A STUDY OF COLIFORM ORGANISMS IN SAMPLES OF "CERTIFIED MILK."

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The presence of coliform organisms in milk has received considerable attention since the introduction into this country of graded milk. Under the Milk Special Designations Order 1923, milk which is to be sold as "Certified" must, among other conditions, not contain coliform organisms in 1/10 c.c. on delivery to the consumer.

Williams (1925) and others state that the *B. coli* test is of value as a check, not only on the work in the cow-shed, but also on the efficiency of the methods employed in the washing and sterilising of the utensils used during the handling of the milk.

Rodgers, Clark and Davies (1914) have shown that coliform organisms of the type occurring in market milk may be divided into two distinct groups. One of these groups is characterised by a low and the other by a high gas ratio, *i.e.* the ratio of carbon dioxide to hydrogen produced in glucose media. Rodgers, Clark and Evans (1914, 1915) showed that the members of the low ratio group are commonly found in the bovine intestine whilst the others are numerous on the surface of dried cereal grains. Although the type most commonly found on grains probably corresponds to *Bacterium lactis aerogenes* many grain cultures would answer to the usual tests for *B. coli communis* and *B. coli communior.*

Clark and Lubs (1915) have shown that those coliform organisms which produce a low gas ratio, attain a greater hydrogen-ion concentration when grown in a standard medium than do those which produce a high gas ratio. Moreover, the limiting hydrogen-ion concentration of the two groups can be distinguished by an indicator such as methyl red.

Levine (1916) correlated the limiting hydrogen-ion concentration with Voges and Proskauer's reaction and showed that those organisms which gave a high concentration of hydrogen-ions generally failed to give the V. and P. reaction and *vice versa.*

The results of these workers therefore show that those lactose fermenting coliform organisms present in milk which are of intestinal origin generally give a low ratio of carbon dioxide to hydrogen, a positive reaction with the methyl red test and a negative reaction with Voges and Proskauer's test.

MacConkey (1906) isolated from milk 107 cultures of coliform organisms which fermented lactose, 29 per cent. of which gave Voges and Proskauer's reaction.

During the Yorkshire Clean Milk Competition held in 1925, 231 samples of milk were examined and the results classified to show the relationship between the *B. coli* contamination and the keeping quality of the samples.

Table I has been constructed by grouping the results according to their total bacterial count and *B. coli* contamination.

Table I. *Relation of* B. coli *to keeping quality.*

Group according to bacterial count per c.c.	*B. coli* absent in 1 c.c.		*B. coli* present in 1 c.c.		*B. coli* present in 1/10 c.c.		*B. coli* present in 1/100 c.c.		*B. coli* pres in 1/1000 c	
	Average bacterial count per c.c.	Keeping quality (days)	Average bacterial count per c.c.	Keeping quality (days)	Average bacterial count per c.c.	Keeping quality (days)	Average bacterial count per c.c.	Keeping quality (days)	Average bacterial count per c.c.	Ke qu (d
100 to 3,000	1,153	4·5	1,170	3·8	1,123	3·6	*220	4·0	—	
3,001 to 10,000	5,111	4·0	5,475	4·0	6,720	3·4	5,450	3·25	*8,350	3
10,001 to 50,000	23,327	3·6	24,050	3·6	19,500	3·25	19,165	3·4	19,100	3
Over 50,000	117,370	3·5	344,660	3·3	341,300	3·0	328,660	2·7	255,000	2

* One sample only.

These results show that as the number of *B. coli* increases the keeping quality tends to be lowered. This result is more noticeable where the bacterial count is low since here the proportion of coliform organisms to others is greater than where the total bacterial count is high.

No detailed study appears to have been made of the coliform organisms generally found in samples of "Certified" milk. It was thought, therefore, that such a study might be of interest and might throw some light on the factors which govern the keeping quality of milk generally.

Methods.

Thirty-two samples of milk, which by the presumptive test did not show the presence of *B. coli* in 1/10 c.c. were examined. The examination consisted of determining the number and type of coliform organisms which grew on bile salt agar, after incubation for 48 hours at 37·5° C.

Every colony which grew on the "1/10 c.c." plate (*i.e.* the plate prepared by adding 1 c.c. of a dilution of the milk of 1 in 10 to 8 c.c. of bile salt lactose, bromcresol agar) was picked off and subcultured into bile salt broth and incubated for 48 hours. Each culture thus produced was then purified by plating out on bile salt agar. Often several platings had to be made before a pure colony was obtained; due, in many cases, to the large capsules which certain types produced. A number of the colonies originally isolated from the "1/10 c.c." plate died before reaching the final platings. Each pure culture was then stained by Gram's method and examined microscopically. Here a large number of cultures were discarded because they were cocci. All the organisms which grew on the final platings on bile salt agar and which were short Gram negative rods were considered to be coliform organisms.

Each organism was then inoculated into the following substances:

1. Taurocholate broth containing:
   - (*a*) Glucose.
   - (*b*) Lactose.
   - (*c*) Saccharose.
   - (*d*) Dulcite.
   - (*e*) Mannite.
   - (*f*) Adonite.
   - (*g*) Inulin.
   - (*h*) Salacin.
   - (*i*) Sorbite.
   - (*j*) Laevulose.
   - (*k*) Galactose.
   - (*l*) Arabinose.
   - (*m*) Maltose.
   - (*n*) Dextrin.
   - (*o*) Glycerin.
2. Litmus milk.
3. Gelatine.
4. Peptone water (for indol formation).
5. Glucose peptone water (for Voges and Proskauer's reaction and methyl red reaction).

NOTES ON THE REACTION AND PREPARATION OF THE MEDIA.

1. *Bile salt-lactose-bromcresol agar.*

This medium had the following composition:

Sodium taurocholate	0·5 %
Peptone (Allen and Hanbury's)	2·0 %
Tap water	100 c.c.
Lactose	1·0 %
Agar agar	2·0 %
*p*H	6·8

Bromcresol purple (0·006 per cent.) was added to the medium after filtering as an aid in identifying those organisms which produced acid from lactose. The medium immediately surrounding those colonies which produced acid became, after 24 hours' incubation, a bright canary yellow, while those colonies which produced alkali turned the medium a deep purple. It should be noted, however, that colonies of *B. coli communis* and *B. coli communior* may become blue again after 48 hours owing probably to the formation of amines and ammonia by the action of these bacteria upon the nitrogenous matter present.

2. *The sugar media.*

In the preparation of this medium, taurocholate broth having the following composition was used:

Sodium taurocholate	0·5 %
Peptone (Allen and Hanbury's)	2·0 %
Tap water	100 c.c.
Sugar (added after filtering)	1·0 %
(In the case of glucose, dulcite and adonite ...	0·5 %)
*p*H	6·8

Bromcresol purple was used as an indicator (0·006 per cent.) and was found to be much superior to acid fuchsin (Holman, 1914) or litmus. In the presence of acid this medium produces various colours between purple and bright yellow, according to the degree of acidity. Alkalinity, on the other hand, is indicated by increasing shades of purple to deep blue. An advantage

of this indicator is that it was not bleached as a result of the growth of any of the organisms studied.

The medium, after inoculation, was incubated at 37·5° C. for three days and the results noted each day. The cultures were then kept at room temperature (15° C.) for three weeks and any changes noted at the end of each week.

3. *Motility.*

Cultures of the organisms grown for 18 to 20 hours on bile salt agar were used to demonstrate motility. This character, however, appeared to be an extremely variable one, and little reliance could be placed on a negative result.

Classification.

The classification adopted is that suggested by Stewart (1918). The number of small differences between many of the organisms made it almost impossible to adopt the classification suggested by Bergey (1923). Stewart's classification, although arbitrary, has been found to work well in practice. It includes "all the small, Gram negative, non-sporing bacilli which grow readily on bile salt media and which give a more or less abundant growth on agar."

The classification is as follows:

(1) B. coli *group:*
Fermentation of glucose and lactose with or without the formation of gas.

(2) B. proteus *group:*
- (*a*) Fermentation of glucose and saccharose with formation of acid and gas.
- (*b*) Non-fermentation of lactose.
- (*c*) Rapid liquefaction of gelatine.
- (*d*) Clotting and bleaching of litmus milk and finally more or less digestion of the clot.

(3) B. morgan, *No.* 1 *group:*
- (*a*) Fermentation of glucose, laevulose and galactose only with the formation of acid and gas.
- (*b*) Formation of indol.

(4) B. faecalis alkaligenes *group:*
- (*a*) Fermentation of none of the carbohydrates tested.
- (*b*) Motility present.
- (*c*) Litmus milk rendered strongly alkaline.
- (*d*) Gelatine not liquefied.

(5) *Group X:*
- (*a*) Fermentation of glucose, laevulose, galactose and inosite, with formation of acid, but no gas.
- (*b*) Non-fermentation of lactose.
- (*c*) Litmus milk rendered acid and then strongly alkaline.
- (*d*) Motility present.
- (*e*) Formation of indol.

(6) *Group Y:*
- (*a*) Fermentation of galactose with formation of acid, but no gas.
- (*b*) Non-fermentation of laevulose.
- (*c*) Motility absent.

(7) B. pyocyaneus.

(8) *Unclassified.*

Two hundred and sixty-eight organisms were isolated from the 32 samples of milk studied. Table II illustrates the group distribution of the organisms.

Table II. *Group distribution of the organisms isolated.*

Group	Number of cultures	Percentage of cultures
B. coli	176	65·7
B. proteus	22	8·2
B. faecalis alkaligenes	21	7·8
Group *X*	17	6·3
Group *Y*	32	12·3

From this table it will be seen that the 268 organisms isolated fall within five of the groups suggested by Stewart. It is interesting to note that, while 65·7 per cent. of the organisms come within the true *B. coli* group, 18·6 per cent. appear in groups *X* and *Y*.

Table III gives in detail the number of cultures of each group which were found in each sample of milk.

Table III. *Number of cultures of each group found in* 1/10 *c.c. in each sample of milk.*

No. of milk sample	*B. coli*	*B. proteus*	*B. faecalis alkaligenes*	Groups
1	3	.	.	2
2	3	.	.	3
3	5	.	.	4
4	4	.	.	3
5	5	.	.	3
6	4	.	.	2
7	6	.	.	2
8	4	.	.	.
9	6	.	.	3
10	4	2	7	2
11	4	2	5	2
12	6	1	2	.
13	4	4	4	.
14	5	7	3	2
15	6	1	.	.
16	5	3	.	2
17	6	2	.	5
18	2	.	.	4
19	6	.	.	.
20	7	.	.	3
21	7	.	.	5
22	8	.	.	2
23	9	.	.	.
24	9	.	.	.
25	8	.	.	.
26	6	.	.	.
27	6	.	.	.
28	9	.	.	.
29	7	.	.	.
30	5	.	.	.
31	4	.	.	.
32	3	.	.	.
	176	22	21	49

Table III shows (1) that organisms of the *B. coli* group were present in all of the samples examined, (2) *B. proteus* occurred in eight of the samples examined but in this connection it should be noted that these samples were

Table IV. *Fermentation reactions of the coliform organisms isolated from samples of certified milk.*
(*Based on Stewart's Classification*, 1918.)

B. coli group	Frequency of occurrence	Glucose	Lactose	Saccharose	Dulcite	Mannite	Adonite	Inulin	Salacin	Sorbite	Laevulose	Galactose	Maltose	Arabinose	Dextrin	Glycerin	Milk E	Milk L	Gelatine	Motility	Indol	V. and P.	M.R.
Subgroup 1:																							
B. vesiculosus	1	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	AC	CB	−	−	+	−	+
	1	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	−	+	−	+
	3	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	−	+	−	+
	2	+	+	−	−	+	−	−	+	+	+	+	+	+	−	+	AC	CB	−	+	+	−	+
	2	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	−	+	−	+
	2	+	+	−	−	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	−	+	−	+
Subgroup 2:																							
B. coli communis	18	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	4	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	2	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	3	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	3	+	+	−	+	+	−	−	+	+	−	+	+	+	+	+	AC	ACB	−	+	+	−	+
	1	+	+	−	+	+	−	−	+	+	−	+	+	+	+	+	AC	ACB	−	+	+	−	+
	4	+	+	−	+	+	−	−	+	+	−	+	+	+	+	+	AC	ACB	−	+	+	−	+
Subgroup 3:																							
B. neapolitanus	56	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	6	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	2	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	1	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	CB	−	+	+	−	+
	3	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	3	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	CB	−	+	+	−	+
	4	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	2	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	ACB	−	+	+	−	+
	1	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	AC	CB	−	+	+	−	+

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Subgroup 4:																							
B. lactis aerogenes	39	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	AC	CB	−	−	−	+	−
	2	+	+	+	−	−	−	−	+	+	+	+	+	+	+	.	AC	CB	−	−	−	+	−
	4	+	+	+	−	+	+	−	+	+	−	+	+	•	+	+	AC	ACB	−	−	−	+	−
	4	+	+	+	−	+	+	−	+	+	+	+	+	−	+	+	AC	CB	+	+	−	+	−
	3	+	+	+	−	+	+	−	+	+	+	+	+	•	+	−	AC	ACB	−	−	−	−	•
B. proteus group:																							
B. proteus vulgaris	15	+	−	+	−	−	−	−	−	−	+	+	−	−	−	+	Alk	CBD	+	+	−	−	+
	2	+	−	+	−	−	−	−	−	−	+	+	−	•	−	+	A	CBD	+	−	−	−	•
	1	+	−	+	−	−	−	−	−	−	+	•	−	−	•	−	Alk	CBD	+	−	−	−	−
	3	+	−	+	−	−	−	−	−	−	+	+	−	•	−	+	Alk	CBD	+	+	−	−	−
	1	+	−	+	−	−	−	−	−	−	+	•	−	−	−	+	Alk	CBD	+	−	−	+	+
B. faecalis alkaligenes group:																							
B. faecalis alkaligenes	17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Alk	Alk	−	+	−	−	−
	2	A	−	−	−	−	−	−	−	−	A	−	−	−	−	−	Alk	B	−	+	−	−	•
	2	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	Alk	Alk	−	−	−	−	−
Group *X*:	11	A	−	A	−	A	−	−	A	−	A	A	A	A	A	−	A	A	−	−	−	−	+
	3	A	−	A	−	A	−	−	A	−	A	A	A	−	A	−	A	A	−	−	−	−	+
	3	A	−	A	−	A	−	−	A	−	A	A	A	A	A	−	A	A	−	−	−	−	−
Group *Y*:	5	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	CB	CBD	+	+	−	−	+
	8	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	CB	CBD	+	−	−	−	−
	7	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	CB	CBD	−	+	−	−	+
	12	A	−	−	−	−	−	−	−	−	−	A	−	−	−	−	CB	CBD	−	+	−	−	−

A = Formation of acid
\+ = Formation of acid and gas or a positive reaction.
AC = Acid and clot in litmus milk.
B = Bleaching.
CB = Clot and bleaching in litmus milk.
ACB = Acid, clot and bleaching in litmus milk.
Alk = Alkalinity.
CBD = Clot, bleaching in litmus milk, digestion of clot.
• = Doubtful reaction.
E = Early.
L = Late.

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received from the same source although on different occasions. Moreover, those samples which contained *B. faecalis alkaligenes* also contained *B. proteus*, (3) that members of groups *X* and *Y* were found in 17 of the 32 samples examined.

The fermentation reactions of the organisms isolated are given in Table IV.

THE *B. COLI* GROUP. This group includes those organisms which "ferment glucose and lactose with or without the formation of gas." The group has been divided into four subgroups, dependent on the fermentation of saccharose and dulcite as suggested by MacConkey (1905), *B. vesiculosus* has been taken as typical of subgroup 1, *B. coli communis* as typical of subgroup 2, *B. neapolitanus* as typical of subgroup 3 and *B. lactis aerogenes* as typical of the fourth subgroup. These subgroups have the following common characteristics (Stewart, 1918):

(1) Fermentation of glucose, lactose, mannite, laevulose, galactose, maltose and arabinose.
(2) Formation of acid and clot in litmus milk.

The characters which tend to divide these subgroups are:

(1) Fermentation of saccharose and dulcite.
(2) Gelatine liquefaction.
(3) Voges and Proskauer's reaction.
(4) The methyl red test.

These characters are summarised in Table V.

Table V. *Characters which separate the subgroups of the* B. coli *group.*

Subgroup	Saccharose	Dulcite	Gelatine	Voges and Proskauer	Methyl red
1	−	−	−	−	+
2	−	+	−	−	+
3	+	+	−	−	+
4	+	−	−	+	−

Subgroup 1. Eleven of the organisms isolated failed to ferment saccharose and dulcite and are therefore classed under subgroup 1. None of the cultures, however, gave the typical reactions of *B. vesiculosus*. Four cultures failed to ferment dextrin, one gave a doubtful reaction with inulin and salacin, one a doubtful reaction with arabinose and one a doubtful reaction with Voges and Proskauer's reaction. Three organisms failed to produce indol and two gave a doubtful reaction with methyl red.

Subgroups 2 *and* 3. These two subgroups will be considered together because, with the exception of the fermentation of saccharose, they have many points in common.

Thirty-five of the organisms isolated are included in subgroup 2 and 78 in subgroup 3. Eighteen of those in subgroup 2 gave the fermentation reactions of *B. coli communis* and 56 of those in subgroup 3 gave the fermentation reactions of *B. neapolitanus*.

The majority of the organisms in these two subgroups are V. and P.

negative and M.R. positive which according to the researches of Levine (1916) and others indicate that the organisms have originally come from faeces. It will be seen that eight of the cultures gave a negative reaction with the V. and P. and M.R. tests, while six gave a negative V. and P. and a doubtful M.R. reaction thus showing that these cultures do not conform to the correlation suggested by Levine (1916).

Four cultures in group 2 liquefied gelatine.

It is interesting to note that seven cultures did not ferment mannite; that laevulose was not fermented by eight cultures and that five cultures gave a doubtful reaction with maltose.

Fourteen cultures gave a doubtful reaction with arabinose.

Subgroup 4. The typical member of this subgroup is *B. lactis aerogenes*. Fifty-two of the 176 organisms in the *B. coli* group fall within this subgroup. Of these, 13 gave atypical reactions. In two cases, mannite was not fermented and in four cases laevulose was not fermented. Four of the organisms failed to ferment arabinose. All of the cultures isolated, with the exception of three, gave the Voges and Proskauer reaction and in no case was indol produced.

Stewart (1918) gives a table which compares the proportions of strains of lactose fermenters from wounds, from human, cow and horse faeces, and from soil and surface waters. It is interesting to add the proportion of strains of *B. coli* obtained from certified milk samples (Table VI).

Table VI. *Distribution of strains of* B. coli *from* (1) *wounds*, (2) *human, cow and horse faeces*, (3) *sewage, soil and surface waters*, (4) *certified milk samples* (*per cent.*).

Lactose fermenters	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
1. From wounds (Stewart)	14·6	12·2	36·6	36·6
2. From human, cow and horse faeces (MacConkey)	17·8	31·9	39·6	10·7
3. From sewage, soil and surface waters (MacConkey)	8·4	4·2	49·3	38·1
4. From certified milk samples	6·2	19·9	44·3	29·6

It will be noticed that the distribution of the organisms found in samples of certified milk bears some resemblance to that found by MacConkey (1909) in sewage, soil and surface waters.

B. PROTEUS GROUP. Fifteen of the 22 organisms included in this group gave fermentation reactions typical of *B. proteus*. Of those organisms which were atypical, five gave doubtful reactions with arabinose, two gave doubtful reactions with galactose, and two gave doubtful reactions with methyl red. Only one culture was found to give the Voges and Proskauer reaction.

B. FAECALIS ALKALIGENES GROUP. Of the 32 samples of milk examined only five samples contained *B. faecalis alkaligenes*. Twenty-one organisms were isolated of which 17 gave typical reactions, *i.e.* did not ferment any of the sugars tested. Two of the atypical cultures produced acid in glucose and laevulose and gave a doubtful reaction with methyl red. Two other cultures

failed to ferment any of the sugars tested but they did not reduce nitrates as did the other organisms in this group.

GROUPS *X* AND *Y*. These groups include those organisms which ferment glucose, galactose, and inosite with the production of acid, but do not ferment lactose. Group *Y* organisms do not ferment laevulose, and they do not produce indol—in these respects they differ from the members of group *X*.

Of the 49 organisms which fall into these two groups, 17 have been classed under group *X*. The reactions given by 11 of these 17 organisms are interesting. They ferment saccharose, glucose, mannite, salacin, laevulose, maltose, galactose, dextrin and arabinose with the production of acid alone. Gelatine was not liquefied even after three months at 15° C. Motility was absent and indol was not produced by any of the strains. Milk was rendered slightly acid but no curdling or bleaching occurred. The Voges and Proskauer reaction was negative. These organisms resemble Stewart's No. 34, the only difference being that they do not ferment lactose which justifies their inclusion in group *X*.

Three of the remaining six organisms in this group did not produce acid in arabinose and three failed to give the methyl red reaction.

All of the 17 organisms reduced nitrates to ammonia.

Group *Y* includes 32 organisms and the only sugars which they fermented were glucose and galactose. These organisms did not give Voges and Proskauer's reaction nor did they produce indol. From Table IV (p. 300) it will be seen that the group can be divided into two subgroups dependent on the liquefaction of gelatine. The organisms therefore resemble those in subgroups 1 and 2 of Stewart's group *Y*. The subgroups can be still further divided according to their methyl red reactions. The subdivisions are given in Table VII.

Table VII. *Subdivisions of subgroups* 1 *and* 2 *of group Y.*

Subgroup	Subdivision	Glucose	Galactose	Gelatine	Methyl red
1	(*a*)	Acid only	Acid only	+	+
	(*b*)	,,	,,	+	−
2	(*a*)	,,	,,	−	+
	(*b*)	,,	,,	−	−

From a consideration of the characters of these organisms it would appear that the members of groups *X* and *Y* isolated from milk and those isolated by Stewart from wounds are closely related and probably originate from the same strain or strains. Stewart suggests that probably these organisms are derived from soil and water.

SUMMARY AND CONCLUSIONS.

The experiments herein described show that:

(1) Of the 268 coliform organisms isolated from 32 samples of certified milk, 65·7 per cent. fall within the true *B. coli* group and 18·6 per cent. in groups *X* and *Y* of Stewart.

(2) 54·1 per cent. of the organisms, *i.e.* those of the *B. faecalis alkaligenes* group and those in subgroups 1, 2 and 3 of MacConkey which fermented lactose, gave a negative V. and P. and a positive M.R. reaction, can be assumed to be of faecal origin.

(3) 45·9 per cent., namely those organisms of the *B. proteus* group, those of subgroup 4 of the *B. coli* group and those of groups *X* and *Y* are of the type generally found in soil and water.

(4) All of the 32 samples examined contained coliform organisms in a dilution of 1 in 10 as shown by bile salt agar plates. None of these samples, however, gave a positive reaction with the presumptive test in two out of three tubes of 1/10 c.c. dilution. The presumptive test cannot, therefore, be relied upon to give a true indication of the presence of coliform organisms in milk. The inaccuracy of the test may be due in part to the structure of the ordinary Durham's tube which fails to ensure the collection of the gas produced.

(5) Although the majority of the coliform organisms present in milk ferment lactose other members of the group which are not lactose fermenters occur and consequently their presence is not demonstrated by the presumptive test.

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