RECENT ADVANCES IN THE DIFFERENTIATION OF LACTOSE-FERMENTING (GAS-PRODUCING) BACILLI, WITH SPECIAL REFERENCE TO THE EXAMINATION OF WATER AND FOOD PRODUCTS.

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In recent years a considerable amount of work has been done by American bacteriologists on the differentiation of lactose-fermenting (gas-producing) organisms.

Rogers, Clark and Davis (1914) showed that by refined technique these organisms could be sharply differentiated into two main types by the estimation of the ratio of carbon dioxide to hydrogen produced in glucose media: those producing gases in the ratio $CO_2 : H_2$ = about 1·06 and characterised by the large proportion of strains producing indol, and those producing gases in the ratio $CO_2 : H_2$ = over 1·5 and characterised by the low proportion of strains producing indol.

Subsequently Rogers, Clark and Evans (1914) showed that practically only those organisms producing gases in the low ratio ($CO_2 : H_2$) were to be found in bovine faeces, while in milk about 50 % belong to the low and 50 % to the high ratio ($CO_2 : H_2$) type, and drew the inference that source of the latter must be sought elsewhere than in bovine faeces.

Later Rogers, Clark and Evans (1915) examined 166 cultures of glucose fermenters derived from various kinds of grain, 160 of these fermented lactose also, and 95 % of the lactose-fermenters were found to belong to the high ratio ($CO_2 : H_2$) type. The further inference was therefore drawn that the high ratio type in milk might be derived from grain.

Clark and Lubs (1915) showed that the gas ratio ($CO_2 : H_2$) varies inversely with the limiting hydrogen ion concentration attained in standard glucose peptone media containing a standard amount of buffer substance[1], the low ratio type producing a hydrogen ion concentration equal to

$$1 \times 10^{-5} \overline{N} - 2 \times 10^{-5} \overline{N},$$

and the high ratio type a hydrogen ion concentration equal to

$$7{\cdot}8 \times 10^{-7} \overline{N} - 0{\cdot}1 \times 10^{-7} \overline{N},$$

[1] Composition of Clark and Lubs medium: glucose 0·5 %, peptone (Witte) 0·5 %, di-potassium phosphate K_2HPO_4 0·5 %.

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and showed moreover that these concentrations can be distinguished by means of the indicators methyl-red and *p*-nitro-phenol, and thereby rendered a simple method available for the differentiation of these two main types suitable for routine bacteriological examinations.

Levine (1916) correlated the Voges-Proskauer reaction with the limiting hydrogen ion concentration, having found that organisms producing a low concentration gave the Voges-Proskauer reaction while those producing a high concentration did not, and later, in conjunction with Weldin and Johnson (1917), devised an improvement in the original reaction accelerating the oxidation process with hydrogen peroxide. Myrtle Greenfield (1916) studied 432 cultures of lactose-fermenters from surface water, ground water, natural and artificial ice, and found that 138 (or 32 %) produced a low concentration of hydrogen ions. All these gave the Voges-Proskauer reaction, while none of those cultures producing a high concentration of hydrogen ions gave a positive V.-P. reaction.

Other workers in America have obtained similar results confirming the correlation of the Voges-Proskauer reaction with the limiting hydrogen ion concentration.

For convenience organisms producing high and low concentrations of hydrogen ions will be referred to in this paper as methyl-red positive ("M.R. +") and methyl-red negative ("M.R. −") respectively, and organisms giving and not giving the Voges-Proskauer reaction as "V.-P. +" and "V.-P. −."

As regards the distribution of the "M.R. +" and "M.R. −" types the following additional observations have been recorded:

Levine (1916) examined lactose-fermenters from the faeces of the horse (19), pig (21), cow (20), sheep (22), and man (25) and found none of the "M.R. −" type out of this total of 107.

Rogers, Clark and Lubs (1916) tested 113 cultures from human faeces and found only six (or 5·6 %) of the "M.R. −" type[1]. Rogers (1916) examined 137 cultures from surface water, 66 % of which were found to belong to the "M.R. −" type. The "M.R. +" types were occasionally found in springs in which there was no evident source of contamination, but were especially abundant in polluted waters. Johnson (1916) examined 363 coli-like organisms derived from soil and found 72 % of the "M.R. −" type.

Burton and Rettger (1917) found the predominant gas formers in soil were of the "V.-P. +" type and liquefied gelatine, *i.e.* were of the *B. cloacae* type. Out of 193 non-sporing lactose-fermenters 76 % were "V.-P. +" and liquefied gelatine, 5 % were "V.-P. +" and did not liquefy gelatine, and 19 % were "V.-P. −". He used the M.R. reaction also but regarded the V.-P. reaction as the more reliable with peptones other than Witte's.

[1] In a recent paper Rogers, Clark and Lubs (1918) record the isolation of 46 "M.R. −" strains from a collection of 177 derived from human faeces. The majority of these however were obtained by special methods, and 31 were obtained from a single specimen.

Levine (1918) gives the relative proportion of strains in soil as *cloacae* 49·7 %, *aerogenes* 30·5 %, and "M.R. +" types 18·7 %.

Observations of the Voges-Proskauer reaction have been recorded by a few investigators in this country, and, although it has not been regarded as altogether reliable, it is interesting to compare the results obtained with those briefly summarised above.

MacConkey (1906) recorded the examination of 107 cultures of non-chromogenic lactose-fermenters isolated from milk 31 of which (or 29 %) gave the Voges-Proskauer reaction. The same author (1909) examined 497 cultures from various sources including human and animal faeces, sewage, pond-water, roof washings, soil, and various grains. Organisms giving the Voges-Proskauer reaction were shown to be rare in human and animal faeces (21 in 334 or 6·3 %), more common in sewage and pond-water, roof-washings and soil (32 in 65 or 49 %), and still more common in grain (17 in 30 or 57 %); a fair number in the last two groups were, however, chromogenic.

Orr (1908) examined 850 cultures of glucose-fermenters isolated from milk and found that 333 (or 39 %) gave the Voges-Proskauer reaction. The number of cultures fermenting lactose was not recorded.

Houston (1911) examined 532 lactose-fermenters isolated from water and found that 10·3 % from raw river water, 5·3 % from stored river water, and 3·2 % from stored and filtered river water gave the Voges-Proskauer reaction: no evidence of increase of the "V.-P. +" types after storage.

In India, Clemesha (1912) found that *B. lactis aerogenes* ("V.-P. +") was rare in recently polluted waters but became extremely common within a period of 5–15 days after pollution; that this organism became very common in surface waters after rainy seasons, and that *B. cloacae* ("V.-P. +") was the predominant type after dry seasons. He examined 104 samples of human faeces and 1207 cultures, only 4·6 % of these gave the Voges-Proskauer reaction, while from 86 samples of bovine faeces and 1029 cultures this reaction was only given by 13·4 %.

Recently experiments have been carried out by Winslow and Cohen (1918) on the viability of "M.R. +" and "M.R. −" types in water. The average result of 11 experiments showed a relative increase in the "M.R. −" type after nine weeks storage from 46 % to 71 %. These investigators were unable to find a proportion of the "M.R. −" type in gas-producers isolated from unpolluted or stored waters greater than the proportion found in polluted and unstored waters.

To sum up: the results of various investigators show that the lactose-fermenting bacilli can be divided into two main types by the methyl-red or Voges-Proskauer tests, that the "M.R. −" "V.-P. +" type are rare in the faeces of man and animals, are more common in surface water and sewage, and are the predominant type in grain and soil. These findings are in favour of the view that they are either the natural survivors of the lactose-fermenters present in excretal matter, or are derived from soil, or possibly from grain,

and consequently their presence in water and food products is to be regarded as of less sanitary significance than the presence of excretal *B. coli*.

The object of the present investigation was (1) to add confirmation to the results outlined above (no observations based on the methyl-red test have as yet been recorded in this country), (2) to form some idea of the frequency with which the "M.R. −" type is likely to be encountered in water examinations, etc. using bile-salt media, and (3) to throw new light on the sanitary significance of this type in water and food stuffs by noting the presence or absence of *streptococci* in water samples in which they are found[1].

The following examinations were made:

A. Human and animal faeces.
B. Cereals, grain and other articles.
C. Milk.
D. Water supplies.

A. THE EXAMINATION OF HUMAN AND ANIMAL FAECES.

The faeces emulsion in sterile water was in most cases plated direct on lactose bile-salt neutral-red agar. In certain cases, however, it was first cultivated in lactose bile-salt broth and then plated out. Subcultures showing gas-production in lactose broth were cultivated in the glucose di-potassium phosphate medium of Clark and Lubs for five days at 37° C., the culture divided into two portions and tested with methyl-red and for the Voges-Proskauer reaction. With the latter, observations were made 24 hours (or within 24 hours if positive) after the addition of the sodium hydroxide.

The following specimens and cultures were examined and gave results as tabulated below:

Table I.

Cultures from human and animal faeces.

Source	No. of Cultures	No. of Individuals	Methyl-red		Voges-Proskauer	
			+	−	+	−
Human faeces	33	11	33	0	0	33
Horse ,,	17	8	16	1	0	17
Cow ,,	13	7	13	0	0	13
Sheep ,,	20	7	20	0	0	20
Rabbit ,,	18	6	15	3	3	15
Mouse ,,	25	7	22	3	4	21
Cat ,,	3	1	3	0	0	3
Guinea-pig faeces	3	1	3	0	0	3
Total	132	48	125	7	7	125
Percentage	—	—	94·7	5·3	5·3	94·7

Notes on Table I.

1. The three "M.R. −" strains from rabbit faeces were from the same individual: a tame rabbit fed on oats.
2. The three "M.R. −" "V.-P. +" strains from mouse faeces and the one "M.R. +" "V.-P. −" strain were from three individuals, all wild mice.
3. "M.R. −" strains (not included in the above series) were isolated from two specimens of cow faeces as the ultimate survivors in water after several weeks' storage in the course of some experiments on the relative viability of *B. coli* and *Streptococci* [Savage and Wood (1918)].

[1] It has been shown by Savage and Read (1916) that *streptococci* are to be found in the majority of waters subject to contamination, and by Savage and Wood (1918) that these organisms die out rather more rapidly than *B. coli* and are useful indicators of recent contamination.

The above represent only a limited number of specimens and cultures, but a sufficient number to confirm the findings of other workers, viz. that the "M.R. −" "V.-P. +" type is very rare in human and animal faeces. It has been noted that organisms of the "M.R. −" type frequently gave a less definite reaction on neutral-red lactose bile-salt agar than those of the "M.R. +" type, the paler colonies were accordingly selected when present in order to favour the isolation of the "M.R. −" type as much as possible.

The correlation between the methyl-red and Voges-Proskauer tests was not quite perfect, though very nearly. Some cultures give neutral tints with methyl-red and a positive Voges-Proskauer reaction when peptone other than Witte's is used for the standard medium. Both tests should therefore be applied. The supply of Witte's peptone was exhausted when the above tests were made and Baird and Tatlock's "Bactopeptone" was used and found to be a very good substitute.

Table II.

Cultural characters of "M.R. −" types isolated from faeces.

Source	No.	Gas in Lactose	Gas in Saccharose	Litmus milk: acid	Litmus milk: clot	Indol	Character of growth on gelatine	Liquefaction of gelatine in 14 days	Voges-Proskauer reaction	Production of capsule
Rabbit	4	$\frac{5}{8}$ in.	$1\frac{1}{2}$ in.	+	+	+	...	+	+	+
,,	5	$\frac{1}{2}$,,	$1\frac{1}{2}$,,	+	+	+	...	+	+	+
,,	6	$\frac{5}{8}$,,	$1\frac{1}{2}$,,	+	+	+	...	+	+	+
Mouse	9	$\frac{5}{8}$,,	$1\frac{1}{2}$,,	+	+	+	...	+	+	+
,,	10	$\frac{3}{4}$,,	2 ,,	+	+	−	...	−	+	+
,,	11	$\frac{1}{4}$,,	2 ,,	+	+	−	...	+	+	+
Cow	1	$\frac{1}{2}$,,	$1\frac{1}{2}$,,	+	+	−	opaque	−	...	+
,,	2	$\frac{1}{2}$,,	$\frac{3}{4}$,,	+	+	−	,,	−	...	+
,,	3	slight	$1\frac{1}{2}$,,	+	+	−	,,	−	...	+
,,	7	$\frac{1}{2}$ in.	$1\frac{1}{2}$,,	+	+	−	,,	−	...	+

Notes on Table II.

1. All cultures fermented saccharose and most of them with abundant gas production.
2. All cultures showed capsule formation in milk.
3. Quite a large proportion produced indol.

B. THE EXAMINATION OF CEREALS, GRAIN AND OTHER ARTICLES.

Sixty specimens were examined, made up as follows:

Whole grain. Oats 12, wheat 14, barley 11.

Flours, etc. Crushed oats 6, barley flour 2, wheat flour 2, maize flour 4, rice flour 1.

Other articles. Straw 2, dried milk 3, egg powder 1, hay 3.

A small quantity was cultivated in lactose bile-salt broth and when acid and gas were produced, plated out on lactose bile-salt agar with neutral-red. In some cases the grains were first allowed to germinate in a sterile moist chamber at 21° C.

Lactose-fermenters were isolated from 20 specimens, from 16 of these only "M.R. −" strains were obtained, while in the remaining four only "M.R. +" strains were found.

The specimens from which the "M.R. −" strains were isolated together with their cultural characters are given in Table III.

Table III.

"M.R. −" strains isolated from cereals, grain, and other articles.

No. of specimen	Source	No. of culture	Gas in Durham's tube in inches: Lactose	Gas in Durham's tube in inches: Saccharose	Litmus milk: acid	Litmus milk: clot	Indol	Character of growth on gelatine		Liquefaction of gelatine in 14 days	Voges-Proskauer reaction	Production of capsule in milk
1	Dried milk	1	¾	¾	+	+	−	opaque*		−†	...	+
		2	¼	1¼	+	+	−	translucent*		−†	...	+
		3	⅛	1¼	+	+	−	,,	*	−†	...	+
2	,,	4	bubble	⅝	+	+	−	,,	*	−†	...	+
		5	⅜	1¼	+	+	+	,,	*	−†	...	+
3	Egg powder	6	½	2	+	+	−	,,		−†	+	+
4	Crushed oats	7	½	¼	+	+	−	,,		−†	+	+
		8	bubble	⅜	+	+	−	,,	*	−†	+	−
		9	¼	½	+	+	−	,,		−†	+	−
5	Barley flour	10	bubble	⅜	+	+	−	,,		−	...	...
6	,,	11	⅝	1¼	+	+	−	,,		−	+	−
7	Maize flour	12	1	⅜	+	+	+	,,		−	+	+
8	,,	13	⅛	1½	+	+	−	,,	*	−	+	−
9	Wheat flour	14	¼	1¾	+	+	−	,,		+	+	−
10	Straw	15	¼	½	+	+	−	,,		+	...	...
11	Oats	16	¾	¾	+	+	−	,,		−	+	−
12	,,	17	¾	1	+	+	−	,,		−	+	+
13	,,	18	¼	...	+	+	−	,,		−	...	−
14	Wheat	19	⅜	1	+	+	−	,,		−	+	+
15	Barley	20	½	1	+	+ slow	−	,,		+	+	+
16	,,	21	½	2								

* These cultures produced a slight yellow pigment.

† These cultures liquefied gelatine very slowly.

It will be noticed from Table III that the "M.R. −" strains isolated from cereals and grain frequently showed very weak lactose fermentation, less than ¼ inch of gas being produced and this sometimes only after several days. After cultivation on gelatine or in milk at 21° C. this faculty can be revived as the following instances showed:

	Gas produced in lactose broth: before cultivation at 21° C.	Gas produced in lactose broth: after cultivation at 21° C.
Barley flour, No. 10	$\frac{1}{16}$ inch	½ inch
Maize flour, No. 13	⅛ ,,	⅞ ,,
Wheat flour, No. 19	¼ ,,	over 1 ,,
Crushed oats, No. 9	¼ ,,	¾ ,,

Grain which had been allowed to sprout in a moist chamber at 21° C. usually showed good fermentation of lactose. The "M.R. −" strains also became very numerous under these conditions, and it is quite conceivable

that surface water in the neighbourhood of grain fields might be considerably affected by germinating grain.

A number of cultures liquefied gelatine, but except in three instances too slowly for this test to be of any diagnostic value. A fair number of cultures were chromogenic, but the majority showed no obvious pigment on gelatine slopes. Saccharose was nearly always fermented and frequently with abundant gas production.

Apparently some organisms of the "M.R. −" type possess remarkable viability in grain and flours. The sample of maize flour No. 7 was re-examined after keeping in a sterile bottle for three months and organisms of the "M.R. −" type were again found.

Excluding all cultures giving less than $\frac{1}{4}$ inch of gas in lactose (but including cultures Nos. 10, 13, 19 and 9), all cultures liquefying gelatine in 14 days or producing pigment, 12 out of the 21 cultures could not certainly be distinguished from *B. coli* of faecal origin except by the methyl-red and Voges-Proskauer tests, though the reaction in litmus lactose broth and on neutral-red lactose agar frequently suggested that they belonged to the "M.R. −" type.

C. EXAMINATIONS OF MILK.

Thirty-two samples of milk were examined and 94 lactose-fermenters isolated. Seventeen of these were found to belong to the "M.R. −" type and 77 to the "M.R. +" type.

In the districts from which these samples were obtained the cows are kept and milked in open fields except in the coldest months of the year when they are brought into sheds. It is interesting to compare the "field" samples with the "shed" samples. Only 8·3 % of the former contained "M.R. −" strains while in 28 % of the shed samples this type was found. These results support the suggestion of Rogers, Clark and Evans that the "M.R. −" types found in milk have their origin in grain and straw, but the possibility that the colder weather favoured the predominance of this type must be borne in mind[1].

The cultural characters of the "M.R. −" strains isolated are given in Table IV.

Table IV.

Cultural characters of "M.R. −" strains isolated from milk.

Indol production	Litmus milk acid and clot	Gelatine: liquefaction	Gelatine: opaque creamy growth	Gelatine: translucent growth	Capsule production in milk	Voges-Proskauer reaction
10 %	90 %	10 %	40 %	60 %	90 %	100 %

No cultures showed obvious pigment on gelatine slopes.

[1] In a recent paper Rogers, Clark and Lubs (1918) have shown that the "M.R. −" types tend to outgrow the "M.R. +" types when milk is allowed to curdle at 20° C.

D. THE EXAMINATION OF WATER SUPPLIES.

These comprise a large number of samples from various sources, 200 of which contained lactose-fermenters. The results are given in Tables V and VI, the former were from sources of good repute and the latter from miscellaneous sources.

For the enumeration of *Streptococci* the method of Savage was employed: cultivation in glucose neutral-red broth and examination of hanging drop preparations after 48 hours, in doubtful cases examining stained films with the $\frac{1}{12}$ inch objective.

Table V.

Methyl-red negative organisms encountered in the routine examination of 200 *water samples containing lactose-fermenting organisms.*

Sources of good repute

No. of sample	Description of source	Methyl-red negative type found in	Methyl-red positive type + = present − = absent	*Streptococci* + = present − = absent	Identification number of "M.R.−" cultures
A. 1620	Well (public supply)	10 c.c.	− 40 c.c.	− 40 c.c.	W. 5
A. 1711	do.	30 ,,	− 40 ,,	− 40 ,,	W. 17
1	Deep well in limestone (public supply)	100 ,,	− 100 ,,	− 100 ,,	W. 19
2	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 20
3	do. do. do.	100 ,,	+ 100 ,,	− 100 ,,	W. 22
x. 1748	Well in field, no source of contamination	30 ,,	− 40 ,,	− 40 ,,	W. 23
4	Deep well in limestone (public supply)	100 ,,	− 100 ,,	− 100 ,,	W. 25
5	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 26
B. 1758	Deep well in limestone	10 ,,	− 40 ,,	− 40 ,,	W. 27
6	Deep well in limestone (public supply)	100 ,,	− 100 ,,	− 100 ,,	W. 29
C. 1809	Deep well sunk through Keuper Marl into Sandstone	10 ,,	− 40 ,,	− 40 ,,	W. 41
7	Deep well in limestone (public supply)	1 ,,	− 100 ,,	− 100 ,,	W. 45, 46, 47
8	do. do. do.	1 ,,	− 100 ,,	− 100 ,,	W. 48, 49, 50
9	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 51
10	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 52
11	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 53
12	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 54
13	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 55
14	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 56
15	do. do. do.	10 ,,	− 100 ,,	− 100 ,,	W. 57, 58
16	do. do. do.	10 ,,	− 100 ,,	− 100 ,,	W. 60, 61
17	do. do. do.	10 ,,	− 100 ,,	− 100 ,,	W. 62, 63
18	do. do. do.	10 ,,	− 100 ,,	− 100 ,,	W. 64, 65
19	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 66
20	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 67
21	do. do. do.	10 ,,	− 100 ,,	− 100 ,,	W. 68
22	do. do. do.	100 ,,	− 100 ,,	− 100 ,,	W. 69
1823	Spring	30 ,,	− 40 ,,	− 40 ,,	W. 70

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Table VI.

Methyl-red negative organisms encountered in the routine examinations of 200 samples of water containing lactose-fermenting organisms.

Miscellaneous sources.

No. of sample	Description of source	Methyl-red negative type found in	Methyl-red positive type +=present −=absent	*Streptococci* +=present −=absent	Identification number of "M.R.−" cultures
1485	Shallow well	30 c.c.	−40 c.c.	−40 c.c.	W. 1
1532	do.	10 ,,	−40 ,,	−40 ,,	W. 2
1610	do.	$\frac{1}{10}$,,	...	$-\frac{1}{10}$,,	W. 3
1618	Deep well	1 ,,	...	+10 ,,	W. 4
1622	Shallow well	1 ,,	...	+ 1 ,,	W. 6
1642	do.	$\frac{1}{10}$,,	...	+ 1 ,,	W. 7
1643	do.	10 ,,	...	−40 ,,	W. 8
1652	do.	10 ,,	...	+30 ,,	W. 9
1658	do.	10 ,,	...	+30 ,,	W. 10
1694	Deep well	10 ,,	−40 ,,	−40 ,,	W. 12
1695	Shallow well	$\frac{1}{10}$,,	+ 1 ,,	+ 1 ,,	W. 13
1706	do.	$\frac{1}{10}$,,	$+\frac{1}{10}$,,	+ 1 ,,	W. 14
1709	do.	10 ,,	+10 ,,	+ 1 ,,	W. 16
1712	do.	10 ,,	+30 ,,	+10 ,,	W. 18
1734	do.	10 ,,	+30 ,,	−40 ,,	W. 21
1691	Spring	30 ,,	−40 ,,	−40 ,,	W. 11
1751	Shallow well	10 ,,	...	+30 ,,	W. 24
1760	do.	10 ,,	...	+10 ,,	W. 28
1792	do.	1 ,,	+10 ,,	+30 ,,	W. 30
1793	do.	30 ,,	−40 ,,	−40 ,,	W. 31
1799	Spring	30 ,,	−40 ,,	−40 ,,	W. 32
1800	do.	30 ,,	−40 ,,	−40 ,,	W. 33
1802	do.	30 ,,	−40 ,,	−40 ,,	W. 34
1803	do.	30 ,,	−40 ,,	−40 ,,	W. 35
1804	do.	1 ,,	−40 ,,	−40 ,,	W. 36, 37, 38
1807	do.	30 ,,	−40 ,,	−40 ,,	W. 39
1808	Well	30 ,,	−40 ,,	−40 ,,	W. 40
1810	Spring	30 ,,	−40 ,,	−40 ,,	W. 42
1813	Well	1 ,,	...	−40 ,,	W. 43
1814	do.	1 ,,	+30 ,,	+30 ,,	W. 44
1821	Shallow well	10 ,,	...	+10 ,,	W. 59
1828	do.	1 ,,	+10 ,,	+30 ,,	W. 73
1836	do.	1 ,,	+10 ,,	−40 ,,	W. 74
1855	do.	$\frac{1}{10}$,,	...	+10 ,,	W. 75
1857	do.	1 ,,	+10 ,,	+30 ,,	W. 76
1858	do.	$\frac{1}{10}$,,	...	$+\frac{1}{10}$,,	W. 77
1870	Spring	10 ,,	...	+10 ,,	W. 78

When *Streptococci* and "M.R. −" strains (the latter in 10 c.c. or less) were both present the evidence of contamination was generally considered sufficiently proven, and the presence of "M.R. +" strains in larger quantities was not as a rule sought. In samples Nos. 1695, 1706, 1709, 1712, 1734, 1792, 1814, 1828, 1836 and 1857 the evidence of contamination, as judged by the presence of both "M.R. −" strains and *Streptococci*, was confirmed by the isolation of "M.R. +" strains from a larger quantity of the sample.

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It will be noticed that 29 of the samples (Table V) were from sources of good repute. In only one of these were organisms of the "M.R. +" type also found, and *Streptococci* were found in none.

Thirteen samples from miscellaneous sources contained "M.R. −" strains but no "M.R. +" strains or *Streptococci*.

Out of the total of 66 samples, therefore, in which "M.R. −" strains were found, as many as 41 contained no "M.R. +" strains or *Streptococci*. Judgment of the water was therefore subject to modification in 62 % of these samples, the results suggesting that no recent excretal contamination had occurred.

Of special interest was the occurrence of organisms of the "M.R. −" strain in the deep wells (numbered 1 to 22). This supply which comprises several wells, sunk through limestone some 250–400 feet into underlying sand, has been kept under observation for several years, and the bacteriological results have been very good. Occasionally, in some 15 % of all samples examined, lactose-fermenters have been found, but seldom in less than 100 c.c., and these have nearly always failed to produce indol. In the spring of 1918 lactose-fermenters (all "Lactose + Indol −") were found in a larger proportion of samples, and the application of the methyl-red and Voges-Proskauer tests showed that these with one exception were of the "M.R. −" "V.-P. +" strain. On no occasion were *Streptococci* found. The simultaneous appearance in all the wells, which are several miles apart, is difficult to account for. The time of the year rather suggests some connection with the sowing of grain. Rogers, Clark and Lubs (1918) have recently shown that the majority of "M.R. −" strains derived from grain do not ferment adonitol. Unfortunately a supply of this alcohol was not available and tests could not be made.

Cultural characters of "M.R. −" strains isolated from water.

Lactose litmus peptone. Eleven cultures (or 18 %) produced $\frac{1}{4}$ inch or less of gas in the Durham's tube. The litmus indicator frequently showed less acidity than that given by "M.R. +" strains.

Saccharose. Only 27 cultures were tested. All fermented saccharose and many with remarkable gas production, the Durham's tube being completely filled in some instances.

Gelatine. Liquefaction was observed with six (or 9 %) but only with three was it sufficiently rapid to be of diagnostic value.

None showed obvious pigment.

25 % showed the opaque creamy growth typical of *B. lactis aerogenes*, the rest being more or less translucent.

Capsule production in milk. 84 % produced capsules.

Litmus milk. 95 % showed acid and clot within a week.

Indol. 15 % produced indol in five days.

Voges-Proskauer reaction. 92 % gave this reaction.

Lactose bile-salt neutral-red agar. Colonies were frequently somewhat paler

than "M.R. +" organisms. The large majority developed mucoid colonies with tendency to become confluent.

English standards as to what is to be considered an excretal type of *B. coli* as distinguished from a coliform organism vary to some extent. Such organisms are required in this laboratory to have the following characters, and it may be taken that this definition would be accepted by most bacteriologists:

Lactose. Acid and gas production (a bubble or less than $\frac{1}{4}$ inch in the Durham's tube excluded)[1].

Litmus milk. Acid and clot production within seven days.

Gelatine. No liquefaction within two weeks, and no pigment formation.

Indol. Not necessarily produced, but a fairly strong point against its being of recent excretal origin if not produced.

The value of the methyl-red and Voges-Proskauer tests really depends upon the extent to which they are capable of further differentiating organisms *with the above characters* into two groups, one of which is truly excretal and the other non-excretal in origin or very resistant. From this point of view only organisms possessing the above characters need be considered. The following table includes only such organisms:

Table VII.

Material	Number of strains tested	Number of strains			Percentages	
		"M.R.+"	"M.R. −" Indol+	"M.R. −" Indol−	"M.R.+"	"M.R. −"
Human faeces	33	33	0	0	100	0
Animal faeces	99	91	4	4	91·9	8·1
Cereals and grain	15	4	1	10	26·5	73·5
Water	231	154	12	65	66·7	33·3
Milk	93	77	2	14	82·5	17·5

The Committee appointed by the Council of the Royal Institute of Public Health in 1914 recommended that in the bacterioscopic examination of waters reports should be based upon the enumeration of "Lactose + Indol +" organisms. If as strict a definition as this be adopted it is evident that only 19 strains from the above series would come under consideration. In judging the purity of a supply from an individual sample, or even from a limited number of samples, however, it is not possible to take so strict a line. In the author's experience a water supply to which an outbreak of typhoid fever was definitely traced yielded only Lactose + Indol − organisms in the first three examinations together with *Streptococci*, subsequent examinations yielding Lactose + Indol + organisms. In the above series of water examinations 13·5 % of the lactose-fermenters did not produce indol and did *not* belong to the "M.R. −" type. Moreover, some of the Lactose + Indol + organisms belong to the "M.R. −" type, Levine (1918) recorded as many as 25 % producing indol.

[1] Some bacteriologists use gelatine shake cultures for observation of lactose fermentation. This is extremely sensitive and cultures producing only a bubble of gas in the Durham's tube would be recorded as positive.

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SUMMARY AND CONCLUSIONS.

1. Investigations by American bacteriologists have shown that the lactose-fermenting (gas-producing) bacilli can be divided into two main types distinguishable by the methyl-red and Voges-Proskauer reactions.

2. The methyl-red − Voges-Proskauer + type are shown to be rare in human and animal faeces, more common in surface water, milk and sewage, and the predominant type in soil and grain, and to be more resistant than the methyl-red + Voges-Proskauer − type.

3. Investigations by the author of this paper confirm their findings as regards human and animal faeces, water, milk and grain. An investigation of the types present in soil is being undertaken and it is hoped to publish an account of this later. Already the "M.R. −" type has been found to predominate in four out of six samples of soil.

4. In the present investigation organisms of the "M.R. −" type were found in 66 samples of water out of a total of 200 containing lactose-fermenters, and in 41 samples containing this type no evidence of recent contamination could be adduced by the search for organisms of the "M.R. +" type or *Streptococci*. Judgment of the water was therefore liable to modification in 20 % of these samples by the recognition of this type.

5. Twenty-nine out of the 66 samples containing organisms of the "M.R. −" type were from sources of good repute, most of them from public supplies.

6. The presence of lactose-fermenters of the "M.R. −" "V.-P. +" type must be regarded with considerably less disfavour than the presence of "M.R. +" "V.-P. −" organisms, and the application of tests for the recognition of these types is important. It is suggested that these tests should be included in all routine examinations of water and food products.

In conclusion I have pleasure in acknowledging my indebtedness to Dr W. G. Savage for calling my attention to the researches of American bacteriologists and for many valuable suggestions he has made in connection with this work.

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