EVALUATION OF SELLERS' MEDIUM FOR THE DIFFERENTIATION OF GRAM-NEGATIVE BACTERIA

ARVIND G. BHAGWAT, M.D. (PATH.),*

AND

JOHN W. KING, M.D., PH.D.

Department of Clinical Pathology

ANTIBIOTICS seem to have altered considerably the patterns of microbial infections[1, 2] in humans. Within the last two decades the literature has clearly shown the importance and the increasing incidence of infections caused by various gram-negative bacteria.[3] The most important of these are *Pseudomonas, Proteus, Alcaligenes, Citrobacter, Escherichia,* and *Aerobacter* which have been variously associated with enteritis,[4] endocarditis,[5] bacteremia,[6, 7] meningitis,[8] and septicemia.[9] Particular interest is being directed toward the group of gram-negative coccobacilli included in the *Mima-Herellea* group by De Bord,[10] and by Daly, Postic, and Kass.[11] The pathogenicity of some of the organisms has been assessed on the basis of their ability to produce specific enzymes.[12-14] The coccobacilliary group includes *Mima polymorpha, Herellea vaginicola,* and species of *Colloides,* which have been associated with conjunctivitis and vaginitis,[11] urethritis,[15] endocarditis,[16, 17] meningitis,[18] and synovitis.[19] In addition to these organisms the bacteria referred to as *Bacterium anitratum* also belong to this group. Its relationship to *Herellea vaginicola* is uncertain. The entire group of organisms has been referred to as autochthonous (indigenous to the host) bacteria, since they are usual commensals of the gastrointestinal, respiratory and genital tracts.[11]

The differentiation of many of these organisms requires time-consuming methods involving the use of a number of special differential media and biochemical tests. Sellers[20, 21] recently described a medium for the differentiation of some of these organisms that he has somewhat loosely referred to as "gram-negative, nonfermenting bacilli of medical interest." The group includes *Mima polymorpha, Herellea vaginicola, Bacterium anitratum, Pseudomonas aeruginosa,* and *Alcaligenes fecalis.* To our knowledge, no confirmatory studies on the usefulness of Sellers' medium have been reported. Our study is an evaluation of Sellers' observations, and an extension of the use of Sellers' medium in the differentiation of other gram-negative bacteria.

* *Fellow, Department of Clinical Pathology.*

Table 1.—*The common constituents of Difco and of BBL Sellers' medium*

Chemical	Amount, gram per liter of distilled water
Sodium chloride	2.000
Sodium nitrate	1.000
Sodium nitrite	0.350
D-mannitol	2.000
L-arginine	1.000
Yeast extract	1.000
Magnesium sulfate	1.500
Dipotassium phosphate	1.000
Brom thymol blue	0.040
Phenol red	0.008
Final pH 6.7 ±	

MATERIAL AND METHODS

In his initial report, Sellers[20] described in detail the constituents of the medium and the rationale for each component, as well as the method of preparation. The medium is now commercially available in two forms,* both of which were used. Difco Sellers' medium contains 20 gm. of Bacto-Peptone and 15 gm. of Bacto-Agar, while BBL Sellers' medium contains 20 gm. of Gelysate and 13.5 gm. of dried agar. All other constituents are identical in both preparations (*Table 1*). Just before using, 0.15 ml. of sterile 50 percent dextrose is added to the medium by letting it run down the side of the culture tube opposite the agar slant. In a large bacteriology laboratory, if use of Sellers' medium is to become a standard procedure, the addition of dextrose in many tubes makes the technic slightly more cumbersome; moreover, the use of the same dextrose solution repeatedly is likely to lead to accidental contamination. Therefore part of the present study investigates the use of Sellers' medium with added dextrose as originally described by Sellers and without added dextrose. The purpose of this was to determine whether or not enough information could be obtained from the use of dextrose-free medium to make this a practical diagnostic procedure.

In this study we used 60 known strains of 20 types of gram-negative bacteria (*Table 2*), which we obtained from the American Type Culture Collection, The Ohio State Department of Health, The Communicable Disease Center, The Western Reserve University bacteriology laboratories and routine isolates from the Cleveland Clinic Hospital.

The investigation comprised two parts: Part 1 related to the evaluation

* *Difco Sellers' medium, from Difco Laboratories; BBL Sellers' medium, from Baltimore Biological Laboratories.*

Table 2.—*Designation of the different types of organisms used in the study*

I.	*Mima-Herellea group*
	Mima polymorpha, Herellea vaginicola, Bacterium anitratum
II.	*Pseudomonadaceae*
	Pseudomonas aeruginosa
III.	*Achromobacteriaceae*
	Alcaligenes fecalis
IV.	*Enterobacteriaceae*
	a. **Coliforms**—*Escherichia coli, Aerobacter, Citrobacter*
	b. **Paracolons**—Arizona, Providence, *Hafnia*, Bethesda-Ballerup
	c. *Proteus—Proteus vulgaris, Proteus mirabilis, Proteus morgani, Proteus rettgeri*
	d. *Salmonella-Shigella—Salmonella typhi, Shigella dystenteriae, Shigella boydi, Shigella flexneri*

of Sellers' medium in relation to the organisms that usually produce an alkaline slant and butt without production of hydrogen sulfide on triple sugar-iron-agar (Groups I, II, and III, in *Table 2*). Part 2 consisted of a study of organisms in the *Enterobacteriaceae* (Group IV, *Table 2*). Part 2 of the study was based on the fact that many of the bacteria included in the *Enterobacteriaceae* are identifiable on the basis of their ability to ferment glucose and mannitol with or without production of gas, and their ability to produce an alkaline reaction if they fail to ferment carbohydrates. The results obtained after inoculation of Difco Sellers' medium and of BBL Sellers' medium with 50 percent dextrose, and BBL Sellers' medium without dextrose are summarized in *Tables 3, 4, 5, and 6.*

The organisms were inoculated on Sellers' medium by a combined streaking-and-stabbing technic preceded by addition of 50 percent dextrose as suggested by Sellers, and also without the addition of 50 percent dextrose. The reasons for this modification have been mentioned earlier.

The changes in the medium were observed, at six-hour intervals for 24 and sometimes 48 hours, in respect of the color changes in the slant and butt, the formation of gas bubbles in the slant and butt, and the fluorescence or its absence in the slant.

The color of the uninoculated Sellers' medium is jade green, the BBL preparation is darker than the Difco medium. It is important when noting the color change in inoculated medium to compare it with an uninoculated control. Also, since gas formation is a crucial feature in identification, especially in the first 18 hours, the butt should be carefully scrutinized for even a single gas bubble. Often in the first 12 hours a single bubble formed in the butt may be missed entirely, especially if the bubble is in the horizontal

plane. After 24 hours the gas formation is obvious, as there are large gas bubbles, and often the entire butt is raised from the bottom of the culture tube.

ANALYSIS OF RESULTS

Part 1

The different patterns obtained on cultures of organisms in Groups I, II, and III, with or without the addition of 50 percent dextrose, are listed in

Table 3.—*Typical patterns produced by gram-negative bacteria on Sellers' medium with 50 percent dextrose*

Pattern	Organism	Color in			Fluorescence	Gas in butt
		Butt	Slant	Band		
1	*Herellea* species*	Green†	Blue	Yellow	±	–
2	*Pseudomonas aeruginosa*	Blue or green†	Dark green	–	Marked (++++)	Present
3	*Mima polymorpha*	Green†	Blue	–	–	–
4	*Alcaligenes fecalis*	Green† or blue	Blue	–	–	Present
5	*Herellea* species*	Yellow	Blue	–	±	–

* *Herellea* species includes *Herellea vaginicola* and *Bacterium anitratum*. *Herellea vaginicola* shows slight fluorescence along the margins of the slant; this was not seen in our *Bacterium anitratum* cultures.

† Green indicates no change in the color of the medium.

Table 4.—*Typical reactions produced on Sellers' medium without 50 percent dextrose*

Organism	Color in			Fluorescence	Gas in butt
	Butt	Slant	Band		
Herellea vaginicola	Green*	Blue	–	±	–
Bacterium anitratum	Yellowish green	Blue	–	–	–
Alcaligenes fecalis	Blue or green*	Blue	–	–	Few bubbles
Pseudomonas aeruginosa	Green*	Dark green or blue	–	Brilliant green	Few bubbles
Mima polymorpha	Green*	Blue	–	–	–

* Green indicates no change in the color of the medium.

Tables 3 and 4. The patterns observed were nearly identical on both commercial media with one exception. The yellow band formation when it occurred with *Herellea* organisms was much more striking because of the sharper color contrast in the BBL Sellers' medium than in the Difco Sellers' medium.

Our results in general confirmed those of Sellers,[21] with one minor exception (*Table 3*). Pattern 1 for *Herellea* species, considered typical by Sellers at 24 hours, in our cultures was best seen consistently between 6 to 12 hours. After that period, pattern 5 was much more common, partly because of the diffusion of acid formed from dextrose into the depths of the butt, and partly because of the slight fermentation of mannitol in the butt. Pattern 1 appeared to be unrelated to the concentration and amount of dextrose solution used, the size of the inoculum, the method of inoculation, and the size of the tube. As mentioned above, to a large extent pattern 1 was related to the time factor, and, to a certain extent, to the height of the

Table 5.—*Typical patterns of* Enterobacteriaceae *on Sellers' medium with 50 percent dextrose*

Group	Organism	Color in		Gas	Band
		Butt	Slant		
Coliform	*Escherichia coli*	Yellow	Yellow	Produced	–
	Citrobacter	Yellow	Yellow or blue	±	–
	Aerobacter	Yellow	Blue	Produced	–
Paracolon	Arizona	Yellow	Yellow or rarely blue	Produced	–
	Providence	Yellow	Blue	–	–
	Hafnia (at 37 C.)	Yellow	Blue	–	–
	Ballerup	Yellow	Blue	±	–
Proteus	*Proteus vulgaris*	Yellow	Blue	–	–
	Proteus mirabilis	Yellow	Blue	±	Occasional yellow
	Proteus morgani	Yellow	Blue	±	–
	Proteus rettgeri	Yellow	Blue	–	–
Salmonella-Shigella	*Salmonella typhi*	Yellow	Yellow	–	–
	Shigella dysenteriae	Yellow	Blue	–	Occasional yellow
	Shigella boydi	Yellow	Yellow or blue	–	–
	Shigella flexneri	Yellow	Blue	±	–

Table 6.—*Typical patterns of* Enterobacteriaceae *on BBL Sellers' medium without 50 percent dextrose*

Group	Organism	Color in		Gas	Band
		Butt	Slant		
Coliform	*Escherichia coli*	Yellow	Yellow	Produced	—
	Citrobacter	Bright yellow	Blue	Produced	—
	Aerobacter	Yellow-green	Blue	±	—
Paracolon	Arizona	Yellow	Yellow	Produced	—
	Providence	Green*	Blue	—	—
	Hafnia (at 37 C.)	Yellow-green	Blue	—	—
	Ballerup	Yellow	Blue	—	—
Proteus	*Proteus vulgaris*	Yellow-green	Yellow-green	—	—
	Proteus mirabilis	Green	Blue	—	—
	Proteus morgani	Green	Blue	—	—
	Proteus rettgeri	Yellow-green	Blue	—	—
Salmonella-Shigella	*Salmonella typhi*	Yellow	Blue	—	—
	Shigella dysenteriae	Green	Blue-green	—	—
	Shigella flexneri	Yellow-green	Blue	—	—

* Green indicates no change in the color of the medium.

butt. Occasionally it was noticed that the same strain of *Herellea* inoculated in two different tubes under practically identical conditions produced patterns 1 or 5 at the same time intervals, pattern 1 finally changing to 5.

Patterns 2, 3, 4, and 5 were most striking between 12 and 18 hours, with the exception of gas formation, which became most easily recognizable after 18 hours and sometimes even after 24 hours.

These organisms can be identified on Sellers' medium nearly as well without as with the use of 50 percent dextrose (*Table 4*). By not using dextrose the growth of organisms is delayed to some extent. In general, the most characteristic patterns were seen after 16 hours. Cultures labeled as *Bacterium anitratum* produced a yellowish-green butt, while *Herellea vaginicola* cultures did not, perhaps because the former was able to ferment mannitol slightly in a relatively anaerobic state. There is no mention in two standard textbooks[22, 23] on the ability of *Herellea* species to ferment mannitol. No mention is made of *Herellea* species or *Bacterium anitratum*

in *Bergey's Manual of Determinative Bacteriology*.[24] Some authors,[17, 19] however, have indicated that the *Herellea* strains in their collections did not ferment mannitol.

Part 2

The only organisms that could be distinguished with reasonable accuracy were *E. coli* from *Aerobacter aerogenes,* Arizona from Providence, and *Salmonella* from *Shigella.* The differentiation was based on the different characteristics in the fermentation of dextrose and mannitol with the formation of acid. Although *Proteus rettgeri* is the only *Proteus* that ferments mannitol as well as dextrose, it produced a color pattern identical to that of other strains of *Proteus.* A possible explanation of this phenomenon is the assumption that under the aerobic conditions prevailing in the slant, the production of a strong alkaline reaction more than compensated for the acid production from mannitol, resulting in a blue or alkaline slant. Sellers[25] suggested that probably the strains of *Proteus rettgeri* and Providence we used "prefer to obtain a greater portion of their energy requirements from glycogenic amino acids in the peptone than do the *E. coli,* Arizona, and *Shigella boydi.*" Probably the same theoretic explanation applies to *Shigella boydi* and *Shigella flexneri,* which usually ferment both mannitol and dextrose, though occasionally certain strains of both organisms may not cause fermentation.

The correlation between the behavior of the organisms of Group IV on Difco Sellers' and BBL Sellers' medium was not so close as between those of Groups I, II, and III. On Difco Sellers' medium, Paracolon, Arizona, *Citrobacter,* and *Shigella boydi* more often produced yellow slants and butt patterns, whereas on BBL Sellers' medium the same strains more often produced yellow butts and blue slant patterns. The gas formation was generally greater on Difco Sellers' medium than on BBL Sellers' medium.

The results obtained by inoculating the Group IV organisms on BBL Sellers' medium without 50 percent dextrose are shown in *Table 6.* This modification again made it possible to differentiate Arizona from Providence and *Citrobacter.* The greatest advantage was in the separation of *Proteus rettgeri* from other *Proteus* species and particularly other *Proteus morgani* not producing hydrogen sulfide; also, *Shigella dysenteriae* could be differentiated from *Shigella boydi.*

SUMMARY AND CONCLUSIONS

A study was made of the various patterns produced by several types of gram-negative bacteria on two commercial forms of Sellers' medium, with and without the addition of 50 percent dextrose. The results in general agree with those of Sellers. The media as supplied by Difco Laboratories and

Baltimore Biological Laboratories are both excellent yet have minor differences.

Evaluation of Sellers' medium for the differentiation of *Enterobacteriaceae* has shown that it is not suitable for their differentiation. This is not intended to be a criticism of Sellers' medium, since it was described for a limited purpose which it seems to serve well. It is therefore suggested that the routine use of Sellers' medium (either with or without added dextrose) in conjunction with triple sugar-iron-agar will hasten the identification of *Pseudomonas aeruginosa, Alcaligenes fecalis,* and the *Mima-Herellea* organisms. However, because the extra effort of adding the dextrose does not seem to produce commensurate advantages, we believe that the medium without dextrose is to be recommended for routine use.

REFERENCES

1. Finland, M.; Jones, W. F., Jr., and Barnes, M. W.: Occurrence of serious bacterial infections since introduction of antibacterial agents. J.A.M.A. **170:** 2188–2197, 1959.
2. Rogers, D. E.: The changing pattern of life-threatening microbial disease. New England J. Med. **261:** 677–683, 1959.
3. Yow, E. M.: Clinical significance of rising incidence of infections due to gram-negative bacilli. Postgrad. Med. **17:** 413–419, 1955.
4. Weinstein, L., and Wasserman, E.: *Bacterium alcaligenes* (*Alcaligenes faecalis*) infections in man. New England J. Med. **244:** 662–665, 1951.
5. Sykes, C.; Beckwith, J. R.; Muller, W. H., and Wood, J. E., Jr.: Postoperative endoauriculitis due to Pseudomonas aeruginosa cured by a second operation. Arch. Int. Med. **110:** 113–116, 1962.
6. Koch, M. L.: Bacteremia due to bacterial species of the genera Aerobacter, Escherichia, Paracolobactrum, Proteus, and Pseudomonas. Antibiotic Med. **2:** 113–121, 1956.
7. Curtain, J. A.: Petersdorf, R. G., and Bennett, I. L., Jr.: Pseudomonas bacteremia: A review of ninety-one cases. Ann. Int. Med. **54:** 1077–1107, 1961.
8. Stanley, M. M.: Bacillus pyocyaneus infections; a review, report of cases and discussion of newer therapy including streptomycin. Am. J. Med. **2:** 253–277; 347–367, 1947.
9. Hodges, R. M., and de Alvarez, R. R.: Puerperal septicemia and endocarditis caused by Pseudomonas aeruginosa. J.A.M.A. **173:** 1081–1088, 1960.
10. De Bord, G. G.: Species of the tribes Mimeae, Neisserieae, and Streptococceae which confuse the diagnosis of gonorrhea by smears. J. Lab. & Clin. Med. **28:** 710–714, 1943.
11. Daly, A. K.; Postic, B., and Kass, E. H.: Infections due to organisms of the genus Herellea; *B5W* and *B. anitratum.* Arch. Int. Med. **110:** 580–591, 1962.
12. Rangam, C. M.; Gupta, J. C.; Bhagwat, R. R., and Bhagwat, A. G.: Fibrinolytic activity of *Ps. aeruginosa.* Ind. J. M. Res. **49:** 232–235, 1961.
13. Rangam, C. M.; Bhagwat, R. R., and Bhagwat, A. G.: Phosphatase activity of *B. coli.* Ind. J. M. Sc. **13:** 511–513, 1959.
14. Bhagwat, A. G.: Letters to the editor. New fibrinolytic and anticoagulant enzyme. Lancet **1:** 487, 1962.
15. Ino, J.; Neugebauer, D. L., and Lucas, R. N.: Isolation of *Mima polymorpha* var. *oxidans* from two patients with urethritis and a clinical syndrome resembling gonorrhea. Am. J. Clin. Path. **32:** 364–366, 1959.

16. Pike, R. M.; Schulze, M. L., and McCullough, M.: Isolation of *Mima polymorpha* from a patient with subacute bacterial endocarditis. Am. J. Clin. Path. **21:** 1094–1096, 1951.

17. Sorrell, W. B., and White, L. V.: Acute bacterial endocarditis caused by a variant of genus Herrellea; report of a case. Am. J. Clin. Path. **23:** 134–138, 1953.

18. Hermann, G., III, and Melnick, T.: *Mima polymorpha* meningitis in the young. Am. J. Dis. Child. **110:** 315–318, 1965.

19. Ino, J., and Neugebauer, D. L.: Isolation of a species of genus Herrellea from a patient with chronic synovitis. Am. J. Clin. Path. **26:** 1486–1489, 1956.

20. Sellers, W.: A medium for differentiating the gram negative non-fermentative bacilli of medical importance. Abs. M43. American Society for Microbiology. Bact. Proc. 1963, Abs. 63rd Ann. Meet. May 5–9, 1963, p. 65, 1963.

21. Sellers, W.: Medium for differentiating the gram-negative, nonfermenting bacilli of medical interest. J. Bact. **87:** 46–48, 1964.

22. Bailey, W. R., and Scott, E. G.: Diagnostic Microbiology, Chap. 31, p. 211–213, *in* A Textbook for the Isolation and Identification of Pathogenic Microorganisms. St. Louis: The C. V. Mosby Company, 1962, 327 p.

23. Smith, D. T., and others: Zinsser Microbiology, 13th ed., Chap. 38, p. 620–621. New York: Appleton-Century-Crofts, 1964, 1214 p.

24. Breed, R. S.; Murray, E. G. D., and Smith, N. R., and others: Bergey's Manual of Determinative Bacteriology, 7th ed. Baltimore: The Williams & Wilkins Co., 1957, 1094 p.

25. Sellers, W.: Personal communication, December 10, 1965.