THE CHEMICAL ACTION ON GLUCOSE OF THE LACTOSE-FERMENTING ORGANISMS OF FAECES.

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In a former series of experiments I found (1901) that *B. coli communis* attacks glucose in a very characteristic manner, each molecular proportion of the sugar yielding half a molecular proportion of acetic acid and of alcohol, and one molecular proportion of lactic acid, together with a small amount of succinic acid, and gaseous carbon dioxide and hydrogen. Although the amounts of the various substances produced vary somewhat with different organisms the molecular ratio of acetic acid to alcohol remains fairly constant and usually approximates to unity.

MacConkey (1905) has recently observed that the lactose-fermenting organisms of the intestine may be subdivided into four groups according to their varying action on cane-sugar and dulcite, and it was with the object of ascertaining whether these differences are correlated with any difference in the mode of attack of the organisms upon glucose that the following experiments were made.

Experimental Method. The organisms examined had all been isolated by MacConkey (1905) and the methods employed for this purpose will be found described in his paper. They were derived from ten separate samples of normal human faeces (N. H. F.), two of milk, four of water, and one each of cow-dung and horse-dung.

Each organism was cultivated in an atmosphere of nitrogen in 500 c.c. of glucose-peptone-water, containing 10 grammes of glucose, 5 grammes of Witte's peptone, and 5 grammes of chalk, for a fortnight at 37° C. The liquid was then filtered from the insoluble residue and the following analyses made.

1. The residual sugar was estimated by Pavy's method.

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2. The total acid produced was estimated from the amount of calcium salt in solution, a correction being applied for dissolved calcium carbonate and calcium in the peptone.

3. Alcohol and acetic acid. The liquid was acidified with excess of oxalic acid, filtered and distilled. The first half of the distillate was collected separately, neutralised by normal caustic soda solution and again distilled, the alcohol being estimated in the distillate by the gravity method.

The total amount of volatile acids was ascertained by titrating the whole distillate, the distillation being continued in a current of steam until 100 c.c. of it only required 0·2 c.c. of normal alkali.

Formic acid, which is usually produced in small amount, was then estimated in the neutralised and evaporated solution by the mercuric chloride method, and the difference between this and the total volatile acids was taken as acetic acid.

4. The non-volatile acids were obtained from the difference between the volatile acids and the total acids produced.

5. The residue from the steam distillation was extracted with ether, the ether evaporated and the residue converted into the zinc salt, the direction of rotation of the plane of polarised light by which was then qualitatively observed.

In all 56 distinct organisms were examined and the results are embodied in the following tables. The groups into which they are divided correspond with the groups 1, 2, 3, and 4 (1) of MacConkey's provisional classification[1].

In addition, the laboratory cultures of *B. coli communis* (Escherich), *B. acidi lactici* (Hueppe), *B. lactis aerogenes* (Escherich), and *B. cloacae* (Jordan) were examined in a similar manner and the results are appended to the tables.

The sugar used is expressed in grammes; the acetic acid and alcohol in molecular proportions per molecule of sugar, and the non-volatile acids (lactic and succinic) in c.c. of normal acid per gramme of sugar.

The rotation of the zinc salt was in every case found to be positive.

[1] *Loc. cit.* p. 352.

GROUP 1.

No.	Origin	Cane-sugar	Dulcite	Sugar used	Acetic acid	Alcohol	Molecular ratio $\frac{\text{alcohol}}{\text{acetic acid}}$	Non-volatile acids
19	N. H. F. i	–	–	3·78	0·43	0·61	1·43	5·79
20	,, i	–	–	3·41	0·54	0·70	1·29	5·66
21	,, ii	–	–	6·67	0·62	0·57	0·92	5·94
22	,, ii	–	–	6·65	0·65	0·63	0·96	3·79
23	,, ii	–	–	4·24	0·45	0·70	1·57	2·41
24	,, x	–	–	6·84	0·60	0·57	0·95	4·93
25	,, iii	–	–	5·85	0·68	0·66	0·98	2·86
26	,, iii	–	–	5·68	0·50	0·73	1·46	3·88
27	,, iv	–	–	4·76	0·71	0·77	1·09	2·71
28	,, iv	–	–	6·35	0·51	0·51	1·00	4·38

GROUP 2.

8	N. H. F. i	–	+	4·05	0·49	0·61	1·24	4·40
9	,, iii	–	+	5·1	0·77	0·72	0·93	2·33
10	,, iv	–	+	5·64	0·66	0·55	0·84	3·83
11	,, iv	–	+	6·13	0·55	0·55	1·00	4·65
12	,, iv	–	+	5·55	0·59	0·50	0·84	4·75
13	,, iv	–	+	5·55	0·57	0·51	0·90	4·73
14	,, iv	–	+	4·36	0·77	0·75	0·98	2·93
15	,, iv	–	+	4·78	0·82	0·75	0·93	3·89
16	,, iv	–	+	6·06	0·56	0·51	0·93	5·11
17	,, iv	–	+	5·82	0·37	0·46	0·81	6·81
18	,, iv	–	+	4·00	0·88	0·76	0·87	4·50

GROUP 3.

1	N. H. F. i	+	+	5·02	0·56	0·62	1·21	5·15
2	,, i	+	+	6·15	0·64	0·56	0·87	4·07
3	,, ii	+	+	5·59	0·71	0·71	1·00	3·02
4	,, ii	+	+	3·74	0·86	0·66	0·77	2·46
5	,, viii	+	+	4·6	0·89	0·73	0·82	2·94
6	,, viii	+	+	4·26	0·77	0·60	0·77	5·12
7	,, ix	+	+	4·81	0·75	0·53	0·71	4·16

GROUP 4. SUB-GROUP 1.

29	Milk I	+	–	5·6	0·59	0·54	0·90	4·0
30	,,	+	–	5·00	0·60	0·59	0·98	4·85
31	,,	+	–	5·51	0·56	0·55	0·98	4·67
32	N. H. F. vi	+	–	6·21	0·81	0·73	0·90	2·23
33	,, vi	+	–	6·00	0·77	0·62	0·81	3·44
34	,, vi	+	–	6·21	0·54	0·60	1·11	3·73
35	Milk II	+	–	7·2	0·67	0·61	0·91	3·32
36	,,	+	–	6·82	0·72	0·65	0·91	3·17
37	,,	+	–	6·72	0·76	0·67	0·88	2·71
38	Horse-Dung	+	–	6·44	0·69	0·70	1·01	3·39
39	,,	+	–	6·25	0·51	0·53	1·04	5·19
40	N. H. F. vii	+	–	6·87	0·61	0·59	0·98	3·70

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GROUP 4. SUB-GROUPS 2 AND 3.

No.	Origin	Cane-sugar	Dulcite	Sugar used	Acetic acid	Alcohol	Molecular ratio $\frac{\text{alcohol}}{\text{acetic acid}}$	Non-volatile acids
41	N. H. F. ii	+	–	9·52	0·05	0·77	13·4	1·20
42	,, iii	+	–	10	0·25	0·73	2·89	1·11
43	,, iii	+	–	10	0·27	0·80	2·93	1·32
44	,, iii	+	–	10	0·24	0·74	3·0	1·39
45	,, v	+	–	10	0·12	0·66	5·5	2·17
46	,, v	+	–	10	0·11	0·64	5·8	2·26
47	,, v	+	–	10	0·10	0·65	6·3	2·14
48	,, v	+	–	9·08	0·10	0·59	5·9	1·52
49	Water I	+	–	10	0·03	0·61	18	1·54
50	,, I	+	–	10	0·13	0·69	5·2	0·19
51	,, I	+	–	10	0·05	0·56	11·3	1·87
52	Water II	+	–	10	0·03	0·65	19	0·84
53	Cow-Dung	+	–	10	0·21	0·62	2·94	1·14
54	Water III	+	–	9·12	0·04	0·70	15·5	1·01
55	,, III	+	–	9·12	0·04	0·71	15·8	0·95
56	,, IV	+	–	6·13	0·12	0·65	5·11	0·77
	LABORATORY CULTURES.							
57	*B. coli com.* (Escherich)	–	+	5·57	0·58	0·64	1·12	3·67
58	*B. acidi lactici* (Hueppe)	–	–	6·37	0·54	0·54	1·0	5·29
59	*B. lactis aero-genes*	+	–	10	0·14	0·72	5·1	1·00
60	*B. cloacae*	+	–	10	0·05	0·65	13·4	0·85

As might be expected from the nature of the experiments a moderate degree of variation is found between the action of the different organisms classed here in the same group. The members of groups 1, 2, 3, and 4 (1), agree, however, in producing acetic acid and alcohol in a ratio which is not far from 1 and never exceeds 1·5 or falls below 0·7, whilst in the great majority of cases (31 out of 40) it does not differ from 1 by more than 0·2 in either direction. The average value of this ratio deduced from the 40 distinct organisms examined is 0·97. No distinctive difference capable of forming the basis of a system of classification is to be found between the action of the organisms of these four divisions.

The organisms of group 4 however are very sharply divided by this method into two classes, (*a*) Those which conform to the molecular ratio

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alcohol/acetic acid = 1 ; (*b*) Those for which this ratio becomes considerably greater than 2·5.

These organisms are grouped together under the heading Group 4, Sub-groups 2 and 3, and it will be observed in the first place that the ratio of alcohol/acetic acid is always greater than 2·5, varying from about 3 to 19. In addition to this the amount of sugar used is generally large, the whole available amount being fermented in nearly every case, whilst the organisms of the other groups only use up 40–70 % of it. Finally the amount of non-volatile acids produced is much smaller than that characteristic of the other groups.

These same features are characteristic of *B. lactis aerogenes* and *B. cloacae*, the deviation being in the main due to a diminished production of acetic acid, the amount of alcohol being at the same time slightly greater. These two organisms have already been differentiated from *B. coli communis* by the amount and composition of the gas evolved (Theobald Smith, 1895). *B. lactis aerogenes* in fact appears to act on glucose in a totally different manner from the bacilli of Groups 1, 2, 3, and 4 (1). The exact nature and quantitative relations of the products formed are at present under investigation. In the cases of several of the organisms of this last group (Group 4, Sub-groups 2 and 3) the ratio alcohol/acetic acid rises from 3–5, the value characteristic of *B. lactis aerogenes*, to 11–19. The ratio given by *B. cloacae* is 13·4, and it is probable that some of these organisms belong to this or some allied type, but as a detailed chemical examination of the characteristics of such organisms has not yet been completed no definite statement can at present be made on this point.

Summary.

(1) The lactose-fermenting organisms of faeces may be divided into two groups according to the molecular ratio alcohol/acetic acid produced from glucose under specified conditions.

(*a*) Those which produce alcohol and acetic acid in approximately equal molecular proportions.

(2) Those which produce more than 2·5 molecular proportions of alcohol to 1 of acetic acid.

(3) The members of the first of these groups comprise the Groups 1, 2, 3, and 4 (1) suggested provisionally by MacConkey from the

consideration of the action of these organisms on cane-sugar and dulcite. The members of the second of the groups correspond with Group 4, Sub-groups 2 and 3, of MacConkey's provisional classification.

(4) *B. lactis aerogenes* acts upon glucose in a totally different manner from *B. coli communis* and is therefore to be regarded as a distinct organism.

REFERENCES.

HARDEN, ARTHUR (1901). *Journ. Chem. Soc.* p. 601.
MACCONKEY, A. (1905). *Journ. of Hygiene*, Vol. v, p. 337.
SMITH, THEOBALD (1895). *Centralbl. f. Bakteriol.* Vol. XVIII, pp. 1, 494, 589.