</i> .	
	I.	II.
	1·63	1·63

This ratio corresponds closely to the molecular proportions:—



But although the alcohol and acetic acid, produced in the fermentations in question, thus stand to each other in virtually the same proportion, the absolute amounts produced are much less in the case of the *Pneumococcus* than in that of the *B. ethaceticus* fermentation. In fact, in the most complete of the latter fermentations practically twice the amount of these products was obtained, as with the *Pneumococcus*, thus:—

Mannitol Fermentations.

	<i>Bacillus ethaceticus</i> .	<i>Pneumococcus</i> .
Alcohol	11·415 grams.	5·06 grams.
Acetic acid	7·008 „	3·16 „

It is very noticeable that mannitol, as the above results show, is more readily fermentable by the *Pneumococcus* than glucose; this point we have not only confirmed qualitatively several times, but we have also compared the relative facility with which the several substances which we have found to be attacked by this organism are fermented.

Thus on inoculating from one and the same cultivation* of the *Pneumococcus* into similar solutions† of glucose, mannitol, cane-sugar, and dextrin, and preserving all at 39°, the

Glucose fermented on the 6th day after inoculation.

Mannitol " " 5th " "

Cane-sugar " " 5th " "

Dextrin " " 5th " "

Again on inoculating from the latter mannitol culture (when it was 13 days old) into similar solutions, the

Glucose fermented on the 4th or 5th day after inoculation.

Mannitol " " 3rd day after inoculation.

Cane-sugar " " 3rd " "

Dextrin " " 3rd " "

Whilst on inoculating from the latter mannitol culture (when it was nine days old), the glucose, mannitol, and cane-sugar all commenced fermenting on the same day, viz., the third after inoculation. (No dextrin solution was inoculated in this case.)

Again, on inoculating from a glucose broth‡ culture (five days old), the

Glucose fermented on the 4th or 5th day after inoculation.

Mannitol " " 3rd day after inoculation.

Cane-sugar " " 2nd " "

Dextrin " " 4th or 5th day after inoculation.

Similarly on inoculating from an ordinary gelatin culture, the

Glucose fermented on the 8th day after inoculation.

Mannitol " " 4th " "

Cane-sugar " " 4th " "

In this case the glucose fermentation was also visibly feebler than the other two.

Thus, although there is some irregularity§ in the several series of experiments as to the precise period which elapses between the time of inoculation and the commencement of fermentation, the balance of

* This culture was in a 3 per cent. solution of mannitol, as given on page 255; it was 6 days old at the time.

† These were all 3 per cent. solutions, similar to the mannitol.

‡ A 3 per cent. solution of glucose in water to which one-tenth of its volume of the ordinary peptone broth has been added.

§ This irregularity doubtless depends upon the quantity of ferment inoculated, and also upon differences in its vitality at the time of inoculation.

evidence points to the glucose being the least, and to the mannitol and cane-sugar being the most readily fermentable.

We have repeatedly endeavoured to ferment dulcitol with the *Pneumococcus*, but have invariably failed. The *Pneumococcus*, therefore, like the *B. ethaceticus*, possesses the extremely interesting property of discriminating between the isomeric bodies mannitol and dulcitol.

Unlike the *B. ethaceticus*, the *Pneumococcus* does not ferment glycerol.

The Fermentation Gases.

The decompositions of glucose and mannitol recorded above are attended with a considerable evolution of gas, the nature of which has been further investigated by one of us. For this purpose, small, narrow-mouthed bottles of a little more than 100 c.c. capacity were employed; 100 c.c. of solutions of glucose and mannitol respectively were introduced into these, which were then plugged with sterile cotton wool, and the whole steam-sterilised on three successive days. These bottles were then inoculated from a fermenting cultivation of the *Pneumococcus*, and the cotton-wool stoppers rapidly exchanged for indiarubber ones fitted with delivery tubes, both of which had been duly sterilised with steam, each delivery tube also contained a small plug of asbestos to prevent the passage of unsterilised air into the bottle.

The inoculated bottles so fitted were then placed in an incubator, kept at about 36°, whilst the delivery-tubes passed through apertures cut in the side of the latter, and dipped into a mercury-bath. The evolved gases were then collected in a series of test-tubes, roughly measured, and several samples representative of the various stages of the fermentation were submitted to careful quantitative analysis.

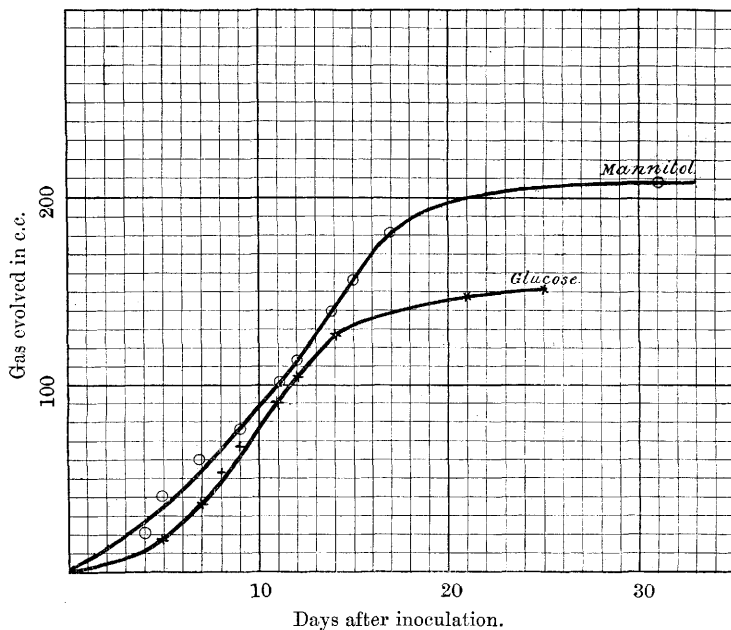
In the following table (p. 261), the volumes of gas collected during the two fermentations are recorded.

Thus the total volume of gas collected in the fermentation of the 3 grams of glucose was 150.5 c.c., and in that of the same quantity of mannitol 209.6 c.c. As the measurements were only approximative, it has not been considered worth while to correct them for temperature and pressure (the temperature was in all cases about 16–18°). It will be seen that the volumes of gas given off in the two cases fully confirms what has been stated above concerning the more ready fermentation of the mannitol.

The progress of the fermentations is more readily followed by means of the following graphic representation, in which the abscissæ represent the lapse of time in days since inoculation, whilst the

No. of days after inocu- lation.	Glucose.		Mannitol.	
	Volume of gas.	Total volume of gas collected.	Volume of gas.	Total volume of gas collected.
4	—	—	Tube (1) 21·6 c.c.	21·6 c.c.
5	Tube (1) 16·3 c.c.	16·3 c.c.	„ (2) 18·3 „	39·9 „
7	„ (2) 21·3 „	37·6 „	„ (3) 20·6 „	60·5 „
8	„ (3) 16·2 „	53·8 „	—	—
9	„ (4) 11·4 „	65·2 „	„ (4) 16·8 „	77·3 „
11	„ * (5) 24·0 „	89·2 „	„ * (5) 23·5 „	100·8 „
12	„ (6) 18·1 „	107·3 „	„ (6) 12·7 „	113·5 „
14	„ (7) 20·6 „	127·9 „	„ (7) 27·1 „	140·6 „
15	—	—	„ (8) 14·7 „	155·3 „
17	—	—	„ * (9) 27·1 „	182·4 „
21	„ * (8) 19·0 „	146·9 „	—	—
25	„ (9) 3·6 „	150·5 „	—	—
31	—	—	„ * (10) 24·5 „	206·9 „
—	—	—	2·7 „	209·6 „

ordinates denote the volume of gas given off, measured in cubic centimetres.



In order to ascertain the composition of the fermentation gases,

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samples of the gas evolved at different stages of the fermentation were submitted to careful analysis. The portions of the evolved gas submitted to analysis are indicated in the above table by a *.

Thus in the case of the glucose fermentation, the 5th and 8th tubes, and, in the case of the mannitol fermentation, the 5th, 9th, and 10th tubes of gas were analysed.

The following are the results of analysis stated in parts per 100 by volume.

Glucose Fermentation.

	Tube 5.			Tube 8.		
	I.	II.	Mean.	I.	II.	Mean.
CO ₂	51·06	51·22	51·14	56·62	56·51	56·57
O	0·09	0·05	0·07	0·00	—	0·00
H	47·26	47·55	47·41	43·20	43·29	43·24
N	1·59	1·18	1·38	0·18	0·20	0·19
	100·00	100·00	100·00	100·00	100·00	100·00

Proportion of H : CO₂ = 1 : 1·08.

Proportion of H : CO₂ = 1 : 1·31.

Mannitol Fermentation.

	Tube 5.			
	I.	II.	III.	Mean.
CO ₂	51·80	51·56	51·45	51·60
O	0·19	0·22	0·00	0·14
H	47·07	47·64	47·88	47·53
N	0·94	0·58	0·67	0·73
	100·00	100·00	100·00	100·00

Proportion of H : CO₂ = 1 : 1·09.

Mannitol Fermentation.

	Tube 9.			Tube 10.*		
	I.	II.	Mean.	I.	II.	Mean.
CO ₂	54·68	54·65	54·67	50·57	50·57	50·57
O	0·06	0·17	0·11	1·70	1·68	1·69
H	45·21	45·18	45·20	42·45	42·44	42·44
N	0·05	0·00	0·02	5·28	5·31	5·30
	100·00	100·00	100·00	100·00	100·00	100·00

Proportion of H : CO₂ = 1 : 1·21. Proportion of H : CO₂ = 1 : 1·19.

The fermentation-gases consist thus exclusively of carbonic anhydride and hydrogen, and in no case was there any trace of methane or other gas yielding carbonic anhydride on explosion with oxygen. The small quantity of nitrogen found in the early tubes in each case is due to the residual air in the fermentation-bottles and delivery-tubes, which contained, of course, a few cubic centimetres of air above the liquid. The analyses, moreover, show that a part of the oxygen of this air had been absorbed by the fermenting liquid, or rather by the organism, which is capable of both aërobic and anaërobic life and activity.

With the exception of the ordinary butyric fermentation, this is, as far as we are aware, the only pure fermentation in which the evolution of hydrogen has been demonstrated. The same gas, as will afterwards be shown, is also evolved in the fermentations excited by the *B. ethaceticus*, which have been described by one of us.

The proportion of hydrogen to carbonic anhydride by volume shows that these gases are given off in approximately the same number of molecules of each, or, more accurately, 10 mols. of hydrogen to 13 mols. of carbonic anhydride in the case of the glucose, and 10 mols. of hydrogen to 12 mols. of carbonic anhydride in that of the mannitol. This larger proportional evolution of hydrogen in the case of the mannitol is what might have been anticipated from a consideration of the larger percentage of hydrogen in mannitol than in glucose.

The carbonic anhydride evolved, it must also be borne in mind, is not wholly obtained from the primary decomposition of the glucose or mannitol, but is also partially a secondary product derived from the decomposition of the carbonate of lime by the acetic and formic acids to which the fermentation gives rise.

* A bubble of air accidentally gained access to this tube in removing it from the mercury-bath; hence the high percentages of oxygen and nitrogen found.

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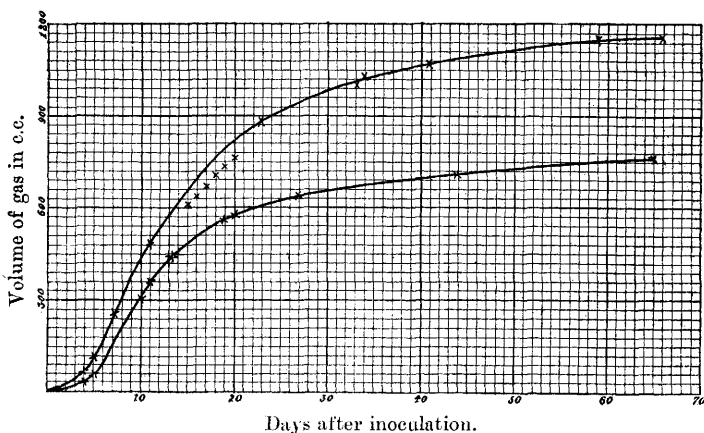
In order to further examine the evolved gases, and determine, not only the ratio between the carbonic anhydride and hydrogen, but also between these and the amounts of alcohol, acetic and formic acids produced, the experiments with the mannitol were repeated on a larger scale (using 400 c.c. of the 3 per cent. solution of mannitol). As we thought that possibly some of the gases might have diffused through the indiarubber stoppers of the fermenting bottles, we took the further precaution of thickly coating the outside of the stoppers with paraffin, thus rendering all diffusion or loss of gas out of the question.

The experiment was performed in duplicate, and the follow quantities of gas were obtained:—

Volume of Gas evolved in the Fermentation of 400 c.c. of Mannitol Solution (3 per cent.).

No. of days after inoculation.	Vol. of gas from Bottle I.	Total vol. of gas collected from Bottle I.	Vol. of gas from Bottle II.	Total vol. of gas collected from Bottle II.
4	64·7	64·7	34·4	34·4
5	35·8*	100·5	15·6*	50·0
7	156·8	257·3	—	—
10	—	—	260·0	310·0
11	224·0	481·3	50·6*	360·6
13	—	—	78·0	438·6
15	120·0	601·3	—	—
16	32·2*	633·5	—	—
17	31·2	664·7	—	—
18	36·0	700·7	—	—
19	40·3	741·0	117·4	556·0
20	26·0	767·0	15·8*	571·8
23	120·0*	887·0	—	—
27	—	—	63·2	635·0
33	126·0	1013·0	—	—
34	17·0*	1030·0	—	—
41	43·0*	1073·0	—	—
44	—	—	82·5	717·5
59	74·0	1147·0	—	—
66	6·8	1153·8	36·0	753·5

The evolution of gas can be more easily followed by reference to the graphic representation on p. 265, in which the ordinates represent the volumes of gas, and the abscissæ the time of fermentation.



Mr. McCowan, B.Sc., Lecturer on Mathematics and Physics in University College, Dundee, has very kindly investigated the nature of these curves, and calculated their equations. He finds that although, as the above figure indicates, they are quantitatively different, yet qualitatively they are very much alike, and can be represented by similar equations. In both cases the initial portion of the curve with the upward concavity has been neglected, as this does not really reflect the progress of the fermentation, owing to the absorption of carbonic anhydride by the liquid in the first instance. It is only after about the 8th day that the curves assume their normal form.

The upper curve, representing the fermentation in which most gas was evolved, is defined by the equation

$$v = \frac{6.73t^2}{1 - 0.006t + 0.00574t^2},$$

in which v = volume, in cubic centimetres, of gas evolved and t = time in days during which the fermentation has been proceeding.

Thus comparing the actually observed results with those obtained by calculation from the above equation, we have—

Days.	Volume (calculated).	Volume (observed).
5	151 c.c.	110 c.c.
10	444 „	435 „
20	847 „	825 „
30	1012 „	987 „
40	1083 „	1060 „
60	1137 „	1145 „

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Thus from the 10th day to the close of the fermentation, the calculated and observed volumes exhibit the most striking concordance.

Again the lower curve, representing the fermentation in which the smaller volume of gas was evolved, is defined by the following equation:—

$$v = \frac{4.96t^2}{1 - 0.004t + 0.0064t^2},$$

in which v and t have the same significance as above.

Thus comparing the calculated with the experimental results we find

Days.	Volume (calculated).	Volume (observed).
4	73.0 c.c.	34 c.c.
6	148.0 "	90 "
7	188.5 "	140 "
8	230.0 "	210 "
9	271.0 "	270 "
10	310.5 "	310 "
15	468.0 "	480 "
19	553.5 "	555 "
20	569.0 "	571 "
30	672.0 "	657 "
60	750.0 "	750 "

Here again, after the 8th day, the coincidence between experiment and calculation becomes very complete.

We believe that the irregularities in the results obtained in such fermentation experiments are due in the first instance to differences in the quantity and vitality of the organisms introduced, and secondly to the pressure under which the fermentations take place being subject to variations. Thus, in the above experiments, the evolved gases were collected over mercury, and the pressure in the fermenting bottles would obviously alter with the considerable variations in the depth of mercury in the trough, and it is more than possible, indeed from our knowledge of the action of carbonic anhydride under pressure, it is highly probable, that any increase of pressure would have the effect of checking the fermentation to an appreciable extent.

We are at present extending our observations on the evolution of gas during fermentation, and we shall in the future introduce precautions to guard against the probability of such irregularities arising.

Specimens of the gas given off at different stages of the two fermentations were again submitted to careful analysis, and in the preceding table the portions thus examined are indicated by a *.

The following results were obtained :—

Fermentation I.

	Composition of gases evolved on				
	5th day.	16th day.	23rd day.	34th day.	41st day.
CO ₂	27·04	51·77	61·29	66·18	65·47
O.....	2·05	0·09	0·15	0·22	0·09
H.....	48·69	47·97	38·26	33·47	34·19
N.....	22·22	0·17	0·30	0·13	0·25
	100·00	100·00	100·00	100·00	100·00

Fermentation II.

	Composition of gases evolved on		
	5th day.	11th day.	20th day.
CO ₂	19·86	50·07	58·45
O.....	3·55	0·07	0·21
H.....	36·74	49·52	41·30
N.....	39·85	0·34	0·04
	100·00	100·00	100·00

These analyses fully confirm the results obtained in the previous series of experiments made on the smaller scale. They show that the oxygen of the air present in the first instance in the fermenting bottle is rapidly consumed during the growth, and that the gas evolved is first richer in hydrogen than carbonic anhydride, which relationship is reversed in the latter part of the fermentations. This reversal is doubtless due to the carbonic anhydride being at first largely absorbed by the water of the fermenting liquid with formation of calcium bicarbonate, so that the real relationship between the carbonic anhydride and hydrogen evolved can only become apparent in the latter part of the fermentation, whilst at the very end the carbonic anhydride again predominates in consequence of the slow decomposition of the calcium bicarbonate at first formed. In round numbers *this relationship may be taken as 6 vols. or molecules of carbonic anhydride to 4 vols. or molecules of hydrogen.*

After the completion of these fermentations we also determined

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the quantity of the other products, alcohol and volatile acids, according to the methods previously described.* We obtained the following results:—

	Fermentation I.	Fermentation II.
<i>Alcohol—</i>		
Barium salts	3·0615 grams.	2·321 grams.
Yielding BaSO ₄	91·39 per cent.	90·89 per cent.
Equivalent to ethyl alcohol	1·106 gram.	0·837 gram.
<i>Volatile acids—</i>		
Barium salts	1·3055 gram.	1·467 gram.
Yielding BaSO ₄	97·17 per cent.	96·29 per cent.
Equivalent to acetic acid	0·653 gram.	0·7276 gram.

On comparing these results with those obtained in the fermentations of mannitol on the larger scale, as recorded on p. 257, it will be seen that in the case of the small fermentations the quantities of alcohol and acetic acid obtained are approximately in the same proportion as those of the large fermentations. In the large fermentations 2000 c.c. of a 3 per cent. solution of mannitol were employed, in the small ones, on the other hand, only 400 c.c. of a similar solution; on multiplying the products obtained in the latter by five, we obtain the following comparative figures:—

	Large fermentations.		Small fermentations.	
	I.	II.	Ia.	IIa.
Alcohol	4·11 grams.	5·06 grams.	5·53 grams.	4·185 grams.
Volatile acids, calculated as acetic acid	2·9921 „	3·1617 „	3·265 „	3·6380 „
Proportion of alcohol to volatile acids, calculated as acetic acid..	1·37	1·60	1·69	1·15

The comparative uniformity of the decompositions brought about by this organism is the more noteworthy, when it is mentioned that

* The mode of procedure was only altered in respect to the determination of the alcohol, which was effected by oxidation with chromic acid (see Frankland and Frew, this vol., p. 83).

the fermentations I and II took place in London in 1888, whilst Ia and IIa were carried out in Dundee in 1890, exactly two years later.

Owing to the close agreement between the results of the fermentations II and Ia, we shall take these as representing the typical course of the fermentative process and in the following deductions make use of these results only; the evolution of gas in the case of Fermentation Ia being considerably in excess of that in Fermentation IIa, also points to the former being the more complete of the two fermentations.

In the case of Fermentation Ia, we have then the following quantities of the several products:—

Alcohol.	Acetic acid.	Carbonic anhydride.	Hydrogen.
1·106 gram.	0·653 gram.	1·3648 gram.	0·0413 gram.

In the calculation of the weights of the carbonic anhydride and hydrogen evolved, we have assumed that the average composition of the 1154 c.c. of evolved gas was 6 vols. of CO₂ to 4 vols. of H, as indicated by the analyses of the later portions of gas given off.

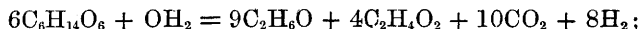
The above weights are found to stand in the following proportion:—

Alcohol.	Acetic acid.	Carbonic anhydride.	Hydrogen.
26·78	: 15·81	: 33·05	: 1

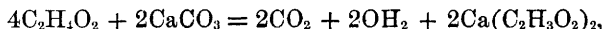
Now these figures are in very close accord with those corresponding to the following molecular proportions:—

9C ₂ H ₆ O	: 4C ₂ H ₄ O ₂	: 12CO ₂	: 8H ₂
(9 × 46)	(4 × 60)	(12 × 44)	(8 × 2)
414	240	528	16
25·9	: 15·0	: 33·0	: 1

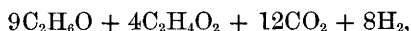
These products, alcohol, acetic acid, carbonic anhydride, and hydrogen, in the above proportions, are most readily referable to the decomposition of the mannitol according to the following equation:—



but the 4 molecules of acetic acid acting upon the excess of calcium carbonate employed in the fermentation will yield two further molecules of carbonic anhydride, thus



the net products determined being thus



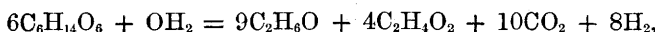
as calculated above from the results of actual experiment.

The results of our investigation may be summarised as follows:—

- 1.—The *Pneumococcus* of Friedländer sets up a fermentative process in suitable solutions of dextrose, cane-sugar, milk-sugar, maltose, raffinose, dextrin, and mannitol.
- 2.—It does not ferment solutions of dulcitol or glycerol, and has thus the power, like the *Bacillus ethaceticus*, of distinguishing between the isomers mannitol and dulcitol.
- 3.—In the fermentation of dextrose and mannitol, the principal products are ethyl alcohol and acetic acid with a smaller proportion of formic acid and traces of a fixed acid, in all probability succinic acid.
- 4.—The gaseous products are carbonic anhydride and hydrogen.
- 5.—The ethyl alcohol, volatile acids (calculated as acetic acid), carbonic anhydride, and hydrogen approximate to the molecular proportions



- 6.—The production of which may be most readily referred to the following equations:—



which is followed by

