

FURTHER STUDIES WITH THE Ph INDICATORS AND ACID PRODUCTION BY THE COLON- TYPHOID GROUP.

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A paper was presented to the Academy in 1922 on the use of Clark and Lubs indicators for the detection of acid production by the Colon-Typhoid group. It was shown at that time that brom cresol purple and brom thymol blue could be used almost interchangeably for the detection of acid by this group, by incorporating the dye in the medium and allowing the organisms to grow, both on the slant, under aerobic conditions and in a deep stab, under anaerobic conditions. By using different sugars the fermentation of each sugar was detected by the production of gas and by a change in the color of the medium. This method furnished one more means for differentiating the members of the group.

This paper is a record of the work done in continuing these experiments using the same sugars but with different dyes. The Ph range of brom thymol blue is approximately 6.0 to 7.6 while brom cresol purple ranges from 5.4 to 7.0. These new indicators, brom cresol green and chlor phenol red, have ranges of 3.4 to 5.0 and 5.0 to 6.6 respectively.

The media used were the same as in the previous experiments and the technique employed was the same as that used in 1922.

The cultures were the same strains carried thru on laboratory media.

The cultures were incubated for 24 hours and 48 hours at 37° C.

Readings were made at the end of the incubation periods and the results are tabulated in the following charts.

The 48 hour readings are not recorded as the change from acid to base had already taken place and the tubes were all back to normal again as far as color was concerned.

TABLE 1. 24 HOUR GROWTH. .1% ARABINOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	++	++	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Pink
		Gas	++	—	++	++	++	—

TABLE 2. 24 HOUR GROWTH. .1% DEXTROSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	++	++	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Yellow
		Gas	++	—	++	++	++	—

TABLE 3. 24 HOUR GROWTH. .1% DULCITOL. .2% DYE

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	+	—	++	+	+	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Pink	Pink	Pink
		Gas	+	—	++	+	+	—

TABLE 4. 24 HOUR GROWTH. .1% GALACTOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	++	++	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Yellow
		Gas	++	—	++	++	++	—

TABLE 5. 24 HOUR GROWTH. .1% LACTOSE. .2% DYE

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	+-	+-	—	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Pink	Pink	Pink	Pink
		Gas	++	—	+-	+-	—	—

TABLE 6. 42 HOUR GROWTH. .1% LEVULOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	++	++	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Yellow
		Gas	++	—	++	++	++	—

TABLE 7. 24 HOUR GROWTH. .1% MALTOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	+	+	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Yellow
		Gas	++	—	++	+	+	—

TABLE 8. 24 HOUR GROWTH. .1% MANNOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	—	++	++	++	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Yellow
		Gas	++	—	++	++	++	—

TABLE 9. 24 HOUR GROWTH. .1% RAFFINOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	+-	—	+-	+-	+-	—
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Pink	Pink	Pink	Pink	Yellow	Pink
		Gas	+-	—	+-	+-	+-	—

TABLE 10. 24 HOUR GROWTH. 1% RHAMNOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	-	++	++	+	-
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Yellow	Yellow	Pink
		Gas	++	-	++	++	+	-

TABLE 11. 24 HOUR GROWTH. 1% SUCROSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	+	-	+-	+-	+-	-
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Pink	Pink	Pink	Pink	Pink	Pink
		Gas	+-	-	+-	+-	+-	-

TABLE 12. 24 HOUR GROWTH. .1% XYLOSE. .2% DYE.

			Colon	Dysentery	Enteritidis	Para A.	Para B.	Typhoid
B. C. G.	Slant	Color	Green	Green	Green	Green	Green	Green
	Butt	Color	Green	Green	Green	Green	Green	Green
		Gas	++	-	++	++	++	-
C. P. R.	Slant	Color	Pink	Pink	Pink	Pink	Pink	Pink
	Butt	Color	Yellow	Pink	Yellow	Pink	Yellow	Yellow
		Gas	++	-	++	++	++	-

Observations. The same general observations might be made as regards these experiments as were made in the former case. It was to be expected that chlor phenol red with its range of 5.0-6.6 would give a better index of the amount of acid produced than did either from cresol purple or brom thymol blue. As the experiments show the amount of acid produced by each member of the group is not sufficient and does not vary enough from the amount produced by the other members to make further differentiation possible. For this reason brom cresol green, with an acid range of 3.6 to 5.0, gave no color change even though there was plenty of fermentation as indicated by the gas produced.

A peculiar thing about this last observation is that in beef bouillon with exactly the same sugars and indicators, there is a color change produced with brom cresol green. The obvious explanations are that in the agar cultures we have in the first place, an anaerobic growth

in the stab while beef bouillon offers better aerobic conditions. On the other hand, the slant offers the best aerobic conditions possible but here the rate of diffusion of the by-products is interfered with by the nature of the medium. In other experiments not connected with this paper a Ph value of 3.6 was recorded for some of these organisms. We must also remember that the acid production by these organisms is soon followed by the production of alkaline by-products. The color change might be on its way when it is overcome by the alkaline reaction.

Chlor phenol red may be classified with brom thymol blue and brom cresol purple as indicators to be used for the differentiation of members of this group using these sugars. It gives slightly better results as the changes are clearer and more marked.

