FURTHER OBSERVATIONS ON THE GROWTH OF BACTERIA ON MEDIA CONTAINING VARIOUS ANILIN DYES, WITH SPECIAL REFERENCE TO AN ENRICHMENT METHOD FOR TYPHOID AND PARATYPHOID BACILLI.*

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In a previous communication (1) we reported the results of a series of observations on the growth of thirty varieties of bacteria on media containing various dyes. We have continued this work using the same series of cultures and forty samples of dyes. The methods employed have been the same. Green dyes are widely used in special media for typhoid and paratyphoid isolation. This led us to include many of these dyes in our present work, in the hope that some might be more selective than those in use. If this were the case, their application to plate and fluid media would be of great value.

In general, the results have been similar to those with other dyes, especially gentian violet and its allies (table I). The inhibition of growth has been most evident among the Gram-positive bacteria, the Gram-negative bacteria as a rule growing freely on the dyed agar.

Certain exceptions are evident in the table. The cholera strain employed was less resistant to the action of green dyes than the allied vibrios. To determine whether this was of differential value, forty cultures of cholera and other vibrios were tried on agar containing *Bittermandelölgrün,* the dye showing the greatest differences. A variable restraining action was noted, but there were no differences according to types.

The diphtheria bacillus and its allies have given apparent differences and irregularities. This is due to the tendency of these cultures to produce flakes which, when inoculated on the agar, produce a limited growth, whereas the separate bacilli fail to grow.

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TABLE I.[1, 2]

	Bacteria tested.	Brilliant green, crystals 366 (Bayer).			Brilliant green.			Anilin green.			Solid green.			Victoria green, crystals 4,833 (Bayer).			Smaragd green.			China green.			Malachite green crystals, "Chlorzink-doppelsalz."			Bittermandelölgrün.			Victoria blue 4 R.			Malachite green.			Malachite green (Höchst).		
		1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000
Gram-positive	Staphylococcus aureus	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	×	−	−	*	−	−	*	−	−	−	−	−	×	−	−	×	−	−
	Streptococcus (green)	−	−	−	×	−	−	−	−	−	−	−	−	−	−	−	∓	−	−	∓	−	−	*	−	−	*	−	−	∓	−	−	×	−	−	×	−	−
	Streptococcus (hemolytic)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	−	−	−	−	−	×	−	−	×	−	−	−	−	−	−	−	−
	Streptococcus mucosus capsulatus	−	−	−	−	−	−	−	−	−	−	−	−	∓	−	−	−	−	−	−	−	−	−	−	−	×	−	−	×	−	−	*	−	−	−	−	−
	Pneumococcus	−	−	−	−	−	−	−	−	−	−	−	−	∓	−	−	−	−	−	×	−	−	−	−	−	*	−	−	*	−	−	−	−	−	−	−	−
	B. diphtheriæ	×	−	−	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	−	−	−	−	−	×	−	−	×	−	−	×	−	−	×	×	−
	Diphtheroid bacilli (Cameron)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	−	−	×	−	−	×	−	−	×	−	−
	Diphtheroid bacilli (Cunningham)	×	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	∓	−	−	∓	−	−	×	−	−	×	−	−
	B. hoffmanni	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	−	−	∓	−	−	∓	−	−	×	*	−	×	−	−
	B. subtilis	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	×	−	−	×	−	−
	B. lactimorbi	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	*	−	−
	B. anthracis	−	−	−	−	−	−	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	*	−	−	∓	−	−
Gram-negative	Cholera	*	−	−	*	−	−	−	−	−	−	−	−	∓	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	+	×	*	+	+	×
	Vibrio (indol-positive)	*	−	−	×	*	−	×	−	−	−	−	−	∓	×	*	×	×	−	+	×	−	+	∓	×	+	∓	×	+	+	∓	+	+	∓	+	+	+
	Vibrio (indol-negative)	∓	−	−	+	×	−	+	−	−	∓	×	−	∓	*	*	×	×	*	+	∓	*	+	∓	×	+	∓	−	+	∓	−	+	+	∓	+	+	+
	B. proteus	∓	*	*	+	×	*	+	*	*	∓	×	−	+	∓	×	+	×	*	+	∓	×	+	∓	∓	+	∓	×	+	∓	×	+	+	∓	+	+	+
	B. pyocyaneus	+	+	+	+	+	+	+	+	+	+	+	+	+	∓	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Shiga)	×	−	−	∓	×	−	∓	−	−	+	*	−	+	−	−	+	*	−	+	*	−	+	−	−	+	−	−	+	+	+	+	∓	*	+	+	+
	B. dysenteriæ (Flexner)	−	−	−	×	×	−	−	−	−	−	−	−	+	−	−	×	−	−	+	*	−	+	−	−	+	−	−	+	+	+	+	∓	*	+	∓	×
	B. dysenteriæ (Mt. Desert)	×	−	−	×	×	−	×	−	−	×	−	−	+	−	−	×	−	−	+	*	−	+	−	−	+	−	−	+	+	∓	+	∓	*	+	∓	∓
	B. typhosus	+	×	−	+	×	×	+	×	−	+	×	−	+	+	×	+	×	×	+	+	×	+	∓	∓	+	+	∓	+	+	+	+	+	+	+	+	+
	B. paratyphosus, A	+	+	∓	+	+	∓	+	+	∓	+	+	∓	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, B	+	+	∓	+	+	∓	+	+	∓	+	+	∓	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. enteritidis (Gaertner)	+	+	+	+	+	∓	+	+	∓	+	+	∓	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

	Bacteria tested.	Brilliant green, crystals 366 (Bayer).			Brilliant green.			Anilin green.			Solid green.			Victoria green, crystals 4.833 (Bayer).			Smaragd green.			China green.			Malachite green crystals, "Chlorzink-doppelsalz."			Bittermandelölgrün.			Victoria blue 4 R.			Malachite green.			Malachite green (Höchst).		
		1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000
Gram-negative	B. coli communis	∓	*	−	+	×	−	+	−	−	+	*	−	+	∓	−	∓	×	−	+	+	×	+	∓	∓	+	+	∓	+	+	∓	+	+	+	+	+	+
	B. coli communior	∓	−	−	+	×	−	+	−	−	+	×	−	+	∓	−	∓	×	−	+	+	×	+	∓	∓	+	+	×	+	+	∓	+	+	+	+	+	+
	Friedländer's bacillus	∓	*	*	+	∓	×	+	×	×	+	∓	×	+	∓	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. lactis aerogenes	×	−	−	+	×	*	+	−	−	∓	×	−	+	∓	*	∓	∓	×	+	+	×	+	∓	∓	+	+	×	+	+	∓	+	+	+	+	+	+
	B. ozænæ	−	−	−	×	−	−	×	−	−	×	−	−	+	*	−	×	*	−	+	∓	×	+	∓	∓	+	∓	*	+	+	∓	+	+	+	+	+	+
	B. pullorum	+	∓	∓	+	∓	−	+	+	∓	+	+	∓	+	+	∓	+	+	∓	+	+	×	+	+	+	+	+	+	+	+	∓	+	+	+	+	+	+

[1] In the tables + = growth like control; ∓ = restrained growth; × = markedly restrained growth; − = no growth; * = some growth developed later; § = several colonies.

[2] In addition to the dyes given in the table, the following were also tested: alkali green, *Säurealizaringrün, Lichtgrün* F. S., *Brillantblau* (*extra grünlich*), naphtol green B., *Echtgrün, Säuregrün,* alizarin green B., methyl blue, trypan blue, anilin blue (water-soluble), China blue, anilin blue-black, *Blauschwarz,* and *Wasserblau.* In all the dilutions employed growth was as vigorous as on the control. Where not otherwise stated, all the dyes employed were obtained from Grübler.

TABLE I.—*Concluded.*

	Bacteria tested.	Malachite green Ia.			Nachtblau.			Methylene blue, med. pure.			Janus green.			Magenta red.			Methyl green, oo rein.			Chlorhydrin blue, or acetin blue.			Pyronin.			Iodin green.			Alkali blue.			Methylene green.			Methyl green.		
		1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000	1:500,000	1:100,000	1:50,000
Gram-positive	Staphylococcus aureus	×	−	−	∓	−	−	∓	×	*	+	*	−	+	∓	×	∓	∓	*	+	+	+	+	+	+	+	∓	×	+	+	+	+	+	+	+	+	×
	Streptococcus (green)	∓	−	−	∓	−	−	+	−	−	+	×	*	+	∓	×	+	+	+	+	+	+	+	×	−	+	+	+	+	+	+	+	+	+	−	+	+
	Streptococcus (hemolytic)	−	−	−	∓	−	−	+	*	−	+	×	−	∓	×	−	+	+	+	+	+	+	+	*	−	+	+	+	+	+	∓	+	∓	*	+	+	∓
	Streptococcus mucosus capsulatus	*	−	−	×	−	−	+	−	−	+	×	−	+	∓	×	+	+	+	+	+	+	+	×	−	+	+	+	+	+	∓	+	+	∓	+	+	+
	Pneumococcus	−	−	−	∓	−	−	+	−	−	+	∓	−	+	∓	∓	+	+	+	+	+	+	+	∓	−	+	+	+	+	+	+	+	+	×	+	+	∓
	B. diphtheriæ	∓	×	−	∓	−	−	×	*	−	+	−	−	×	−	−	∓	×	−	×	×	−	+	+	∓	+	∓	×	+	∓	×	+	∓	×	+	+	∓
	Diphtheroid bacilli (Cameron)	×	−	−	−	−	−	−	−	−	+	−	−	+	−	−	×	−	−	×	−	−	+	+	∓	+	×	×	+	×	×	+	+	∓	+	+	∓
	Diphtheroid bacilli (Cunningham)	×	−	−	−	−	−	−	−	−	−	−	−	×	−	−	∓	*	−	×	−	−	+	+	+	+	∓	×	+	×	×	+	+	∓	+	∓	×
	B. hoffmanni	×	−	−	−	−	−	−	−	−	−	−	−	×	−	−	+	*	−	×	−	−	+	+	+	+	∓	×	∓	×	×	+	+	*	+	∓	×
	B. subtilis	×	−	−	∓	−	−	∓	∓	×	+	−	−	+	×	−	+	×	−	+	+	+	+	+	+	+	∓	*	+	+	+	+	+	+	+	+	×
	B. lactimorbi	*	−	−	×	−	−	∓	*	−	∓	−	−	+	×	−	+	∓	*	+	+	+	+	+	+	+	∓	∓	+	+	+	+	+	×	+	+	×
	B. anthracis	×	−	−	∓	−	−	+	+	∓	+	−	−	+	∓	×	+	∓	−	+	+	+	+	+	+	+	∓	×	+	+	+	+	+	+	+	+	∓
Gram-negative	Cholera	+	+	×	+	+	+	+	×	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	×	+	+	+
	Vibrio (indol-positive)	+	+	+	+	+	+	+	×	×	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Vibrio (indol-negative)	+	+	+	+	+	+	∓	∓	×	+	+	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. proteus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. pyocyaneus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Shiga)	+	+	+	+	+	+	+	∓	∓	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Flexner)	+	∓	×	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. dysenteriæ (Mt. Desert)	+	∓	×	+	+	+	+	+	∓	+	∓	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. typhosus	+	+	+	+	+	+	+	+	+	+	+	∓	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. paratyphosus, B	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. enteritidis (Gaertner)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. coli communior	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Friedländer's bacillus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. lactis aerogenes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. ozænæ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B. pullorum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

With one exception the results obtained have failed to show variations which could be used for practical differentiation. They are given, however, as similar detailed observations are not available in the literature.

The observations on the green dyes show marked variations in the resistance of members of the typhoid-paratyphoid-colon group. In general the paratyphoid-enteritidis types are highly resistant, the typhoid less so, but somewhat more resistant than the coli types. Various green dyes (2) have been employed in media to suppress the colon types found in feces. Loeffler employed malachite green agar, Werbitzki China green, and Conradi brilliant green agar, adding picric acid which reduces the activity of the dye. The media have been of some use, especially for preliminary plating for enrichment, for paratyphoid more than for typhoid. On these media the difference in resistance of the typhoid and colon types was slight. If very few typhoid bacilli were present or if less resistant than the average, failure resulted. Peabody and Pratt and others attempted to use malachite green in fluid media as an enrichment medium.

Of the dyes we tried, ten revealed variations among the typhoid-colon group. A large number of cultures of the group were obtained[3] and grouped according to their sugar fermentations, and tried on agar containing some of these dyes. This was done to determine whether one dye showed wider differences than another and as a preliminary to the application of the results already obtained, to a selective plating medium, or a fluid enrichment medium. Agar is an easier medium to employ as an index, and was therefore used, as we had found that the results on agar could be duplicated in broth with slight variations in dilutions. To reveal slight differences in reaction, a small number of bacteria were inoculated. A loopful of a broth culture was carried to a second tube and loop inoculations were made from this (table II).

The results show that the different dyes possess a similar differential action. This difference, however, appears at different dilutions according to the dye employed. At the appropriate dilutions no one dye is perceptibly more differential than another.

[3] We are indebted to Dr. Torrey and Prof. C.-E. A. Winslow for some of the cultures employed.

TABLE II.[4, 5]

Type tested.	No. of strains.	Glucose.	Dulcite.	Lactose.	Raffinose.	Saccharose.	Anilin green.			Brilliant green (Grübler).			Brilliant green (Bayer).			Smaragd green.			Solid green.			Victoria green (Bayer).			China green.		
							1:300,000	1:100,000	1:10,000	1:300,000	1:100,000	1:10,000	1:300,000	1:100,000	1:50,000	1:100,000	1:50,000	1:10,000	1:100,000	1:50,000	1:10,000	1:100,000	1:50,000	1:10,000	1:100,000	1:50,000	1:10,000
B. alkaligenes	2	−	−	−	−	−	−	−	−	−	−	−	..	..	..	−	−	−	−	−	−	..	..	..	..	..	..
B. typhosus	3	+	−	−	−	−	ǂ	*	−	×	*	−	ǂ	*	−	−	−	−	×	−	−	ǂ	×	*	ǂ	×	×
B. paratyphosus, A	6	†	†	−	−	−	+	ǂ	×	+	+	×	+	ǂ	×	ǂ	ǂ	×	+	+	×	+	+	+	+	+	+
							ǂ	*	−	ǂ	×	−	ǂ	×	*	−	−	−	§	−	−	ǂ	ǂ	−	+	ǂ	ǂ
B. paratyphosus, B	4	†	†	−	−	−	+	+	ǂ	+	+	ǂ	+	+	ǂ	ǂ	ǂ	ǂ	+	ǂ	ǂ	+	+	+	+	+	+
							+	ǂ	−	+	ǂ	−	ǂ	ǂ	×	×	*	−	ǂ	×	*	+	+	ǂ	+	+	ǂ
B. enteritidis	2	†	†	−	−	−	+	+	×	+	ǂ	×	+	ǂ	ǂ	+	ǂ	×	ǂ	ǂ	×	+	+	+	+	+	ǂ
							+	ǂ	−	+	ǂ	−	ǂ	×	−	×	*	−	ǂ	ǂ	−	+	+	−	ǂ	ǂ	×
Intermediate types	4	†	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ǂ	−	−	ǂ	ǂ	×
	1	†	−	−	+	−	−	−	−	−	−	−	..	..	..	−	−	−	−	−	−	..	..	..	..	..	..
	1	†	†	+	†	†	−	−	−	*	−	−	−	−	−	−	−	−	−	−	−	ǂ	×	§	+	ǂ	×
	1	†	†	+	†	+	−	−	−	−	−	−	..	..	..	−	−	−	−	−	−	..	..	..	..	..	..
B. acidi lactici	6	†	−	†	−	−	−	−	−	−	−	−	..	..	..	−	−	−	−	−	−	..	..	..	..	..	..
B. coli communis	3	†	†	†	† or −	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
B. coli communior	6	†	†	†	†	†	+	+	×	+	ǂ	ǂ	+	ǂ	ǂ	ǂ	×	×	ǂ	ǂ	×	+	+	+	+	+	+
							−	−	−	×	−	−	×	−	−	−	−	−	−	−	−	×	*	−	ǂ	×	*
B. aerogenes types	6	†	−	†	†	†	+	+	ǂ	+	ǂ	ǂ	+	+	ǂ	ǂ	ǂ	ǂ	+	ǂ	ǂ	+	+	+	+	+	+
							−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[4] Where two sets of observations are given they are the extremes on that type of culture.

[5] − = no fermentation; + = acid production; † = production of acid and gas; .. = no observations. Other symbols are as in preceding table.

Five cultures were not included in the preceding table. These are the cultures used by Churchman and Michael (3). We have taken their observations on the growth of these cultures on gentian violet agar and their interagglutinations, and added our observations of the sugar fermentations of the same cultures and of the growth on media containing green dyes. Table III shows the possible application of dye media to the differentiation of some members of the enteritidis group. The question that immediately arises is: Has the reaction to dyes any relation to their source or pathogenicity? For instance, two cultures, E 25 and E 26, were isolated from a drainage canal by Jordan. Unfortunately the source of the other cultures is not available. If the strains which did not grow came from human beings suffering from infection by these organisms, there will be times when the application of these dyes for enrichment methods will fail.

TABLE III.

Organism.	Growth on gentian violet agar.	Growth on agar, or in broth with green dyes.[6]	Glucose.	Dulcite.	Summary of agglutination reactions. Sera.			
					E 18.	E 25.	E 26.	E J.H.H., same as E 234.
E 18 (67).....	+	+	†	†	+++	0	0	++
E 25 (68).....	+	−	†	−	0	+++	0	0
E 26 (14).....	+	−[7]	†	†	0	0	+++	0
E 132 (70).....	+	+	†	†	+++	0	0	++
E 234 (64).....	−	−[7]	†	†	++	0	0	+++

Experiments were then begun with fluid and solid media for differential enrichment. The use of solid media was not as successful as fluid media. As we had not found any one dye more selective than another and because of two reports giving results similar to our own which appeared at this time, we continued to work with brilliant green.

Torrey (4) found that by the use of Grübler's brilliant green in fluid media he could enrich the paratyphoid-enteritidis group and restrain the common fecal bacteria. Browning, Gilmour, and Mackie (5) were able, by the use of Bayer's brilliant green in fluid

[6] Dyes given in table II.

[7] Very slight delayed growth on agar containing 1 : 500,000 of some of the dyes.

media, to isolate typhoid or paratyphoid bacilli from stools, where direct plating was unsuccessful.

Preliminary tests were made to determine what medium to use. Peptone and meat extract broth with and without glucose and containing the dye in various dilutions were tried. Counts were made of the number of paratyphoid bacilli inoculated and the number per loop of the broth after incubation. The glucose extract broth gave the most abundant growth. This medium was therefore used in our subsequent work. For all our work we have used media neutral to phenolphthalein.

Some experiments had shown that slight changes would lower the threshold of growth for the typhoid bacillus but make little change in the restraining action of the dye towards the coli types; for instance, small additions of proteid substances change the action of the dye. A test was made, therefore, of the influence of added feces as a preliminary to selecting dilutions for work with stools. Typhoid bacilli, being less resistant, were used for this test. The results with Bayer's brilliant green (table IV) show that about one third of the activity of the dye is lost.

TABLE IV.

	No. per loop after 18 hours' incubation.		
	500,000.	400,000.	300,000.
Typhoid (213 bacilli inoculated)	218	0	0
Typhoid + 0.1 c.c. of boiled feces (213 typhoid bacilli inoculated)	+++[8]	+++	+++
0.1 c.c. of boiled feces (control)	0	0	0

As a further index to the selection of dilutions, the possible variation of different strains to the dye and to different types of the dye was determined. Further strains were tried in broth containing Bayer's brilliant green. The number of bacilli inoculated ranged from one to seventy. No growth, as determined by plating a loopful of the media, occurred in dilutions after and including 1 to 400,000. In 1 to 500,000 two showed multiplication. A loop plated from these tubes showed only 24 and 500 colonies. Repeating this with six strains with Grübler's dye gave essentially similar results.

[8] Like plain broth control.

TABLE V.[9]

Results of Examination of Stools.

Name of case.	Direct plates (Conradi).	Conradi plates after enrichment in broth containing brilliant green.			Source of dye.
		1:500,000	1:400,000	1:300,000	
R.	Less than 1% typhoid	10% typhoid[10]	Over 50% typhoid; many bluish colonies not typhoid	Sterile (lower dilutions also sterile)	Bayer.
M.	Negative (overgrowth by red colonies)	60% typhoid	75% typhoid	Overgrown by aerogenes types (lower dilutions the same)	Bayer.
O.	30% typhoid	Overgrown by aerogenes types	Many hundred typhoid colonies wherever plate is free from aerogenes types	Overgrown by aerogenes types; typhoid isolated from plate (lower dilutions gave aerogenes types only)	Bayer.
M.	7% typhoid	90% typhoid	95% typhoid	95% typhoid	Grübler.
M.	7% typhoid	Many typhoid colonies where plate is clear of heavy red growth; plate red from diffusion	Clear parts of plate peppered with typhoid colonies; heavy red growth with diffusion of acid through plate	Moderate number of typhoid colonies; plate overgrown by aerogenes types	Bayer.
O.	50% typhoid	Very many typhoid colonies where plate is free from overgrowth of aerogenes types	Few typhoid; practically overgrown by aerogenes types	Moderate number of typhoid colonies; plate overgrown by aerogenes types	Grübler.
O.	50% typhoid	Very many typhoid colonies where plate is free from overgrowth of aerogenes types	Few typhoid; practically overgrown by aerogenes types	Plate overgrown by mucosus types	Bayer.
R.	Five typhoid colonies or three plates	Confluent growth, red margin and bluish center, many fine bluish colonies between confluent areas; impossible to identify typhoid by direct agglutination from plate; fishings gave slow lactose-fermenting aerogenes types.			Grübler.
R.	Five typhoid colonies or three plates	Confluent growth, red margin and bluish center, many fine bluish colonies between confluent areas; impossible to identify typhoid by direct agglutination from plate; fishings gave slow lactose-fermenting aerogenes types.			Bayer.

[9] Eight other stools were examined and were negative both by direct plating and after growth in dye broth.

[10] Verified by direct agglutinations of growth from plate or by fishing onto Russell's medium with subsequent agglutination.

Two strains, however, showed slight multiplication at the 1 to 400,000 dilution.

Because of these results dilutions of 1 to 500,000 to 1 to 100,000 were tried in testing the stools from typhoid carriers. The stools were from three cases and were examined on two occasions, once with Bayer's dye only, and once with both makes of dye. The broth was used in ten cubic centimeter amounts, and 0.1 of a cubic centimeter of thinned feces was added to each tube. The results are given in table V.

That we can enrich typhoid by this method is evident. The results confirm those of Browning, Gilmour, and Mackie. We also agree with their suggestion to use graded dilutions. When we add feces we are introducing a variable factor and the typhoid strains also vary somewhat in their resistance to the dye. Success depends on the right adjustment. The value of the method must rest on the results of routine examinations over a period of time. From our results we would suggest the use of the three dilutions as given, and the addition of 0.1 of a cubic centimeter of thinned feces. The overgrowth of some of the plates suggests the advisability of streaking two plates from each tube, without burning the wire between the plates. Unsuccessful results from overgrowth are apparently due most frequently to *Bacillus aerogenes* types, which are extremely resistant to the dye and usurp the plate where seeding is too heavy.

The range of variability of the available paratyphoid-enteritidis strains was determined. Table VI gives the results.

The results show a wide range of variability. Certain members of this group, as already noted, failed completely to grow. How far such variations will be present in strains in human feces will be shown only by the results of routine examinations.

The results with our cultures make us doubt the advisability of using only one dilution,—1 to 6,600, as advised by Torrey. Although he grades the amount of feces, we doubt whether this would reduce the activity of the dye sufficiently to allow the growth of the less resistant types, assuming that such strains will occur in naturally infected feces. Furthermore, such low dilutions may not weed out the more resistant fecal types of bacteria any more than would the

TABLE VI.[11]

Culture and type.		No. inoculated.	Brilliant green (Bayer). No. per loop after 18 hours. 1 : 100,000	Brilliant green (Bayer). No. per loop after 18 hours. 1 : 10,000	No. inoculated.	Brilliant green (Grübler). No. per loop after 18 hours. 1 : 100,000	Brilliant green (Grübler). No. per loop after 18 hours. 1 : 10,000
Paratyphoid, A	3	53	+|	+	92	++	1320
	54	228	++	242	300	+|	99
	56	364	++	++	496	++	++
	57	392	++	+|	248	+|	85
	58	314	++	+|	640	++	++
	60	304	++	++	47	++	++
	62	59	++	0	112	0[12]	0
	63	234	2[12]	0	400	2[12]	0
	66	200	++	++	240	++	++
Paratyphoid, B	52	600	++	+|	412	+|	214
	53	282	++	++	55	++	++
	59	85	++	0	18	0[11]	0
	61	140	++	+|	228	++	++
	65	142	++	+|	160	++	++
Enteritidis	4	384	4[12]	0	668	552[12]	129
	47	270	++	++	160	++	+|
	67	360	++	++	352	++	++
	70	298	++	++	480	++	+|

higher dilutions. Some aerogenes types are more resistant than the most resistant paratyphoid-enteritidis strains. In fact two of Torrey's strains showed decidedly less resistance than the others, and acted more like some of our strains. His results with feces of dogs artificially infected prove the value of the method, but are no index to the correct dilutions to employ. The strains used for feeding are not stated, but were undoubtedly fully resistant to the dye. This proves nothing as to the results to be expected in routine examinations where strains may vary.

As no naturally infected stools were at our disposal, we did not continue work in this direction. From our results with cultures, graded dilutions seem to us to be indicated in stool examinations for paratyphoid and enteritidis. Taking into consideration the fact that the feces itself will lower the activity of the dye, a series of dilutions ranging from 1 to 100,000 to 1 to 500,000 will probably cover the variations we have noted, with the exception of those

[11] ++ = closely crowded colonies, like control; +| = closely crowded colonies, less than control; + = closely crowded colonies, less than preceding.

[12] Quantitatively similar restraint in 1 : 200,000.

strains which were completely inhibited, and restrain the fecal organisms that can be restrained.

Our results in the attempt to apply these results to a solid medium have been only partially successful. The green dyes can be used as by Conradi, but this has the disadvantage of the absence of an indicator for lactose fermentors. The use of sodium sulphite with the green dye or some other added dye, as fuchsin, has the disadvantage that the sulphite lowers the activity of the dye and a balance is obtained with difficully. The attempt to find an indicator which would be sharp but not influence the activity of the green dye has so far been unsuccessful.

CONCLUSIONS.

Several green dyes show a marked selective action for members of the typhoid-paratyphoid-colon group. This can be used for the enrichment of typhoid and paratyphoid bacilli present in feces. Forty dyes were tested with thirty strains covering all types of pathogenic bacteria. In general the dyes restrained the growth of the Gram-positive bacteria but had no effect on the growth of the Gram-negative group.

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