Differentiation of Enterobacteriaceae by Biochemical Reactions

Revised and Emended, 1970

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Summary. Tabular data on the biochemical reactions of Enterobacteriaceae are presented, which, if used properly, will enable investigators to identify ninety-five percent or more of cultures isolated in daily practice. The data, including percentages, are given in twenty-one tables. The majority of these contain the results of tests that are of particular usefulness in differentiation of members of the various genera and most species within the family. An emended outline of the nomenclatural system employed is presented in an appendix.

For many years the author and co-workers have studied the biochemical reactions of relatively large numbers of cultures of each of the genera of Enterobacteriaceae in an attempt to produce tabular data, with percentages, that might be of value to investigators in laboratories of all kinds. Although some of these studies still are unpublished, the work led to revision of definitions for the family Enterobacteriaceae, its tribes, and genera (Ewing, 1967), and to recognition of additional tests and methods of value in the differentiation of members of the various genera and species within the family.

The biochemical methods employed were the recommended or standard methods given in the 1958 Report of the International Subcommittee on Enterobacteriaceae, as revised and extended by Ewing (1960, 1962) and by Ewing and Davis (1970).

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The numerical data given in the tables in this and other publications on biochemical reactions by the author and colleagues are based upon the results obtained from examinations of thousands of cultures from all States and Territories of the United States, and from many other parts of the world, over a period of more than 20 years. Since these strains were, with few exceptions, submitted directly or indirectly from clinical laboratories, and since these cultures were, with very few exceptions, quite typical it is believed that the numerical data are representative and objective.

It is the author's opinion that unless percentages are included in tabular data, the resulting tables are of little value. As in the past, 90 percent levels were employed in determining the signs that would be applied. Some arbitrary level must be selected, and the author and colleagues believe that the 90 percent level is the most reasonable and practical. Thus a + sign means that 90 to 100 percent of strains tested on a particular substrate gave positive results within one or two days of incubation. The addition of the actual percentage of such reactions (e.g., 91 percent or 98 percent) yields valuable, useful information. Conversely the same is true of negative results. Further, if percentages are included, the sign "d" (for different reactions) assumes meaning in most instances. For example, if 60 to 90 percent of results obtained in a test are positive, then the "d" sign is meaningful since the majority of cultures yielded positive results. Similarly, the "d" sign is useful if the majority of

cultures give negative reactions on a particular substrate (e.g., 11 to 25 or 30 percent). When the percentages obtained are in the area between about 30 and 60 percent the "d" sign usually indicates that tests that yield such figures are of little differential value, but this cannot be determined if the actual percentages of positive results are not listed. However, there are instances in which a "d" sign followed by about 40 percent positive for example, is of value as in the case of the differentiation of S. cholerae-suis, inositol, -:0%+, and S. enteritidis, inositol, 42.8%+, or in the differentiation of commonly occurring salmonellae from members of the genus Arizona.

The nomenclatural system employed herein is that proposed by Ewing (1963) and emended in 1966 and 1967. This system is given in outline form in the Appendix for reference. The genus Pectobacterium was incorporated into the tribe Klebsielleae for the reasons given by Graham (1964) and Ewing (1967). An emendation of a name now is required, and some citations must be changed. Because of changes made in the rules of nomenclature approved during the IX International Congress of Microbiology (see International Code of Nomenclature of Bacteria, 1966), it became necessary to change the specific epithet arizonae in Arizona arizonae. The specific epithet hinshawii was proposed (Ewing, 1969) in recognition of Dr. William R. Hinshaw who did much of the pioneer work with members of the genus Arizona. Therefore the correct species name for these microorganisms now is Arizona hinshawii (Appendix).

In 1966 the author submitted a request for an opinion regarding a proposal for validation of the species name Arizona arizonae (Kauffmann and Edwards, 1952). As mentioned above it was necessary to emend the specific epithet. More recently, the author was informed (personal communication, 1969) by Dr. P.H.A. Sneath (Chairman, Judicial Commission of the International Committee on Nomenclature of Bacteria) that the Judicial Commission had ruled that the generic name Arizona was not validly published by Kauffmann and Edwards (1952) because the classification and names used by these investigators were suggested, not recommended (Rule 12c). However, the generic name Arizona was legitimately and validly published by Ewing (1963), Ewing, et al. (1965), Ewing and Fife (1966), and Ewing (1967). Since the genus Arizona was characterized and defined by Ewing et al. (1965) and Ewing and Fife (1966), and since the specific epithet was changed legitimately to hinshawii, the correct citations should be Arizona hinshawii (Ewing and Fife) Ewing (Appendix).

If Arizona Kauffmann and Edwards (1952) was illegitimate, then Providencia Kauffmann and Edwards (1952) also was. However, the genus Providencia was validly published, characterized, and defined by Ewing, 1962. Therefore, the citation for this genus should be Providencia Ewing (Appendix).

The 21 tables that follow largely are self-explanatory, but notes are included where necessary to explain certain important exceptional reactions.

It is suggested that the data given in these tables might prove to be of greatest value when used in conjunction with those presented in two other publications (Ewing, 1969, Ewing et al., 1970).

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TABLE 1

Differentiation of the Tribes of Enterobacteriaceae By Biochemical Methods[1]

Substrate or test	TRIBES				
	Escherich-ieae	Edwards-ielleae	Salmon-elleae	Klebs-ielleae	Proteeae
Hydrogen sulfide (TSI or PI agar)	-	+	+	-	+ or -
Urease	-	-	-	- or (+)	+ or -
Indol	+ or -	+	-	-	+ or -
Methyl red	+	+	+	-	+
Voges-Proskauer	-	-	-	+	-
Citrate (Simmons')	-	-	+	+	d
KCN	-	-	- or +	+	+
Phenylalanine deaminase	-	-	-	-	+
Tartrate (Jordan's)	+ or -	-	d	+ or -	+ or -
Mucate	d	-	d	+ or -	-
Mannitol	+ or -	-	+	+	- or +

[1]Adapted from Ewing (1966)

N.B. Salmonella typhi, Salmonella enteritidis bioserotype Paratyphi A and some rare biotypes fail to utilize citrate. Cultures of S. enteritidis bioser. Paratyphi A and some rare biotypes may fail to produce hydrogen sulfide. Some cultures of P. mirabilis may yield a positive Voges-Proskauer test.

+ 90% or more positive within 1 or 2 days

- 90% or more, no reaction

(+)delayed positive 3 or more days

d different biochemical reactions, +, (+), -.

+ or - majority of strains +, some cultures negative

- or + majority of cultures negative, some strains positive.

TABLE 2

Differentiation within the Tribe ESCHERICHIEAE[1]

Substrate or Test	Escherichia			Shigella		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Gas from glucose	+	90.7		-	2.1[a]	
Lactose	+	90.8	(5.1)	-	0.3	(11.4)[b]
Sucrose	d	48.9	(5.6)	-	0.9	(31.1)[b]
Salicin	d	40	(14)	-	0	
Motility	+ or -	69.1		-	0	
Indol	+	99.2		- or +	39.8	
Lysine decarboxylase	d	87.9	(1.2)	-	0	
Arginine dihydrolase	d	17.2	(44.8)	d	9.5	(17.3)
Ornithine decarboxylase	d	63.4	(7.1)	-	20[b]	
Esculin	d	30.9	(19.7)	-	0	
Sodium acetate	+ or(+)	83.9	(9.7)	-	0[c]	
Christensen's citrate	d	24.4	(21.2)	-	0	
Mucate	+	96.3		-	0[b]	

[1]Adapted from Ewing (1966) and Ewing et al., in preparation.

*Figures in parentheses indicate percentages of delayed reactions (3 days or more)

[a]Certain biotypes of S. flexneri 6 form gas

[b]S. sonnei strains usually ferment lactose and sucrose slowly and cultures of this species decarboxylate ornithine. Some strains of S. sonnei utilize mucate.

[c]See table 4

N.B. Obviously there is no difficulty in the differentiation of typical E. coli cultures and shigellae. However, the anaerogenic nonmotile, varieties of E. coli, some of which are often referred to as Alkalescens-Dispar types, may require closer examination before they can be definitely classified as E. coli. In attempting to classify a particular strain as E. coli or as a member of the genus Shigella, the biochemical reactivities of the culture should be considered as a whole. Shigellae are much less reactive than E. coli strains and a culture that produces acid promptly (i.e., within 24 hrs.) from all, or most of a wide variety of carbohydrates, such as maltose, rhamnose, xylose, sorbitol, and dulcitol, undoubtedly is not a member of the genus Shigella.

+90% or more positive within 1 or 2 days. -90% or more, no reaction. (+) Positive reaction 3 or more days. d Different biochemical reactions, +, (+), -. +or- Majority of strains +, some cultures negative. -or+ Majority of cultures negative, some strains positive.

TABLE 3

The decarboxylase reactions of shigellae and E. coli including nonmotile anaerogenic biotypes such as the "Alkalescens-Dispar" biotypes

Genera and species	No. tested	Lysine			Arginine			Ornithine		
		Sign	%+ *	(%+) **	Sign	%+ *	(%+) **	Sign	%+ *	(%+) **
S. dysenteriae	352	-	0	0	d	5.4	(42.3)	-	0	0
S. flexneri	1817	-	0	0	d	8.3	(2)	-	0	0
S. boydii	363	-	0	0	d	21	(35.7)	-	2.5[a]	0
S. sonnei	633	-	0	0	d	8.8	(41.9)	+	99.8[b]	0
E. coli	505	d	87.9	(1.2)	d	17.2	(44.8)	d	63.4	(7.1)
"Alkalescens-Dispar" biotypes	190	d	72.1[c]	(12.6)	d	10.5	(50.5)	d	7.4	(37)

* %+ Percentage of positive reactions within 1 or 2 days

** (%+) Percentage of positive reactions after 3 or 4 days

[a] These few cultures all were S. boydii ser. 13, a very rare serotype

[b] Decarboxylation of ornithine is a characteristic of S. sonnei.
Note that the Shigella cultures tested did not decarboxylate lysine whereas the majority of strains of E. coli did and that only a small percentage of S. flexneri possessed an arginine dihydrolase system (10.3%+ in 1 to 4 days).

[c] 84.7% of "A-D" O group 1 (E. coli biotype, O group 1) gave positive reactions in lysine medium within 4 days).

TABLE 4a

The reactions of shigellae and E. coli in acetate,

Christensen's citrate, and mucate media

Genera and species	Sodium acetate				Christensen's citrate				Sodium mucate			
	No. tested	Sign	%+	(%+)*	No. tested	Sign	%+	(%+)*	No. tested	Sign	%+	(%+)*
S. dysenteriae	50	-	0	0	294	-	0	0	63	-	0	0
S. flexneri	100[1]	-	0	0	1375	-	0	0	423	-	0	0
S. boydii	50	-	0	0	442	-	0	0	123	-	0	0
S. sonnei	100	-	0	0	209	-	0	0	165	d	6.4[w]	(30.3[w])
E. coli	186	+or(+)	83.8	(9.7)	423	d	15.8	(18.4)	344	+	91.6	(1.4)
"Alkalescens-Dispar" biotypes	238	+or(+)	89.6	(4.7)	200	d	75	(12.5)	61	d	29.5	(27.9)

Adapted from Trabulsi and Ewing (1962), and unpublished data.

[1] Includes all S. flexneri serotypes (see also table 4b).

* Figures in parentheses indicate percentages of delayed reactions (3 to 7 days).

[w] Weakly positive reaction.

TABLE 4b

Reactions of cultures of S. flexneri serotype 4 in sodium acetate medium

Subserotype or biotype of S. flexneri 4	No. tested	%+	(%+)*
4a	52	0	(8[w])
4a (mannitol negative)	50	0	(43[w])
4b	52	0	0

*Positive in 2 to 7 days.
[w]Weakly positive reaction.

TABLE 5

Differentiation of Escherichia and Edwardsiella[1]

Substrate or test	Escherichia			Edwardsiella		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Hydrogen sulfide (TSI)	-	0		+	99.7	(0.3)
Mucate	+	91.6	(1.4)	-	0	(0.3)
Tartrate (Jordan's)	+	97.6	(1.9)	-	0	
Sodium acetate	+ or(+)	83.8	(9.7)	-	0	
Mannitol	+	97		-	0	
Sorbitol	+	93.4	(0.5)	-	0.3	
Rhamnose	d	81.8	(2.8)	-	0	
Xylose	d	82.4	(6.7)	-	0	
Trehalose	+	98.6	(1)	-	0.3	

[1]Adapted from Ewing et al. (1965, 1967, 1969, and unpublished data).

* Figures in parentheses indicate percentages of delayed reactions (3 days or more)
+ 90% or more positive within 1 or 2 days
- 90% or more, no reaction
(+)Delayed positive 3 or more days
d Different biochemical reactions, +, (+), -.
+ or - Majority of strains +, some cultures negative.
- or + Majority of cultures negative, some strains positive.

TABLE 6

Differentiation within the Tribe SALMONELLEAE

Test or substrate	Salmonella[1]			Arizona[2]			Citrobacter[3]		
	Sign	%+	(%+)*	Sign	%+	(%+)*	Sign	%+	(%+)*
Urease	-	0		-	0		d	69.4	(6.9)
KCN	-	0.3	(0.3)	-	8.7		+	96.2	(0.9)
Gelatin (22 C)	-		(1.3)	(+)		(92)	-		(0.9)
Lysine decarboxylase	+	97.7		+	100		-	0	
Ornithine decarboxylase	+	100		+	100		d	17.2	(0.2)
Lactose	-	1		d	61.3	(16.7)	(+)or+	39.3	(50.9)
Sucrose	-	0.7		-	4.7		d	15.3	(9.4)
Dulcitol	+	98.3		-	0		d	59.4	(0.7)
Inositol	d	42.8	(1)	-	0		-	3.3	(1.9)
Cellobiose	(+)or+	5.4	(88.1)	d	1	(72)	+or(+)	60.8	(38)
Malonate	-	0.7		+	92.6	(0.7)	d	21.8	(0.7)
Jordan's tartrate	+	92.5	(1.1)	-	5.3	(19.3)[4]	+	100	
Beta galactosidase	-	1.5		+	92.8		+or-	74.4	
Organic acids**									
citrate	+	96	(4)	+or(+)	78.7	(19.3)	(+)or+	49.2	(49.5)
D-tartrate	+	91	(5.3)	(+)or-		(83.3)	(+)		(90.9)

*Figures in parentheses indicate percentages of delayed reactions (3 or more days)
**Organic acid media of Kauffmann and Petersen, 1956 (see also table 8)
+Positive within one or two days' incubation (90% or more)
(+)Positive reaction after 3 or more days
-No reaction (90% or more)
+or- Majority of strains positive, some cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
dDifferent reactions: +, (+), -.

[1]Adapted from Ewing and Ball (1966)
[2]Adapted from Ewing and Fife (1966)
[3]Adapted from Davis and Ewing (1965)
[4]Cognizance should not be given to delayed reactions. Final readings of Jordan's tartrate medium should be made at 48 hours.

N.B. The majority of salmonellae ferment dulcitol promptly, but S. typhi, S. enteritidis biosero. Paratyphi A and Pullorum, S. cholerae-suis, and a few others do not. Members of the genus Arizona are uniformly negative on this substrate. Bioser. Paratyphi A is lysine negative. S. typhi is ornithine negative.

TABLE 7

Differentiation of Salmonella, Arizona, and Citrobacter

Test or substrate	Salmonella enteritidis[1]			Arizona hinshawii[2]			Citrobacter freundii[3]		
	Sign	%+	(%+)*	Sign	%+	(%+)*	Sign	%+	(%+)*
Urease	-	0		-	0		d	69.4	(6.9)
KCN	-	0.1	(0.1)	-	8.7		+	96.2	(0.9)
Gelatin (22C)	-	0.4[4]		(+)		(92)	-		(0.9)
Lysine decarboxylase	+	94.4	(0.1)	+	100		-	0	
Ornithine decarboxylase	+	100		+	100		d	17.2	(0.2)
Lactose	-	0.3		d	61.3	(16.7)	(+)or+	39.3	(50.9)
Sucrose	-	0.2		-	4.7		d	15.3	(9.4)
Dulcitol	+	97.7		-	0		d	59.8	(0.7)
Inositol	d	43.8	(2)	-	0		-	3.3	(1.9)
Malonate	-	1	(0.1)	+	92.6	(0.7)	d	21.8	(0.7)
Jordan's tartrate	d	84.2	(1)	-	5.3	(19.3)[5]	+	100	
Beta galactosidase	-	2.1		+	92.8		+or-	74.4	
D-tartrate**	+	92.3	(4)	(+)or-		(83.3)	(+)		(90.9)

\+ 90% or more positive within 1 or 2 days
\- 90% or more, no reaction
(+)Delayed positive 3 or more days
d Different biochemical reactions, +, (+), -.
\+ or - Majority of strains positive, some cultures negative.
\- or + Majority of cultures negative, some strains positive.

*Figures in parentheses indicate percentages of delayed reactions (3 or more days).

[1]Summary of reactions given by 787 cultures: Ewing and Ball, 1966 (tables 8 and 9) and Martin et al., in press. Compare percentages with those given in table 6.

[2]Adapted from Ewing and Fife, 1966 (specific epithet emended, 1969).

[3]Adapted from Davis and Ewing (1966).

[4]Two cultures among 526 strains examined by Martin et al. (1969)

[5]Cognizance should not be given to delayed reactions. Final readings of reactions in Jordan's tartrate should be made at 48 hours.

**Method of Kauffmann and Petersen (1956).

N.B. The names of tests and substrates that are of particular value in the differentiation of Salmonella and Arizona are underscored.

TABLE 8

Reactions of members of the tribe SALMONELLEAE in organic acid media of Kauffmann and Petersen (1956)*

Genus	No.	Citrate					D-tartrate				
		1**	2	5	14	-	1**	2	5	14	-
Salmonella	299	245 (81.9)	42 (14.1)	8 (2.7)	4 (1.3)	0	272 (91)	4 (1.3)	3 (1)	9 (3)	11 (3.7)
Arizona	150	7 (4.7)	111 (74)	23 (15.3)	6 (4)	3 (2)	0	0	68 (45.3)	57 (38)	25 (16.7)
Citrobacter	268	4 (1.5)	143 (53.3)	117 (43.7)	0	4 (1.5)	0	0	119 (44.4)	122 (45.5)	27 (10.1)

*From Ewing, 1965, and Ewing, Fife, and Davis (1965)

**Days of incubation

N.B. Figures in parentheses indicate percentages.

TABLE 9

Differentiation of species of *Salmonella*[1]

Test or substrate	*S.* cholerae-suis			*S.* typhi			*S.* enteritidis		
	Sign	%+	(%+)*	Sign	%+	(%+)*	Sign	%+	(%+)*
Hydrogen sulfide (TSI)	d	60	(10)	+[W]	94.3		+	98	
Citrate (Simmons')	(+)		(90)	-	0		+	99.3	(0.7)
Ornithine decaroboxylase	+	100		-	0		+	100	
Gas from glucose	+	100		-	0		+	97.7	
Dulcitol	d	5	(15)	-or(+)	0	(31.3)	+	98.3	
Inositol	-	0		-	0		d	42.8	(1)
Trehalose	-	0		+	100		+	100	
Arabinose	-	0		-		(6.3)	+	99.3	
Rhamnose	+	100		-	0		+	95	
Cellobiose	-	0		d	37.5		(+)	5	(92.8)
Erythritol	(+[W])or-		(85)	-	0		-	0.6	
Sodium acetate	-or(+[W])		(20)	-	0		+	92.4	(2.2)
Mucate	-	0		-	0		+or(+)	88.3	(1.7)
Stern's glycerol fuchsin	-	0		-	0		+	98.2	(0.6)
Organic acids**									
citrate	-	10		-	10		+1or2da	96	(4)
D-tartrate	+1or2da	95		+1or2da	87.5	(6.3)	+	91	(5.3)
i-tartrate	(+)or-	0	(85)	-	0		d	4.7	(57.5)
l-tartrate	-or(+)	0	(35)	-	0		d	11.8	(75.2)

[1]Adapted from Ewing and Ball (1966)

*Figures in parentheses indicate percentages of delayed reactions (3 days or more)

**Method of Kauffmann and Petersen, 1956. (Twenty hour readings except where indicated, see also table 8)

[W] Weakly positive reactions
+ Positive within one or two days' incubation
(+)Positive reaction after 3 or more days
- No reaction
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -

TABLE 10

Biochemical reactions of S. enteritidis bioserotype Paratyphi A

Substrate or test	Bioserotype Paratyphi A[1] Sign	%+	(%+)*	S. enteritidis[2] Sign	%+	(%+)*
Hydrogen sulfide (TSI)	-or+[w]	12.5		+	98	
Citrate (Simmons')	-or(+)	0	(25)	+	99.3	(0.7)
Lysine decarboxylase	-	0		+	99.7	
Inositol	-	0		d	42.8	(1)
Xylose	-	0		+	99	
Cellobiose	d	12.5	(6.2)	(+)	5	(92.8)
Glycerol	(+)	0	(100)	d	5.7	(7.2)
Stern's glycerol fuchsin	-	0		+	98.2	(0.6)
Jordan's tartrate	-	0		+	92.5	(1.1)
Sodium acetate	-		(6.2)	+	92.4	(2.2)
Mucate	-	0		+or(+)	88.3	(1.7)
Organic Acids**						
citrate	-	0		+	96	(4)
D-tartrate	-	0		+	92.3	(4)
i-tartrate	-	0		d	4.7	(57.5)
l-tartrate	-	0		d	11.8	(75.2)

[1]Adapted from Ewing and Ball (1966)
[2]From table 21 and Ewing and Ball, 1966
*Figures in parentheses indicate percentages of delayed reactions (3 or more days)
**Method of Kauffmann and Petersen, 1956

N.B. Bioserotype Paratyphi A does not occur commonly in the United States, but investigators should be able to recognize it.

+ Positive within 1 or 2 days' incubation (90 percent or more)
(+)Positive reaction after 3 or more days
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -
w Weakly positive reaction

TABLE 11

Differentiation of Salmonella enteritidis bioserotypes Pullorum and Gallinarum[1]

Substrate or test	Bioserotype Pullorum			Bioserotype Gallinarum		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Jordan's tartrate	-	0		+	100	
Ornithine decarboxylase	+	100		-	0	
Mucate	-	0		+	90.3	
Gas from glucose	+	95.1		-	0	
Dulcitol	-	0		+	99	(1)
Maltose	-or(+)		(35)	+	98.1	(1.9)
Cellobiose	-	0		d	60	(30)
Glycerol	-	0		(+)		(90)
Cysteine-gelatin	-	0		+	98.1	(1.6)
Organic acids**						
citrate	-	0		-or(+)		(40)
D-tartrate	-	0		+	92.2	(3.9)
i-tartrate	-	0		(+)		(100)
l-tartrate	-	0		(+)or-		(80)

[1]Modified from Trabulsi and Edwards (1962) and Ewing and Ball (1966)

*Figures in parentheses indicate percentages of delayed reactions (3 days or more)
**Method of Kauffmann and Petersen, 1956

N.B. This table is included primarily for the use of workers in veterinary bacteriology. However, others should remember that bioserotype Pullorum occasionally occurs in human infections.

+ Positive within 1 or 2 days' incubation (90 percent or more)
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or- Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -

TABLE 12

The reactions given by certain cultures of *Arizona hinshawii* in selected tests[1]

Test or substrate	Sign	%+	(%+)*
Hydrogen sulfide (TSI)	+	100	
Indol	-	8.2	
Citrate (Simmons')	+	91.8	(8.2)
Urease	-	0	
Motility	+	100	
Gelatin (Kohn's)	+or(+)	59.2	(40.8)
Lysine decarboxylase	+	100	
Arginine dihydrolase	(+)or+	16.3	(83.7)
Ornithine decarboxylase	+	100	
Malonate	+	100	
Jordan's tartrate	-	0	
Gas from glucose	+	100	
Lactose	+or(+)	81.6	(18.4)
Sucrose	-	0	
Dulcitol	-	0	
Salicin	-	0	
Inositol	-	0	
Beta galactosidase	+	100	

[1]These data are included for comparison with that in tables 6 and 7. This group of 49 cultures was received in a single shipment. Forty-seven strains were recovered from intestinal contents of wild reptiles and two were isolated from the stools of humans.

*Figures in parentheses indicate percentages of delayed reactions (3 days or more)

+ Positive within 1 or 2 days' incubation (90 percent or more). - 90% or more, no reaction. (+) Positive reaction 3 or more days. d Different biochemical reactions: +, (+), -. +or- Majority of strains +, some cultures negative. -or+ Majority of cultures negative, some strains positive.

TABLE 13

Differentiation within the genus Klebsiella[1]

Test or substrate	K. pneumoniae Sign	K. pneumoniae %+ (%+)*	K. ozaenae Sign	K. ozaenae %+ (%+)*	K. rhinoschleromatis Sign	K. rhinoschleromatis %+ (%+)*
Urease	+	94.5	d	9.5 (10.3)	-	0
Methyl red	-or+	13.3	+	99.1	+	100
Voges-Proskauer	+	91.1	-	0	-	0
Citrate (Simmons')	+	97.7	d	31.9 (31)	-	0
Organic acids**						
citrate	+or-	64.4	-or+	18	-	0
D-tartrate	+or-	67.1	-or+	36	-	0
Malonate	+	92.5	-	4	+	95.5
Mucate	+	92.8	-or+	24	-	0
Lysine decarboxylase	+	97.2	-or+	48	-	0
Gas from glucose	+	96.5	d	64 (2)	-	0
Lactose	+	98.2 (1.4)	(+)or+	24.1 (70.7)	(+)or-	(72.8)
Dulcitol	-or+	31.5	-	0	-	0

[1]Adapted from Fife, Ewing, and Davis (1965)
+ Positive within 1 or 2 days' incubation (90 percent or more)
- No reaction (90 percent or more)
(+) Positive reaction after 3 or more days
+or- Majority of strains positive, occasional cultures negative.
-or+ Majority of cultures negative, occasional strains positive.
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days.
d Different reactions: +, (+), -

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)
**Method of Kauffmann and Petersen (1956)

TABLE 14

Differentiation of *Klebsiella pneumoniae* and *Enterobacter cloacae*[1]

Test or substrate	*K. pneumoniae*			*E. cloacae*		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Gas from:						
Inositol	+	91.9		-	4.5	
Glycerol	+	92.5		d	5.5	(15.9)
Adonitol	+or-	83.7		-or+	28.4	
Esculin	+	98.9	(1.1)	-or+	29.3	
Lysine decarboxylase	+	97.2		-	0.5	
Arginine dihydrolase	-	0.9		+	96.5	
Ornithine decarboxylase	-	0		+	96	
Urease	+	94.5		+or-	64.7	
Gelatin (22 C)	-	3.3		(+)		(96)
Motility	-	0		+	94.6	
Growth on synthetic alginate medium	+or(+)	88.5	(9.2)	-	0	

[1] Adapted from Fife, Ewing, and Davis (1965)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

+ Positive within one or two days' incubation (90% or more)
(+) Positive reaction after 3 or more days
- No reaction (90% or more)
+or- Majority of strains positive, some cultures negative.
-or+ Majority of cultures negative, some strains positive.
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days.
d Different reactions: +, (+), -.

TABLE 15

Differentiation of Enterobacter cloacae and Enterobacter aerogenes[1]

Test or substrate	E. cloacae Sign	E. cloacae %+	E. cloacae (%+)*	E. aerogenes Sign	E. aerogenes %+	E. aerogenes (%+)*
Urease	+or-	64.7		-	2.7	
Lysine decarboxylase	-	0.5		+	98.7	
Arginine dihydrolase	+	96.5		-	0	
Jordan's tartrate	-or+	27.8		+or-	89.3	
Adonitol acid	-or+	28.4		+	98.7	
gas	-or+	28.4		+	98.7	
Inositol acid	d	21.9	(12.4)	+	100	
gas	-	4.5		+	100	
Glycerol acid	d	43.3	(44.8)	+	100	
gas	d	5.5	(15.9)	+	98.7	(1.3)
Esculin	-or+	29.3		+	98	

[1]Adapted from Fife, Ewing, and Davis (1965)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

+ Positive within 1 or 2 days' incubation (90% or more)
(+) Positive reaction after 3 or more days
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative.
-or+ Majority of cultures negative, occasional strain positive.
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days.
d Different reactions: +, (+), -.

TABLE 16

Differentiation of *Enterobacter aerogenes* and *Enterobacter hafniae*[1]

Substrate or test	*E. aerogenes* Sign	*E. aerogenes* %+	*E. aerogenes* (%+)*	*E. hafniae* Sign	*E. hafniae* %+	*E. hafniae* (%+)*
Adonitol						
acid	+	98.7		-	0	
gas	+	98.7		-	0	
Inositol						
acid	+	100		-	0	
gas	+	100		-	0	
Sorbitol	+	100		-	0	
Raffinose	+	96		-	0	
Salicin	+	98.7	(1.3)	d	13	(8)
Alpha methyl glucoside	+	96	(2)	-	0	
Esculin	+	98		-	6	(2)
Methyl red						
37 C	-	0		+or-	54	
22 C				-	1	
Voges-Proskauer						
37 C	+	100		+or-	65	
22 C				+	99	
Citrate (Simmons')						
37 C	+	93.7		(+)or-		(58)
22 C				d	3	(79)
Gelatin (22 C)	(+)or-		(77.3)	-	0	
Mucate	+	94.7		-	0	

[1] Adapted from Ewing and Fife (1968)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

\+ Positive within 1 or 2 days' incubation (90 percent or more)
\- No reaction (90 percent or more)
(+) Positive reaction after 3 or more days.
+or- Majority of strains positive, occasional cultures negative.
-or+ Majority of cultures negative, occasional strains positive.
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days.
d Different reactions: +, (+), -.

TABLE 17

Differentiation of *Enterobacter liquefaciens*[1] and *Serratia marcescens* subspecies *marcescens*

Substrate or test	*E. liquefaciens* Sign	%+	(%+)*	*S. marcescens*, *marcescens* Sign	%+	(%+)*
Glucose						
Acid	+	100		+	100	
Gas	+	94.1		+[b]or-	52.6	
Inositol						
Acid	+	97	(1.5)	d	78.5	(8.2)
Gas	d	1.5	(22)	-	0	
Glycerol						
Acid	+	98.5	(1.5)	+	97	(2.6)
Gas	d	45.6	(38.2)	-	0	
Cellobiose						
Acid	d	26.5	(44.1)	d	20.8	(33.4)
Gas	d	5.9	(33.8)	-	0	
Esculin						
Acid	d	75	(1.6)	+	90.8	
Gas	-or+[a]	37.5		-	0	
Raffinose						
Acid	d	86.8	(2.9)	-	1.7	(1.2)
Gas	d	17.6	(60.3)	-	0	
Arabinose						
Acid	+	92.6		-	0	
Gas	d	23.5	(14.7)	-	0	
Xylose						
Acid	+	92.6	(1.5)	d	8	(18.3)
Gas	d	30.9	(23.5)	-	0	
Erythritol						
Acid	-	0		d	1.7	(22.8)
Alpha methyl glucoside						
Acid	-or+	21.7		-	0.9	(0.6)
Methyl red						
(37 C)	+or-	75		-or+	17.7	
(22 C)	-or+	33.3		-or+	8.8	
Voges-Proskauer						
(37 C)	-or+	30.9		+	100	
(22 C)	+or-	79.4		+	100	

[1]Adapted from Ewing et al. (1959), Ewing, Johnson, and Davis (1962), Fife, Ewing, and Davis (1965), and Ewing and Davis (unpublished data).

[a]Gas volumes: bubble to 10 percent

[b]Gas volumes: 10 percent or less

*Figures in parentheses indicate percentage of delayed reactions (3 or more days).

+ Positive within 1 or 2 days' incubation (90 percent or more). - 90% or more, no reaction (+) Positive reaction 3 or more days. d Different biochemical reactions: +, (+), -. +or- Majority of strains +, some cultures negative. -or+ Majority of cultures negative, some strains positive.

N.B. The only important difference between *S. marcescens* subspecies *marcescens* and *S. marcescens* subspecies *kiliensis* is their reactions in the Voges-Proskauer test. Cultures of the later are V-P negative.

TABLE 18

Differentiation of *Proteus vulgaris* and *Proteus mirabilis* from *Proteus morganii* and *Proteus rettgeri*

Substrate or test	*P. vulgaris* and *P. mirabilis*			*P. morganii* and *P. rettgeri*		
	Sign	%+	(%+)*	Sign	%+	(%+)
Hydrogen sulfide (TSI)	+	94.5	(2.6)	-	0	
Gelatin (22 C)	+	91.6	(6.4)	-	0	
Lipase (corn oil)	+	89.6	(5.2)	-	0	
Swarm (2% agar)	+	94	(1)	-	0	

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

+ Positive within 1 or 2 days' incubation (90 percent or more)
(+) Positive reaction after 3 or more days
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -

TABLE 19

Differentiation of Proteus vulgaris and Proteus mirabilis[1]

Test or substrate	P. vulgaris			P. mirabilis		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Indol	+	98.2		-	1.9	
Voges-Proskauer						
37 C	-	0		-or+	15.6	
22 C	-or+	11.3		+or-	51.6	
Citrate (Simmons')	d	10.5	(14.1)	+or(+)	58.7	(37.1)
Ornithine decarboxylase	-	0		+	99.2	
Sucrose	+	94.7		d	18.9	(63.3)
Maltose	+	96.2	(1.9)	-	0.9	(0.4)
Salicin	d	58.2	(10.9)	d	0.8	(29.8)
Alpha methyl glucoside	d	79.5	(5.1)	-	0	
Esculin	d	59	(2.6)	-		(0.9)
DNase	+or-	60		-	0	

[1]Adapted from Ewing, Suassuna, and Suassuna (1960), Ewing (1962), and Ewing et al. (in preparation)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

+ Positive within 1 or 2 days' incubation (90 percent or more)
(+) Positive reaction after 3 or more days
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -

TABLE 20

Differentiation of Proteus morganii and Proteus rettgeri[1]

Test or substrate	P. morganii			P. rettgeri		
	Sign	%+	(%+)*	Sign	%+	(%+)*
Citrate (Simmons')	-	0		+	95.6	(3.3)
Ornithine decarboxylase	+	97.1		-	0	
Gas from glucose	d	84.9	(0.9)	-or+	12.2	
Sucrose	-	1	(2.9)	d	13.3	(56.7)
Mannitol	-	0		+or-	88.5	
Adonitol	-	0		d	80.9	(5.6)
Inositol	-	0		+	93.3	(4.5)
Salicin	-	0		d	30	(6.6)
Erythritol	-	0		d	78.3	(6.5)
Esculin	-	0		d	30.4	(8.7)
Xylose	-	0		-or+	15.1	
Cellobiose	-	0	(1.9)	d	3.7	(30.4)

[1]Adapted from Ewing, Suassuna, and Suassuna (1960) and Ewing et al. (in preparation)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

+ Positive within 1 or 2 days'incubation (90 percent or more)
(+) Positive reaction after 3 or more days
- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative
-or+ Majority of cultures negative, occasional strains positive
(+)or+ Majority of reactions delayed, some occur within 1 or 2 days
d Different reactions: +, (+), -

TABLE 21

Differentiation of *Proteus morganii* and *Proteus rettgeri* from *Providencia alcalifaciens* and *Providencia stuartii*[1]

Substrate or test	*P. morganii*		*P. rettgeri*		*P. alcalifaciens*		*P. stuartii*	
	Sign	%+ (%+)*	Sign	%+ (%+)*	Sign	%+ (%+)*	Sign	%+ (%+)*
Urease	+	98.2(0.9)	+	100	-	0	-	0
Ornithine decarboxylase	+	97.1	-	0	-	0	-	0
Gas from glucose	d	84.9(0.9)	-or+	12.2	d	85.8(0.6)	-	0
Mannitol	-	0	+or-	88.5	-	2(0.2)	d	13.3(1.3)
Adonitol	-	0	d	80.9(5.6)	+	94.5(0.2)	-	3.8
Inositol	-	0	+	93.3(4.5)	-	0.6	+	97.5(2.5)
Erythritol	-	0	d	78.3(6.5)	-	0	-	0
Esculin	-	0	d	30.4(8.7)	-	0	-	0
Cellobiose	-	0(0.9)	d	3.7(30.4)	-	1.5(3)	d	12.5(68.7)

[1]Adapted from Ewing, Tanner, and Dennard (1954), Ewing, Suassuna and Suassuna (1960), Ewing, et al. (in preparation)

*Figures in parentheses indicate percentage of delayed reactions (3 or more days)

\+ Positive reaction within 1 or 2 days' incubation (90 percent or more)
\- No reaction (90 percent or more)
+or- Majority of strains positive, occasional cultures negative.
-or+ Majority of cultures negative, occasional strains positive.
(+)or+ Majority of reactions dealyed, some occur within 1 or 2 days.
d Different reactions: +, (+), -

APPENDIX

The Nomenclature of the Family Enterobacteriaceae in Outline

Family ENTEROBACTERIACEAE Rahn

Tribe I ESCHERICHIEAE Bergey, Breed, and Murray

Genus I *Escherichia* Castellani and Chalmers
1. *Escherichia coli* (Migula) Castellani and Chalmers

Genus II *Shigella* Castellani and Chalmers
1. *Shigella dysenteriae* (Shiga) Castellani and Chalmers
2. *Shigella flexneri* Castellani and Chalmers
3. *Shigella boydii* Ewing
4. *Shigella sonnei* (Levin) Weldin

Tribe II EDWARDSIELLEAE Ewing and McWhorter

Genus I *Edwardsiella* Ewing and McWhorter
1. *Edwardsiella tarda* Ewing and McWhorter

Tribe III SALMONELLEAE Bergey, Breed, and Murray

Genus I *Salmonella* Lignières
1. *Salmonella cholerae-suis* (Smith) Weldin
2. *Salmonella typhi* (Schroeter) Warren and Scott
3. *Salmonella enteritidis* (Gaertner) Castellani and Chalmers

Genus II *Arizona* Ewing and Fife
1. *Arizona hinshawii* (Ewing and Fife) Ewing

Genus III *Citrobacter* Werkman and Gillen
1. *Citrobacter* freundii (Braak) Werkman and Gillen

Tribe IV KLEBSIELLEAE Trevisan

Genus I *Klebsiella* Trevisan
1. *Klebsiella pneumoniae* (Schroeter) Trevisan
2. *Klebsiella ozaenae* (Abel) Bergey, Breed, and Murray
3. *Klebsiella rhinoschleromatis* Trevisan

APPENDIX (Continued)

Genus II *Enterobacter* Hormaeche and Edwards
1. *Enterobacter cloacae* (Jordan) Hormaeche and Edwards
2. *Enterobacter aerogenes* (Kruse) Hormaeche and Edwards
3. *Enterobacter hafniae* (Moeller) Ewing
4. *Enterobacter liquefaciens* (Grimes and Hennerty) Ewing

Genus III *Pectobacterium* Waldee
1. *Pectobacterium carotovorum* (Jones) Waldee

Genus IV *Serratia* Bizio
1. *Serratia marcescens* Bizio (*Serratia marcescens* subspecies *marcescens*)
   1a. *Serratia marcescens* subspecies *kiliensis* (Lehmann and Neumann) Ewing, et al.

Tribe V PROTEEAE Castellani and Chalmers

Genus I *Proteus* Hauser
1. *Proteus vulgaris* Hauser
2. *Proteus mirabilis* Hauser
3. *Proteus morganii* (Winslow et al.) Rauss
4. *Proteus rettgeri* (Hadley et al.) Rustigian and Stuart

Genus II *Providencia* Ewing
1. *Providencia alcalifaciens* (DeSalles Gomes) Ewing
2. *Providencia stuartii* (Buttiaux et al.) Ewing

N.B. The first species listed in each genus is the type species.