METABOLIC CHARACTERIZATION OF THE GENUS *BRUCELLA*

I. Statistical Evaluation of the Oxidative Rates by Which Type I of Each Species Can Be Identified

MARGARET E. MEYER[1] AND H. S. CAMERON

School of Veterinary Medicine, University of California, Davis, California

Received for publication March 2, 1961

ABSTRACT

MEYER, MARGARET E. (University of California, Davis), AND H. S. CAMERON. Metabolic characterization of the genus *Brucella*. I. Statistical evaluation of the oxidative rates by which type I of each species can be identified. J. Bacteriol. **82**:387-395. 1961.—The oxidative uptake rates on 11 amino acid and seven carbohydrate substrates were determined for 75 strains of brucellae that had been identified by the conventional determinative methods as *Brucella melitensis* type I, *Brucella abortus* type I, or *Brucella suis* type I. By calculating the standard deviation of the oxidative rates, it was demonstrated that a metabolic pattern that is characteristic and definitive for each of the species was formed by their differential oxidative utilization of substrate groups, and that qualitative as well as quantitative metabolic differences exist among the *Brucella* species. *B. melitensis* oxidized L-alanine, L-asparagine, and L-glutamic acid, but not L-arginine, DL-citrulline, L-lysine, DL-ornithine, L-arabinose, D-galactose, D-ribose, or D-xylose. *B. abortus* differed qualitatively from *B. melitensis* in that it oxidized the carbohydrate substrates. *B. suis* differed quantitatively from both of these species in its consistently low oxidative rates of L-alanine, L-asparagine, and L-glutamic acid, and its high rates of utilization of the carbohydrate substrates. It differed qualitatively in that it oxidized the four amino acid substrates that are components of the urea cycle.

The correct identification of organisms classified within the genus *Brucella* is of more than academic interest. Members of this genus induce abortion in domesticated livestock, particularly sheep, goats, cattle, and swine. Each of these livestock species can act as a reservoir of infection for man. Since there is a loose but none the less apparent host preference exhibited by each of the *Brucella* species, it is of epidemiological significance to identify correctly these organisms. It is of importance, therefore, that the impasse among investigators concerning the reliability of the determinative methods and the recognition of the species be resolved (Meyer and Eddie, 1930; Huddleson, 1929, 1931, 1957; Meyer and ZoBell, 1932; Taylor, Lisbonne, and Roman, 1932; Wilson, 1933; Veazie and Meyer, 1936; Kabler and MacLanahan, 1936; Castenada, Tovar, and Velez, 1942; Brim, Morris, and Sunkes, 1950; Hoyer, 1950; Pacheco and Thiago de Mello, 1950; Renoux and Quatrefages, 1951; McCullough and Beal, 1951; Renoux, 1952; Sanders and Warner, 1953; Pickett, Nelson, and Liberman, 1953; Bruni and de Felip, 1954; Pickett and Nelson, 1955; von Sprockhoff and Strauch, 1955; Spink, 1956; Pital, Cooper, and Leise, 1958; Meyer and Cameron, 1959; Stableforth and Galloway, 1959; Renoux, 1960).

There is no hope for resolution of the problems of species identification until the groups of organisms within this genus are defined precisely. The investigations reported herein were undertaken to determine if the oxidative metabolic patterns, observed by Meyer and Cameron (1959) on 27 strains of brucellae, could be used to define quantitatively the species classified within this genus. To ascertain this, the oxidative rates on 18 substrates were first determined on 25 strains of each of the three recognized species, identified as typical *Brucella melitensis*, *Brucella abortus*, and *Brucella suis* by the determinative methods of Huddleson (1929, 1957). The use of manometric techniques as an approach to species delineation will not only provide precise quan-

[1] These papers represent part of a thesis submitted by the senior author to the Graduate Division of the University of California, in partial fulfillment of the requirements of the Ph.D. degree.

titative data for defining a species, but should also detect qualitative differences if such differences do, in fact, exist.

A second objective of this investigation was to subject to critical examination Huddleson's determinative methods of species identification and ascertain if the results of these methods can be correlated with metabolic patterns.

MATERIALS AND METHODS

The cultures of brucellae were obtained from a number of laboratories (*see* "Acknowledgments"). A numerical listing of the cultures and their geographical and host origins are included with the tables of data in the section on results. Each culture has been isolated from a separate host; none of the cultures represents repeated isolations from the same individual.

The organisms were employed only in their smooth phase, as determined by the methods of Braun and Bonestell (1947) and White and Wilson (1951). The growth medium was tryptose agar (Difco) dispensed in Roux flasks, Blake bottles, slants, and plates.

Conventional procedures for ascertaining CO_2 requirements, H_2S production, and the bacteriostatic action of basic fuchsin and thionin on the growth of the organisms were carried out as recommended by the World Health Organization Expert Committee on Brucellosis (1953, 1958). In

TABLE 1. *Strain number, geographic and host origins, and results of conventional determinative methods of identification of 25 strains of Brucella melitensis*

Strain no.*	Geographic origin	Host origin	Dye bacteriostasis						
			Basic fuchsin			Thionin			
			10	15	20	10	15	20	50
			concn in μg/ml media						
Silva	U. S. A.	Human	+	+	+	+	+	T†	−
Green	U. S. A.	Human	+	+	+	+	+	+	−
1025	U. S. A.	Human	+	+	+	+	+	T	−
5938	U. S. A.	Human	+	+	+	+	+	+	−
4103	U. S. A.	Human	+	+	+	+	+	+	−
7-29-2-59	U. S. A.	Human	+	+	+	+	+	T	−
T-52	Turkey	Human	+	+	+	+	+	+	−
T-68	Turkey	Human	+	+	+	+	+	+	−
T-78	Turkey	Human	+	+	+	+	+	+	−
T-83	Turkey	Human	+	+	+	+	+	T	−
B-22-57	Uganda	Human	+	+	+	+	+	T	−
6015	Unknown	Unknown	+	+	+	+	+	T	−
16M		FAO‡/World Health Organization reference strain	+	+	+	+	+	T	−
53-H-38	Mexico	Goat	+	+	+	+	+	T	−
Goat	Malta	Goat	+	+	+	+	+	+	−
10747	Mexico	Unknown	+	+	+	+	+	T	−
10483	Mexico	Unknown	+	+	+	+	+	T	−
57-C-43	Tunis	Goat	+	+	+	+	+	+	−
57-C-44	Tunis	Goat	+	+	+	+	+	+	−
57-C-51	Tunis	Goat	+	+	+	+	+	+	−
57-C-52	Tunis	Goat	+	+	+	+	+	+	−
59-C-56	Tunis	Goat	+	+	+	+	+	+	−
59-C-57	Italy	Goat	+	+	+	+	+	T	−
59-C-58	Italy	Goat	+	+	+	+	+	+	−
59-C-59	Italy	Goat	+	+	+	+	+	T	−

* None of these strains required CO_2 for initial isolation. The maximal amount of H_2S produced by all strains was a trace amount on the 4th day.

† T = trace.

‡ FAO = Food and Agriculture Organization (United Nations).

the results, the dye concentrations are expressed as micrograms of dye per ml of media. The medium used for determining dye sensitivity and H_2S production was tryptose agar (Difco).

The nomenclature and classification of species and types used in this paper are as described by Huddleson (1957).

Resting cell suspensions for manometric determinations were prepared as described previously by Cameron and Meyer (1953, 1955). The substrates were dissolved in Sorenson's 0.06 M phosphate buffer and, when necessary, the pH of the solution was adjusted to 7.0 by the addition of sodium hydroxide. Conventional manometric techniques were employed to determine oxygen uptake (Umbreit, Burris, and Stauffer, 1957). Each flask contained cell suspension, 1.0 ml; solution containing 5 mg of the desired substrate, 0.5 ml; buffer, 1.4 ml; and 20% KOH, 0.1 ml. Endogenous respiration rates were determined for each experiment. The figures given in "Results and Discussion" are (QO_2N) values with the endogenous rates subtracted.

RESULTS AND DISCUSSION

Tables 1, 2, and 3 show 75 strains of brucellae that have been classified into the three recognized

TABLE 2. *Strain number, geographic and host origins, and results of conventional determinative methods of identification of 25 strains of Brucella abortus type I*

Strain no.*	Geographic origin	Host origin	Dye bacteriostasis						
			Basic fuchsin			Thionin			
			10	15	20	5	10	15	20
			concn in μg/ml media						
2308	U. S. A.	Bovine	+	+	+	+	T†	−	−
19	U. S. A.	Bovine	+	+	+	+	T	−	−
544	England	FAO‡/World Health Organization reference strain	+	+	+	+	−	−	−
53	U. S. A.	Bovine	+	+	+	+	T	−	−
1706	U. S. A.	Bovine	+	+	+	+	+	−	−
1628	U. S. A.	Bovine	+	+	+	+	T	−	−
8093	U. S. A.	Man	+	+	+	+	−	−	−
138	U. S. A.	Bovine	+	+	+	+	−	−	−
986	U. S. A.	Bovine	+	+	−	+	−	−	−
987	U. S. A.	Bovine	+	+	+	+	+	−	−
T-69	Turkey	Bovine	+	+	+	+	+	−	−
C-39	Argentina	Bovine	+	+	+	+	T	−	−
C-40	Argentina	Bovine	+	+	+	+	−	−	−
C-41	Argentina	Bovine	+	+	+	+	−	−	−
C-43	Argentina	Bovine	+	+	+	+	−	−	−
C-44	Argentina	Bovine	+	+	+	+	−	−	−
C-45	Argentina	Bovine	+	+	+	T	−	−	−
C-46	Argentina	Bovine	+	+	+	+	T	−	−
C-47	Argentina	Bovine	+	+	+	+	−	−	−
Bull 7*	Unknown	Bovine	+	+	+	+	−	−	−
R-1442*	U. S. A.	Bovine	+	+	+	+	T	−	−
R-1461*	U. S. A.	Bovine	+	+	+	+	T	−	−
F-1401	U. S. A.	Bovine	+	+	+	+	T	−	−
B-539	Teheran	Bovine	+	+	+	+	+	−	−
B-519	Teheran	Bovine	+	+	+	+	+	−	−

* All of these strains required CO_2 for initial isolation. Strains marked with the asterisk required CO_2 for continued subculture. The remaining strains were non-CO_2-requiring when used for this study. Strains 2308 and 53 produced H_2S for 2 days; all other strains for 4 days.

† T = trace.

‡ FAO = Food and Agriculture Organization (United Nations).

TABLE 3. *Strain number, geographic and host origins, and results of conventional determinative methods of identification of 25 strains of Brucella suis type I*

Strain no.*	Geographic origin	Host origin	Dye bacteriostasis					
			Basic fuchsin				Thionin	
			1	5	10	15	20	50
			conc in μg/ml media					
148A	U. S. A.	Human	+	+	T†	−	+	+
Kearney	U. S. A.	Human	+	+	T	−	+	+
Jeff	U. S. A.	Human	+	+	T	−	+	+
Lutz	U. S. A.	Human	+	+	T	−	+	+
2785	U. S. A.	Human	+	+	−	−	+	+
4824	U. S. A.	Human	+	−	−	−	+	+
187-1-60	U. S. A.	Human	+	+	−	−	+	+
1103	U. S. A.	Human	+	+	T	−	+	+
4532	U. S. A.	Human	+	+	−	−	+	+
1246-2-60	U. S. A.	Human	+	+	+	−	+	+
55	U. S. A.	Porcine	+	+	+	−	+	+
1330		FAO‡/World Health Organization reference strain	+	T	−	−	+	+
2B	U. S. A.	Bovine	+	+	T	−		
301	U. S. A.	Bovine	+	+	T	−	+	+
1754	U. S. A.	Porcine	+	+	−	−	+	+
362	Argentina	Porcine	+	+	−	−	+	+
233	Argentina	Porcine	+	+	T	−	+	+
1030	Argentina	Porcine	+	+	T	−	+	+
1025	Argentina	Porcine	+	+	−	−	+	+
1026	Argentina	Porcine	+	+	−	−	+	+
1823	Unknown	Unknown	+	+	−	−	+	+
1820	Unknown	Unknown	+	+	−	−	+	+
1776	Unknown	Unknown	+	+	−	−	+	+
1744	Unknown	Unknown	+	+	T	−	+	+
1778	Unknown	Unknown	+	+	−	−	+	+

* None of these strains required CO_2 for initial isolation and all strains produced H_2S for 4 days.
† T = trace.
‡ FAO = Food and Agriculture Organization (United Nations).

species by ascertaining their identity according to the conventional determinative methods. These 75 strains could be identified with certainty, as each strain conformed to its species description on each of the tests, and are classified as type I within their respective species (Huddleson, 1957).

As can be seen by comparing Tables 1, 2, and 3, when the brucellae organisms are plated out on graded concentrations of both dyes, the dye tolerances among the species are more readily observable than when only the recommended concentration of 10 μg per ml of media is employed.

B. melitensis and *B. abortus* both grow abundantly at 20 μg per ml of basic fuchsin, whereas not a single strain of *B. suis* grew at 15 μg per ml. On thionin, the differences were even greater. All strains of *B. suis* grew in the presence of 50 μg of thionin per ml of media. The highest concentration on which all strains of *B. abortus* would grow was 5 μg per ml, and the highest concentration on which all strains of *B. melitensis* would grow was 15 μg per ml.

The oxidative rates on nine amino acid and four carbohydrate substrates are presented for 25 strains of *B. melitensis* type I in Table 4, for 25 strains of *B. abortus* type I in Table 5, and for 25 strains of *B. suis* type I in Table 6.

In addition to the substrates shown in Tables 4, 5, and 6, oxidative rates also were determined on D-asparagine, L-proline, adonitol, and fructose.

TABLE 4. *Oxidative rates (QO_2N) of 25 strains of Brucella melitensis on nine amino acid and four carbohydrate substrates*

Strain no.	Substrates												
	Amino acids									Carbohydrates			
	D-Alanine	L-Alanine	L-Arginine	L-Asparagine	L-Aspartic acid	DL-Citrulline	L-Glutamic acid	L-Lysine	DL-Ornithine	L-Arabinose	D-Galactose	D-Ribose	D-Xylose
Silva	77	76	15	89	124	19	210	18	30	0	0	48	0
Green	398	151	0	90	237	30	305	20	10	20	20	31	6
1025	74	61	9	90	21	12	104	20	30	11	0	2	0
5938	169	100	0	60	113	0	101	4	0	7	0	13	0
4103	166	120	10	166	116	0	310	10	0	0	12	19	45
7-29-2-59	91	123	0	141	25	12	204	10	15	7	14	24	5
T-52	140	84	5	118	81	14	293	0	46	0	22	0	0
T-68	125	98	20	100	160	16	190	16	23	17	0	4	4
T-78	130	72	0	125	48	8	160	0	12	0	42	36	48
T-83	200	150	8	107	100	0	163	14	6	15	20	0	23
B-22-57	390	192	27	151	43	25	322	27	39	44	38	16	74
6015	111	100	0	150	14	14	188	10	18	1	6	31	12
16M	250	198	8	222	122	4	412	10	19	0	0	30	11
53-H-38	124	104	4	110	24	11	177	2	16	10	17	34	24
Goat	116	69	9	133	50	24	265	11	3	40	21	45	29
10747	130	101	16	98	45	14	168	10	24	0	6	10	20
10483	103	90	6	90	30	10	115	8	10	23	18	7	41
57-C-43	117	107	0	163	40	4	450	4	2	33	29	41	0
57-C-44	120	140	0	160	34	9	203	5	8	17	20	34	7
57-C-51	276	200	12	90	30	1	234	11	18	19	17	52	23
57-C-52	370	216	10	88	29	6	218	10	21	17	8	10	0
59-C-56	136	132	25	130	68	27	209	20	42	35	37	37	45
59-C-57	179	150	40	140	50	11	266	27	16	10	23	8	30
59-C-58	154	141	27	157	22	40	194	50	46	30	20	16	53
59-C-59	144	168	12	167	55	30	401	49	39	16	15	0	9

These substrates are not useful for purposes of speciation as there was no consistent pattern of utilization displayed by any of the species. Glucose also was used as a substrate. Since it was oxidized at comparable rates by all strains of each species, the rates on this substrate are of no value for defining a species.

From Tables 4, 5, and 6 it can be observed, however, that, on nine amino acid and four carbohydrate substrates, each strain within a species displayed a quantitatively similar oxidative rate on substrates that can be divided into three distinct groups: D-alanine, L-alanine, L-asparagine, L-glutamic acid, and L-aspartic acid, which are intermediates in transamination; L-arginine, DL-citrulline, L-lysine, and DL-ornithine, which are components of the urea cycle; and the carbohydrates, L-arabinose, D-galactose, D-ribose, and D-xylose.

In order to establish whether the observed rate differences on these substrate groups represented significant differences among the species, the standard deviation of the oxidative rate was calculated for the nine amino acid and four carbohydrate substrates on which the three species displayed differential rates of utilization. These data are presented in Table 7.

From Table 7 it can be seen that, except on the substrate of D-alanine, each species oxidized each substrate within a rate range that can be statistically delineated, and these ranges were significantly different for each species on the three

TABLE 5. *Oxidative rates (QO_2N) of 25 strains of Brucella abortus type I on nine amino acid and four carbohydrate substrates*

Strain no.	Substrates												
	Amino acids									Carbohydrates			
	D-Alanine	L-Alanine	L-Arginine	L-Asparagine	L-Aspartic acid	DL-Citrulline	L-Glutamic acid	L-Lysine	DL-Ornithine	L-Arabinose	D-Galactose	D-Ribose	D-Xylose
2308	180	172	18	182	60	30	296	0	30	56	112	285	33
19	83	72	40	198	110	50	490	6	40	45	50	158	22
544	147	113	17	151	90	40	290	22	50	189	175	348	93
53	113	86	18	120	70	0	420	0	0	122	156	263	21
1760	163	85	20	152	98	36	260	14	39	58	101	103	33
1628	169	70	38	110	79	43	400	14	36	50	114	200	25
8093	136	65	40	153	13	40	220	19	36	111	162	349	78
138	169	170	31	145	11	25	236	14	34	113	152	295	16
986	172	141	24	80	21	30	202	8	18	114	230	326	61
987	158	130	35	150	8	26	290	13	35	140	217	326	10
T-69	108	95	16	98	0	12	140	11	17	108	211	198	39
C-39	96	113	28	143	22	10	165	17	38	119	219	272	40
C-40	101	70	25	101	24	25	140	25	23	94	124	269	31
C-41	126	99	28	100	6	18	242	6	25	92	200	286	29
C-43	130	134	40	140	31	40	197	25	50	77	148	265	27
C-44	110	86	21	141	22	17	225	16	22	83	135	295	22
C-45	111	117	32	107	14	20	158	16	32	108	212	297	27
C-46	139	92	17	95	7	10	251	0	24	86	208	331	50
C-47	97	102	22	160	21	8	190	23	22	105	163	280	28
Bull 7	139	86	48	158	17	33	226	9	33	45	116	296	47
R-1442	100	85	14	90	20	18	201	16	44	60	180	290	36
R-1461	161	90	15	110	65	16	216	10	36	72	168	201	26
F-1401	113	78	27	111	25	20	189	16	25	48	95	268	27
B-539	153	90	32	136	10	14	234	16	36	103	124	253	46
B-519	159	92	19	86	13	16	241	10	33	81	78	203	34

substrate groups. Thus, a metabolic pattern characteristic and definitive for each species was formed by its differential oxidative utilization of the substrate groups.

B. melitensis oxidatively utilized L-alanine, L-asparagine, and L-glutamic acid. It did not oxidize L-arginine, DL-citrulline, L-lysine, DL-ornithine, L-arabinose, D-galactose, D-ribose, or D-xylose.

B. abortus oxidized L-alanine, L-asparagine, and L-glutamic acid, but did not utilize L-arginine, DL-citrulline, L-lysine, or DL-ornithine. It differed qualitatively from *B. melitensis* in that it oxidized L-arabinose, D-galactose, and D-ribose.

B. suis differed significantly from *B. abortus* and *B. melitensis* in its consistently low rates of oxidation of L-alanine, L-asparagine, and L-glutamic acid. *B. suis* differed qualitatively from the other two species in that it oxidized the four amino acid substrates of the urea cycle. *B. suis* also consistently displayed the highest rates of utilization on all of the substrates in the carbohydrate group.

ACKNOWLEDGMENTS

The cooperation of the following individuals, all of whom generously donated cultures for use in this study, is gratefully acknowledged: David Berman, Gordon Janney, Lois M. Jones, and J. B. Wilson, University of Wisconsin; Alcor Browne, California State Department of Public Health; M. R. Castenada, Mexico; Victorio C. F.

TABLE 6. *Oxidative rates (QO_2N) of 25 strains of Brucella suis type I on nine amino acid and four carbohydrate substrates*

Strain no.	Substrates												
	Amino acids									Carbohydrates			
	D-Alanine	L-Alanine	L-Arginine	L-Asparagine	L-Aspartic acid	DL-Citrulline	L-Glutamic acid	L-Lysine	DL-Ornithine	L-Arabinose	D-Galactose	D-Ribose	D-Xylose
148A	64	50	60	15	6	65	41	50	90	257	136	294	80
Kearney	49	26	40	0	10	60	56	43	75	370	239	420	121
Jeff	91	38	107	9	0	115	60	87	158	317	202	288	170
Lutz	81	21	106	0	6	137	34	150	230	384	317	370	195
2785	48	33	95	14	16	175	60	155	221	167	101	296	83
4824	110	101	116	20	15	189	48	180	217	190	138	200	90
187-1-60	115	25	102	0	0	152	41	117	173	214	158	419	151
1103	100	29	90	2	0	60	36	71	158	268	187	297	164
4532	67	40	67	23	0	95	14	94	156	304	158	322	157
1246-2-60	170	41	71	7	4	104	13	119	200	178	369	351	127
55	137	57	70	10	20	118	52	115	163	402	326	343	213
1330	165	30	50	8	4	99	17	66	123	210	115	342	122
2B	78	21	60	0	0	113	20	62	120	497	494	400	293
301	95	56	70	24	27	114	52	98	168	273	147	388	162
1754	92	20	68	14	6	139	37	84	146	274	156	283	270
362	74	41	86	10	0	148	31	109	151	441	442	410	196
233	64	40	57	0	0	83	20	79	100	290	121	300	158
1030	88	26	89	10	10	134	6	113	137	224	168	334	157
1025	98	54	97	30	15	137	32	120	210	300	190	390	158
1026	146	44	73	6	5	120	18	119	133	378	266	412	236
1823	83	21	51	13	9	124	14	108	155	323	201	297	164
1820	157	35	55	19	18	125	14	78	122	331	155	466	170
1776	101	56	112	26	33	219	58	145	264	560	526	509	500
1744	71	38	124	16	15	114	53	141	193	416	340	428	297
1778	90	36	91	19	19	154	48	90	180	441	195	446	270

TABLE 7. *Comparison of expected range of oxidative rates (value of $M - 2\sigma$ and $M + 2\sigma$) with observed range of oxidative rates on nine amino acid and four carbohydrate substrates for type I of each species of Brucella*

Substrate groups	*B. melitensis* type *I*			*B. abortus* type *I*			*B. suis* type *I*		
	Expected range*	Observed range	Mean	Expected range	Observed range	Mean	Expected range	Observed range	Mean
D-Alanine	0–358	74–398	171	76–190	83–180	133	37–162	49–170	97
L-Alanine	38–214	61–216	126	53–149	65–172	101	5–73	21–101	39
L-Asparagine	51–199	60–222	125	69–189	80–198	129	0–30	0–30	12
L-Glutamic acid	48–420	101–450	234	74–414	140–420	244	0–71	6–60	35
L-Aspartic acid	0–173	21–237	68	0–98	0–110	34	0–28	0–33	10
L-Arginine	0–31	0–40	10	7–45	15–48	26	34–126	40–124	80
DL-Citrulline	0–34	0–40	14	0–48	0–50	24	48–200	60–219	124
L-Lysine	0–39	0–50	15	0–37	0–25	13	50–166	43–150	108
DL-Ornithine	0–48	0–46	20	9–53	0–50	31	60–264	75–264	162
L-Arabinose	0–41	0–44	15	30–164	45–189	97	116–524	167–560	320
D-Galactose	0–40	0–42	16	95–291	50–230	193	0–475	101–526	232
D-Ribose	0–54	0–52	22	148–384	103–349	266	219–501	200–509	360
D-Xylose	0–62	0–53	20	29–43	10–93	36	72–306	80–500	189

* The confidence limit of 2σ is 95%.

Cedro, Argentina; S. Bilal Golem, Turkey; I. F. Huddleson, Michigan State College, and Gerard Renoux, Tunis.

This study was supported in part by a grant (E-2463) from the National Institutes of Health.

LITERATURE CITED

BRAUN, W., AND A. E. BONESTELL. 1947. Independent variation of characteristics in *Brucella abortus* variants and their detection. Am. J. Vet. Research **8**:386–390.

BRIM, A., J. F. MORRIS, AND E. J. SUNKES. 1950. Methods of isolation and incidence of *Brucella* types found in Georgia. J. Lab. Clin. Med. **35**:483–487.

BRUNI, A., AND G. DE FELIP. 1954. Sulla differenziazone delle specie del genere *Brucella* con la prova di Renoux. Boll. soc. ital. biol. sper. **30**:793–795.

CAMERON, H. S., AND M. E. MEYER. 1953. Comparative metabolic studies on the genus *Brucella*. II. Metabolism of amino acids that occur in the urea cycle. J. Bacteriol. **67**:34–37.

CAMERON, H. S., AND M. E. MEYER. 1955. Synthesis of amino acids from urea by the genus *Brucella*. Am. J. Vet. Research **16**:149–151.

CASTENADA, M. R., R. TOVAR, AND R. VELEZ. 1942. Studies on brucellosis in Mexico. Comparative study of various diagnostic tests and classification of the isolated bacteria. J. Infectious Diseases **70**:97–102.

HOYER, B. H. 1950. Some aspects of the physiology of *Brucella* organisms. *In* Brucellosis. A Symposium. American Association for the Advancement of Science, Washington, D. C.

HUDDLESON, I. F. 1929. The differentiation of the species in the genus *Brucella*. Mich. State Univ. Agr. Expt. Sta. Tech. Bull. 100.

HUDDLESON, I. F. 1931. Differentiation of the species of the genus *Brucella*. Am. J. Public Health **21**:491–498.

HUDDLESON, I. F. 1957. *In* R. S. BREED, E. G. D. MURRAY, AND N. R. SMITH. Bergey's manual of determinative bacteriology, p. 404–406, 7th ed. Williams & Wilkins Company, Baltimore.

KABLER, P., AND M. MACLANAHAN. 1936. A differential study of forty *Brucella* strains isolated in Minnesota. J. Infectious Diseases **58**:293–298.

MCCULLOUGH, N. B., AND G. A. BEAL. 1951. Growth and manometric studies on carbohydrate utilization of *Brucella*. J. Infectious Diseases **89**:266–271.

MEYER, K. F., AND B. EDDIE. 1930. Notes on the bacteriology of the *Brucella* group. J. Lab. Clin. Med. **15**:447–456.

MEYER, K. F., AND C. E. ZOBELL. 1932. Metabolism studies on the *Brucella* group. IV. The bacteriostatic action of dyes. J. Infectious Diseases **51**:72–90.

MEYER, M. E., AND H. S. CAMERON. 1959. Comparative metabolism of species and types of organisms within the genus *Brucella*. J. Bacteriol. **78**:130–136.

PACHECO, G., AND M. THIAGO DE MELLO. 1950. A urease test for the differentiation of *Brucella suis*. J. Bacteriol. **59**:689–691.

PICKETT, M. J., AND E. L. NELSON. 1955. Speciation in the genus *Brucella*. IV. Fermentation of carbohydrates. J. Bacteriol. **69**:333–336.

PICKETT, M. J., E. L. NELSON, AND J. D. LIBERMAN. 1953. Speciation within the genus *Brucella*. II. Evaluation of differential dye, biochemical, and serological tests. J. Bacteriol. **66**:210–219.

PITAL, A., R. E. COOPER, AND J. M. LEISE. 1958. Rapid method for determining carbohydrate utilization by brucellae. J. Bacteriol. **75**:422–425.

RENOUX, G. 1952. Une nouvelle methode de differenciation des varietes de *Brucella*. Action du diethyldithiocarbamate de souche. Ann. inst. Pasteur **82**:556–562.

RENOUX, G. 1960. Nouvelles epreuves bacteriostatiques pour differencier les *Brucella*. Resultats et consequences. Arch. inst. Pasteur Tunis **37**:23–25.

RENOUX, G., AND H. QUATREFAGES. 1951. L'Identification des *Brucella* par leur activite ureasique. Comparison avec autres methods de differenciation. Ann. inst. Pasteur **80**:182–188.

SANDERS, E., AND J. WARNER. 1953. Urease and catalase activities of *Brucella melitensis* from different geographical regions. Am. J. Vet. Research **14**:388–391.

SPINK, W. W. 1956. The nature of brucellosis. The University of Minnesota Press, Minneapolis.

STABLEFORTH, A. W., AND I. A. GALLOWAY. 1959. Diseases due to bacteria, p. 53–159. *In* Infectious diseases of animals. v. 1. Academic Press, Inc., New York.

TAYLOR, R. M., M. LISBONNE, AND G. ROMAN. 1932. Recherches sur l'identification des *Brucella* isolees en France. Par l'action bacteriostatique des matieres colorantes et la production d'hydrogene sulfure (Huddleson). Ann. inst. Pasteur **49**:284–302.

UMBREIT, W. W., R. H. BURRIS, AND J. F. STAUFFER. 1957. Manometric techniques and related methods for the study of tissue metabolism. Burgess Publishing Company, Minneapolis.

VEAZIE, L., AND K. F. MEYER. 1936. The serologic

classification of the *Brucella* group. J. Infectious Diseases **58**:280–292.

VON SPROCKHOFF, H., AND D. STRAUCH. 1955. Untersuchungen von Brucellastammen auf ihren Gehalt an *Abortus* and *Melitensis* antigen. Zentr. Veterinärmed. **2**:66–75.

WHITE, P. G., AND J. B. WILSON. 1951. Differentiation of smooth and nonsmooth colonies of *Brucella*. J. Bacteriol. **61**:239–240.

WILSON, G. S. 1933. The classification of the *Brucella* group: a systematic study. J. Hyg. **33**:516–541.

World Health Organization. 1953. Joint Food and Agricultural Organization (United Nations) World Health Organization Expert Committee on Brucellosis. Second Report Food and Agricultural Organization (United Nations) Agricultural Studies, no. **24**:22–26.

World Health Organization. 1958. Joint Food and Agricultural Organization (United Nations) World Health Organization Expert Committee on Brucellosis. Third Report Food and Agricultural Organization (United Nations) Agricultural Studies, no. **45**:24–26.