A COMPARISON BETWEEN MacCONKEY BROTH AND GLUTAMIC ACID MEDIA FOR THE DETECTION OF COLIFORM ORGANISMS IN WATER

BY THE PUBLIC HEALTH LABORATORY SERVICE WATER SUB-COMMITTEE*

(With 1 Figure in the Text)

INTRODUCTION

Of the culture media introduced by MacConkey (1900, 1905, 1908) the bile-salt lactose peptone-water variety, now popularly termed MacConkey broth, has long been the standard medium in this country for the primary isolation of coliform bacteria from water, and is still officially recommended for this purpose (Ministry of Health, 1956). Its advantages in the presumptive coliform test are well known: a low proportion of false positive reactions (P.H.L.S. Water Sub-Committee, 1953) and the fact that most strains of *Bact. coli* produce a positive reaction within 24 hr. (Thresh, Beale & Suckling, 1943). Its disadvantages, not so commonly appreciated, lie in the variability of two of its ingredients: peptone and bile salt. For satisfactory gas-production a good quality peptone is essential, and only certain brands fulfil this criterion. Bile salts vary considerably in inhibiting the growth of non-coliform flora; some preparations appear to lack this property altogether, while others are only partially effective. To overcome this latter difficulty, Windle Taylor introduced in the Metropolitan Water Board Laboratories a form of standardization, described by Burman (1955), for testing the efficiency of new samples of bile salt against a known satisfactory sample. Such precautions in the choice of peptone and bile salt are essential for the preparation of MacConkey broth of optimal character but, even if they are observed, no culture medium containing such variable biological products can be expected to give a uniform performance in different laboratories. For this reason it has long been the aim of water bacteriologists to devise a chemically defined medium which would function at least as well as MacConkey broth at its best and would, at the same time, give consistently reproducible results.

The glucose glutamic acid medium described by Folpmers (1948) for anaerobic use in routine water examination was investigated by Burman & Oliver (1952). These authors found that the anaerobic method used by Folpmers had too many practical disadvantages for the routine examination of large numbers of samples; the medium, however, could be distributed in tubes and incubated aerobically, as is customary with MacConkey broth. In a trial in which Folpmers's medium, used in this way, was compared with MacConkey broth in the presumptive coliform test

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of 168 samples of water, the glutamic acid medium produced the higher yield, both of presumptive positive results and of *Bact. coli*. The authors then substituted lactose for the glucose in Folpmers's formula and compared this medium with MacConkey broth in the presumptive coliform test of 120 samples of water. They found that *Bact. coli* developed more slowly in lactose glutamic acid than in *MacConkey broth, but that the ultimate yield was greater, and was obtained from* fewer presumptive positive tubes. They concluded that, as regards isolation of *Bact. coli*, both types of glutamic acid medium were slightly superior to MacConkey broth, but that lactose glutamic acid provided a lower yield of the other coliform organisms and was, for this reason, inferior to both MacConkey broth and glucose glutamic acid. They emphasized, however, the preliminary nature of the trials and the desirability of conducting more extensive and more carefully controlled comparisons between the three media. The results of such an investigation are described below.

METHODS

Preparation of media

MacConkey broth (single-strength and double-strength) was prepared as recommended (Ministry of Health, 1956); the routine practice at each laboratory being to employ a good quality peptone, and a bile salt of satisfactory inhibitory power.

The glutamic acid media were prepared as described by Burman & Oliver (1952), viz.

	Single-strength	Double-strength
Glucose or lactose	10 g.	20 g.
L (+)-Glutamic acid	5 g.	10 g.
Potassium phosphate (K_2HPO_4)	3 g.	6 g.
Ammonium lactate 50% (w/w) solution	10 ml.	20 ml.
Tap water	1000 ml.	1000 ml.

The glutamic acid was dissolved in the water and neutralized with sodium hydroxide, the lactate and phosphate were added and the whole was heated by steaming. After filtration the glucose (or lactose) was added and the medium adjusted to pH 6·0. Brom-cresol purple (1% alcoholic solution) was added as indicator in amounts of 1 ml./l. for single-strength and 2 ml./l. for double-strength medium. The double-strength medium was distributed in 10 ml. quantities in 6 in. × $\frac{3}{4}$ in. test-tubes and the single-strength medium in 5 ml. quantities in 6 in. × $\frac{1}{2}$ in. test-tubes. Inverted inner (Durham) tubes were inserted in all tubes, and sterilization was effected by steaming for 45 min. on each of 2 successive days.

Water samples and procedure

Six laboratories co-operated in the investigation: the Metropolitan Water Board (M.W.B.) laboratories and the P.H.L.S. laboratories in Birkenhead, Conway, Manchester, Newport (Mon.) and Oxford. At these laboratories the media were compared on routine samples of water in which coliform organisms were expected to be present. In addition to the routine test set up with MacConkey broth for the presumptive coliform examination, one tube of each of the double-strength glutamic acid media was inoculated with 10 ml. of the sample. One of the five routine 10 ml.

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tubes of double-strength MacConkey broth was marked before incubation to serve as a single MacConkey broth tube for comparison with the single tubes inoculated with each of the glutamic acid media. When a coliform count of more than 25 per 100 ml. was expected, one tube of each of the single-strength glutamic acid media was inoculated with 1 ml. of the sample and one of the five routine tubes of single-strength MacConkey broth (inoculated with 1 ml. of sample) was marked for the comparison.

The glutamic acid tubes were thus studied in parallel with the routine tests at each laboratory. All tubes were inspected after 18, 24 and 48 hr. incubation. Tubes showing production of acid and gas (if necessary after gentle tapping) within these times were recorded as presumptive positives. These were further tested by: (1) subculture in MacConkey broth or brilliant-green bile broth for incubation at 44° C. for 24 hr., (2) subculture in peptone water at 44° C. for 24 hr. (for indole production), and (3) plating on MacConkey agar. Any presumptive positive tube giving a positive indole reaction and positive fermentation test at 44° C. was regarded as containing *Bact. coli* Type I. When no fermentation occurred at 44° C. or no indole was produced, one or more colonies were picked from the MacConkey plate and subcultured in lactose peptone-water. These tubes were then incubated at 37° C. for 48 hr. The appearance of acid and gas in the lactose peptone-water tube was accepted as proving the presence of true coliform organisms in the presumptive positive tube. When the lactose peptone-water tube failed to show acid and gas the presumptive positive reaction was classified as a false positive reaction, i.e., a presumptive positive tube from which no lactose-fermenting coliform bacteria could be isolated.

Incubation of the presumptive coliform test

The five P.H.L.S. laboratories adhered to the recommended procedure (Ministry of Health, 1956) whereby the inoculated tubes, without preliminary warming, were incubated at 37° C. for 48 hr. In the Metropolitan Water Board laboratories, however, the routine practice was different. The inoculated tubes, first warmed by immersion in a 42° C. water-bath, were incubated for 18 hr. at 42° C. and then transferred to the 37° C. incubator for the remainder of the 48 hr. During the course of the investigation differences were observed between the results obtained in London (M.W.B. laboratory) and those recorded elsewhere. An additional comparison was therefore made in the P.H.L.S. laboratories between initially warmed and unwarmed ('cold') tubes incubated at 37° C. for 48 hr. Similarly, an additional comparison was made in the M.W.B. laboratory, namely between initially warmed tubes incubated according to their routine procedure and at 37° C. for 48 hr. The non-routine method of incubation in each laboratory, namely in initially warmed tubes, at 37° C. for 48 hr., provided a link between the results in the various laboratories.

RESULTS

Comparison between the media after 48 hr. incubation

Of a much larger total of samples tested, 4421 showed differences between the media. All three media were tested in parallel for part of the investigation only, on 1997 of these samples. MacConkey broth and lactose glutamic acid were also

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compared on a further 1920 of these samples (giving a total of 3917 samples for this comparison) and MacConkey broth and glucose glutamic acid on the remaining 504 samples (giving a total of 2501 samples for this comparison).

The findings after incubation for 48 hr. are summarized in Table 1, in which the top two lines record a comparison between MacConkey broth and lactose glutamic acid on 3917 samples. Lactose glutamic acid produced 207 fewer presumptive coliform reactions than MacConkey broth, but gave a considerably greater yield of *Bact. coli* I—an increase of 156 isolations, or 9·5 % of the isolations in MacConkey broth. The smaller total of presumptive coliform reactions represented a reduction of 61 (36 %) in the number of false positive reactions, and a reduction of 146 (5·8 %) in the number of true coliform reactions. The gain of 156 *Bact. coli* I reactions with lactose glutamic acid was thus outweighed by a loss of 302 (35 %) of the true coliform organisms other than *Bact. coli* I.

The next comparison in Table 1 is between MacConkey broth and glucose glutamic acid on 2501 samples. Glucose glutamic acid yielded more positive reactions than MacConkey broth in each category. Significantly higher yields of *Bact. coli* I (an increase of 86 isolations, or 8·1 % of the isolations in MacConkey broth) and of other true coliform organisms (an increase of 34 isolations, or 5·9 %) were associated with a very substantial increase of 248 (232 %) in false positive reactions.

The third comparison in Table 1, for the 1997 samples tested on all three media, allows the relative efficacy of the two glutamic acid media to be assessed. Compared with lactose glutamic acid, glucose glutamic acid medium produced a slightly (but not significantly) higher yield of *Bact. coli* I, a substantial increase (183, or 59 %) of other true coliform organisms and a very great surplus (257, or 325 %) of false positive reactions.

The greatest absolute yields, both of true coliform organisms and of *Bact. coli* I, were thus obtained with glucose glutamic acid, but these extra yields were associated with such an excess of false positive reactions as to disturb the specific nature of the presumptive coliform test expected in this country where MacConkey broth is routinely used. In the yield of *Bact. coli* I lactose glutamic acid rivalled glucose glutamic acid and outstripped MacConkey broth, but appeared to suppress the production not only of false positive reactions but also, and to a greater extent, the growth of true coliform organisms other than *Bact. coli* I. As compared with glucose glutamic acid, MacConkey broth also exerted a suppressive effect on false positive reactions and a lesser suppression of true coliform organisms (including both *Bact. coli* I and other types).

Rapidity of appearance of the reactions in the three media

Apart from the fact, already noted by Burman & Oliver (1952), that the quantity of gas produced in the glutamic acid media was usually much less than that usually obtained in MacConkey broth, it was the general experience that the initial appearance of gas was relatively delayed in the glutamic acid media. This is shown in the figure which reproduces graphically the lower part of Table 1, indicating at the same time the numbers of reactions obtained after 18 and 24 hr. incubation.

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Table 1. *Isolation of coliform organisms after* 48 *hr. incubation*

	No. of samples	Medium	Total presumptive coliform reactions	False positive reactions	True coliform reactions	True coliform reactions (other than *Bact. coli* I)	*Bact. coli* I reactions
Samples tested simultaneously in two media	3917	MacConkey broth	2672	170	2502	861	1641
		Lactose glutamic acid	2465	109	2356	559	1797
	2501	MacConkey broth	1742	107	1635	577	1058
		Glucose glutamic acid	2110	355	1755	611	1144
Samples tested simultaneously in three media (included in comparisons above)	1997	MacConkey broth	1320	90	1230	423	807
		Lactose glutamic acid	1252	79	1173	309	864
		Glucose glutamic acid	1715	336	1379	492	887

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After 18 hr. incubation MacConkey broth produced not only the greatest number of presumptive coliform reactions but also the greatest number of true coliform organisms and the most *Bact. coli* I. By 24 hr., however, this lag in the synthetic media had been largely overcome and the yields were more nearly equal than at 18 hr. Between 24 and 48 hr. all the lines again diverged. In respect of *Bact. coli* I the two glutamic acid media behaved almost identically and both produced

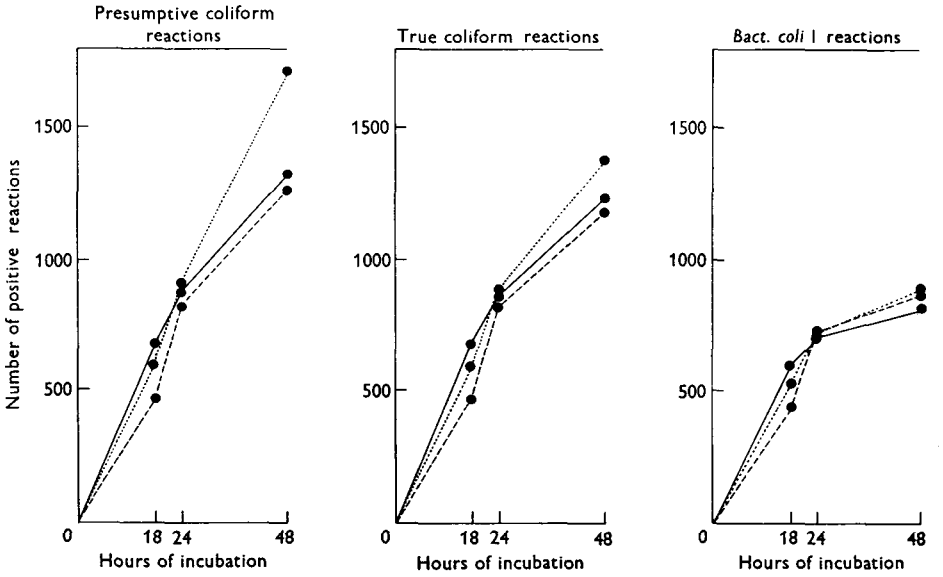

Fig. 1. Speed of appearance of coliform reactions in the three media. —, MacConkey broth; - - -, lactose glutamic acid; . . . ., glucose glutamic acid.

an appreciable number of extra isolations. As regards the total of true coliform organisms, glucose glutamic acid gave more, and lactose glutamic acid fewer, isolations than MacConkey broth. The graph for the presumptive coliform reactions shows the great increase in the number of glucose glutamic acid isolations between 24 and 48 hr., compared with the other two media; it is noteworthy that of the 336 false positive reactions produced in glucose glutamic acid 307 (91 %) appeared between 24 and 48 hr.

Results in the individual laboratories

The results obtained in successive periods of incubation at each of the participating laboratories are set out in Table 2. It is evident that there are considerable differences between the laboratories in the types of organism isolated. For example, false positive reactions occurred rarely, if at all, at the Birkenhead, Conway, and Oxford laboratories, but were common elsewhere. Again, true coliform reactions, other than *Bact. coli* I, were particularly common at the Birkenhead, Newport and Oxford laboratories. Such differences presumably indicate the different character of the waters examined in the various laboratories.

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Table 2. *Numbers of coliform organisms in successive periods of incubation in the various laboratories*

Medium	Laboratory	No. of samples	False positive reactions				True coliform reactions (other than *Bact. coli* I)				*Bact. coli* I reactions			
			0–18 hr.	18–24 hr.	24–48 hr.	Total	0–18 hr.	18–24 hr.	24–48 hr.	Total	0–18 hr.	18–24 hr.	24–48 hr.	Total
MacConkey broth	Birkenhead	180	0	0	0	0	38	30	13	81	50	18	6	74
	Conway	558	0	0	0	0	10	28	84	122	209	164	41	414
	Manchester	856	2	5	63	70	14	27	132	173	194	84	101	379
	M.W.B.	1407	1	6	30	37	46	48	101	195	473	5	6	484
	Newport	1074	5	14	58	77	50	50	216	316	373	14	3	390
	Oxford	346	0	0	3	3	36	30	62	128	132	5	14	151
	Total	4421	8	25	154	187	194	213	608	1015	1431	290	171	1892
Lactose glutamic acid	Birkenhead	147	0	0	0	0	7	9	4	20	37	20	9	66
	Conway	355	0	0	0	0	12	15	37	64	132	124	16	272
	Manchester	693	0	0	9	9	6	8	60	74	82	90	145	317
	M.W.B.	1407	0	1	53	54	16	55	109	180	525	28	21	574
	Newport	1074	0	0	46	46	5	20	159	184	90	296	56	442
	Oxford	241	0	0	0	0	2	11	24	37	48	52	26	126
	Total	3917	0	1	108	109	48	118	393	559	914	610	273	1797
Glucose glutamic acid	Birkenhead	115	1	0	0	1	19	6	5	30	26	13	6	45
	Conway	336	0	0	0	0	3	19	61	83	78	85	70	233
	Manchester	495	0	0	36	36	5	3	101	109	93	40	128	261
	M.W.B.	735	4	10	132	146	29	47	70	146	279	12	9	300
	Newport	690	3	9	150	162	9	44	156	209	148	89	16	253
	Oxford	130	0	4	6	10	1	16	17	34	7	29	16	52
	Total	2501	8	23	324	355	66	135	410	611	631	268	245	1144

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There are also substantial differences in the rapidity with which the reactions appeared in the various laboratories. With each of the three media, for example, more than 90% of the *Bact. coli* I reactions appeared in the first 18 hr. at the M.W.B. laboratories, compared with very much lower percentages elsewhere. At the other extreme (Manchester laboratory) between a quarter and a half of the *Bact. coli* I reactions appeared only in the second 24 hr. The majority of the true coliform reactions (other than *Bact. coli* I) appeared in the second 24 hr., except in the Birkenhead laboratory, where substantial numbers were isolated in the first 18 hr. Many of these differences too will arise from the different character of the waters examined in the various laboratories, but a special study was made of differences in technique between the laboratories, to assess what part they had played. Apart from the major variations in incubation practice which are referred to below, the differences in technique were only slight, and did not appear to have contributed materially to the differences either in the types of organism isolated, or in the rapidity with which the reactions appeared, in the various laboratories.

Effect of warming the inoculated tube

During the course of the investigation a number of the samples at the laboratories outside London were tested in parallel in a 'cold' tube and in a 'warmed' tube of each medium. The latter tubes, after inoculation, were warmed by immersion in a 37° C. water-bath for at least half an hour; during this time the 'cold' tube, also inoculated, was retained on the bench. Thereafter both tubes were placed in the 37° C. incubator for 48 hr. The results for these samples are presented in Table 3. It had been hoped that the preparatory warming of the tubes before incubation would hasten the appearance of the reactions. With presumptive coliform reactions and true coliform reactions there was a slight benefit to the warmed tube in the first 18 hr. of incubation, which was significant only in glucose glutamic acid and was of no practical importance in terms of the final yield. With *Bact. coli* I reactions, however, the effect of the preparatory warming was not only to hasten the growth of the organisms, but also to increase the total yield. In MacConkey broth the total yield of *Bact. coli* I was not significantly greater in warmed than in cold tubes (306 compared with 291). In glucose glutamic acid (331 compared with 308) and particularly in lactose glutamic acid (389 compared with 353), the gains in total yield of *Bact. coli* I in the warmed tubes were statistically significant.

Effect of different temperatures of incubation for the same samples of water

The samples at the M.W.B. laboratories were for a time tested in parallel in warmed tubes incubated either at 37° C. for 48 hr., or at 42° C. for 18 hr. followed by 37° C. for 30 hr. The results for the two methods of incubation are compared in Table 4. In MacConkey broth the effect of the early period of incubation at 42° C. was a very substantial inhibition in the total yield of presumptive coliform reactions (305 compared with 498) and of true coliform reactions (288 compared with 419). The number of *Bact. coli* I reactions was only slightly depressed (211 compared with 223). In lactose glutamic acid there was a similar, though less marked, inhibitory effect on the total yield of presumptive coliform reactions (338

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Table 3. *Results with cold tubes and warmed tubes for the same samples of water.* (*Laboratories outside London.*)
Numbers of coliform organisms in successive periods of incubation at 37° C.

Medium	No. of samples	Cold or warmed tubes	Presumptive coliform reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total	True coliform reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total	*Bact. coli* I reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total
MacConkey broth	627	Cold	259	112	143	514	257	109	107	473	221	59	11	291
		Warmed	274	94	128	496	273	92	109	474	232	46	28	306
Lactose glutamic acid	681	Cold	157	165	145	467	157	165	135	457	140	146	67	353
		Warmed	174	157	145	476	174	157	138	469	165	144	80	389
Glucose glutamic acid	627	Cold	115	159	227	501	115	156	194	465	108	118	82	308
		Warmed	138	160	217	515	137	155	192	484	129	117	85	331

Table 4. *Results with different temperatures of incubation for the same samples of water.* (*M.W.B. laboratories only.*)
Numbers of coliform organisms in successive periods of incubation

Medium	No. of samples	Temperature of incubation	Presumptive coliform reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total	True coliform reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total	*Bact. coli* I reactions 0–18 hr.	18–24 hr.	24–48 hr.	Total
MacConkey broth	672	37° C.	305	78	115	498	288	55	76	419	214	7	2	223
		42° C. followed by 37° C.	220	20	65	305	219	18	51	288	206	0	5	211
Lactose glutamic acid	672	37° C.	208	45	134	387	207	38	106	351	199	23	17	239
		42° C. followed by 37° C.	237	32	69	338	237	32	57	326	234	17	15	266
Glucose glutamic acid	333	37° C.	131	60	98	289	128	55	62	245	113	19	4	136
		42° C. followed by 37° C.	112	30	100	242	111	26	61	198	105	12	16	133

compared with 387) and on true coliform reactions (326 compared with 351); the total of *Bact. coli* I reactions, however, was slightly but not significantly increased (266 compared with 239). In glucose glutamic acid the effect of early incubation at 42° C. was again an inhibition of the total yield of presumptive coliform reactions (242 compared with 289) and of true coliform reactions (198 compared with 245), but with no effect upon the yield of *Bact. coli* I. The higher temperature of early incubation appears to have had no important effect other than the inhibition of presumptive and true coliform reactions. In particular, the speed with which the *Bact. coli* I reactions appeared showed no consistent differences with temperature of early incubation in the three media.

Initial incubation at 42° C. thus showed in all three media a definite suppressive effect on presumptive and on true coliform reactions without conferring any advantage in the yield of *Bact. coli* I, except with lactose glutamic acid. However, these findings relate to lightly polluted waters from the London area, all expected to have fewer than 25 coliform organisms per 100 ml., and it is thus possible that the same conclusions might not hold for more heavily polluted waters, or in other areas.

DISCUSSION

The results of this extended trial confirm the earlier findings of Burman & Oliver (1952) that, as compared with MacConkey broth, both the glutamic acid media give a higher yield of *Bact. coli* I and that lactose glutamic acid gives a lower yield of the other coliform organisms. The fuller follow-up of the presumptive positive tubes not yielding *Bact. coli* I has, however, shown a disadvantage not previously noticed with the glucose glutamic acid medium, namely the heavy excess of false positive presumptive results. The results summarized in Table 1 show that the number of false positive reactions produced by glucose glutamic acid is more than three times that produced by MacConkey broth; the false positive reactions represent respectively 17 and 6% of the total positive presumptive reactions. In this respect, therefore, the recent findings are less encouraging. Whereas the introduction of glucose glutamic acid could thus be expected to increase by about 7% the yield of all true coliform organisms (both *Bact. coli* I and other types), the specificity of the presumptive coliform test would be decreased from a level of 94 to 83%. It remains a matter of opinion whether the advantages outweigh this disadvantage.

For example, in laboratories where large numbers of routine samples are examined daily for works-control it is customary to incubate tubes for 24 hr. only. For this shorter incubation time it will be seen from Fig. 1 that there was close agreement between MacConkey broth and glucose glutamic acid medium without any excess of false positives. Used in this way the glutamic acid medium shows all the advantages without its major disadvantage.

The coliform test in water examinations is primarily designed for the detection of lactose fermenting organisms; a medium containing lactose as the fermentable substance is, therefore, to be preferred in the presumptive coliform test. It is disappointing that the lactose glutamic acid medium, which combined the optimum yield of *Bact. coli* I with a satisfactorily low incidence of false positive reactions,

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should be significantly inhibitory to the other coliform types. If waters were assessed purely on the *Bact. coli* I results, the lactose glutamic acid medium would provide all the necessary findings with the minimum of effort. Since, however, it is generally agreed that the other true coliform organisms ought to be considered in the assessment of water supplies the present lactose glutamic acid medium cannot be recommended as a satisfactory substitute for MacConkey broth.

It would appear from the results recorded above that any medium which is inhibitory to the non-coliform flora must to a certain extent inhibit the coliform organisms also. Of the media tested glucose glutamic acid permits the fullest growth of coliform organisms, but with a heavy burden of other organisms. The lactose glutamic acid medium permits a full growth of *Bact. coli* I but suppresses some of the other coliform organisms. Perhaps the most noteworthy finding—one which has been long suspected, but not definitely proved—is that MacConkey broth in the presumptive coliform test does exert a slight suppressive effect both on *Bact. coli* I and on other true coliform organisms.

Although neither of the glutamic acid media described can be unreservedly recommended as a satisfactory substitute for the older medium, MacConkey broth itself has been shown to lack perfection in its performance. The results with the other media suggest that the ultimate solution may lie in some modification of the glucose glutamic acid medium by which it is made slightly more inhibitory to the non-coliform flora or, preferably, whereby lactose glutamic acid medium can be made less inhibitory to true coliform organisms.

SUMMARY

Folpmers's glutamic acid medium and the lactose modification described by Burman & Oliver (1952) have been compared with MacConkey broth in the presumptive coliform test of 4421 samples of water examined at six different laboratories in England and Wales. As compared with MacConkey broth, both glutamic acid media gave between 8 and 10% more isolations of *Bact. coli* I; the glucose medium additionally gave an increase of 6% in other coliform organisms, but this was associated with a very heavy excess (232%) of false positive results; lactose glutamic acid satisfactorily controlled false positive reactions, giving a reduction of 36%, but also suppressed by 35% the isolations of coliform organisms other than *Bact. coli* I.

MacConkey broth gave the largest early (18 hr.) yield of positive results, but the results at the end of 24 hr. were approximately the same with all three media. Preliminary warming of the inoculated tube had only a trivial effect on the rapidity with which the organisms grew, but the warming resulted in a slightly higher total yield of *Bact. coli* I in the glutamic acid media. Incubation at 42° C. for 18 hr. followed by 37° C. for 30 hr. was found to be inferior to incubation at 37° C. for 48 hr. in the case of lightly polluted waters, in that true coliform organisms were suppressed without any compensating advantage in the yield of *Bact. coli* I.

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