METABOLIC STUDIES ON *BRUCELLA NEOTOMAE* (STOENNER AND LACKMAN)

H. S. CAMERON AND MARGARET E. MEYER

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

Received for publication June 16, 1958

Stoenner and Lackman (1957) isolated an organism from the wood rat *Neotoma lepida* and, on the basis of its behavior on differential dye media, CO_2 requirements, and H_2S production, regarded it as a new species of *Brucella*, for which they proposed the name *Brucella neotomae*. Although recognition of a new species may be justified on the basis of these conventional techniques, it was considered advisable to investigate the oxidative metabolic pattern of the proposed new species on substrates of amino acids, carbohydrates, and intermediates in the Kreb's cycle. Meyer and Cameron (1958) have reported that each of the three recognized species display metabolic patterns that would aid in defining the species. Cultures of the proposed new species were obtained from Dr. Stoenner and subjected to manometric studies to determine if the oxidative pattern differed significantly from those exhibited by the three recognized species.

MATERIALS AND METHODS

Seven strains of *B. neotomae* were examined concurrently with the following typical species: *B. abortus* strain 2308, a standard virulent strain; *B. suis* strain 148A and *B. melitensis* strain S90, both of which were isolated from human cases of brucellosis by the California State Department of Public Health Laboratory. Cultures were smooth, as determined by the methods of Braun and Bonestell (1947) and White and Wilson (1951).

Tryptose agar (Difco) dispensed in Roux flasks, slants, and plates was used as the growth medium throughout the investigation. Conventional procedures for differentiating species were as recommended by Huddleson (1957). To obtain resting cells for manometry, Roux flasks were inoculated with 2.5 ml of a saline suspension of the desired culture, incubated 24 hr at 37.5 C, and the resultant growth harvested, washed, and suspended in Sorenson's 0.066 M phosphate buffer at pH 7.0. Cell concentrations were adjusted on a spectrophotometer and cellular nitrogen determined as described earlier by Cameron and Meyer (1953, 1955).

The substrates were dissolved in Sorenson's 0.066 M phosphate buffer and, where necessary, the pH of the solution adjusted to 7.0 by the addition of sodium hydroxide. Conventional manometric technique was employed to determine oxygen uptake (Umbreit *et al.* 1945). Each flask contained 1.0 ml cells, 0.5 ml solution containing 5 mg of the desired substrate, 1.4 ml buffer, and 0.1 ml alkali. Endogenous respiration rates were determined for each experiment and all experiments were repeated on several harvestings of cells from various lots of media. The figures given in the results are $Q_{O_2}(N)$ values with the endogenous rates subtracted.

RESULTS

The H_2S production and the effect of basic fuchsin and thionin on the growth of seven strains of the proposed new species as compared to a representative strain of each of the recognized species is shown on table 1. No growth of the atypical species was observed on either dye; the H_2S production resembled that associated with the growth of *B. suis*.

Table 2 shows the oxidative metabolism of typical strains of the three species on amino acid and carbohydrate substrates as compared to the unclassified organism. Included in the table are only those substrates which contributed to a pattern differing significantly from that observed with recognized species. The following substrates were also used: D- and L-alanine, L-lysine, and L-proline, fructose, glucose, D-ribose, and D-xylose. All exhibited activity usually associated with the genus but in a pattern that would not serve to differentiate a species. The oxidative pattern on substrates of intermediates in the Krebs' cycle was similar to that observed in the three species.

As a result of inoculating dye plates heavily,

TABLE 1

Dye bacteriostasis and hydrogen sulfide production of Brucella neotomae compared with typical strains of the three species of Brucella

Species and Strains of *Brucella*	Dye Conc						H_2S Production
	Basic fuchsin			Thionin			
	10*	20	40	10	20	40	
B. neotomae strain 5K33	−	−	−	−	−	−	++++
B. neotomae strain 6D152	−	−	−	−	−	−	++++
B. neotomae strain 5E1169	−	−	−	−	−	−	++++
B. neotomae strain 5G239	−	−	−	−	−	−	++++
B. neotomae strain 5E1266	−	−	−	−	−	−	++++
B. neotomae strain 6H8488	−	−	−	−	−	−	++++
B. neotomae strain 7E164	−	−	−	−	−	−	++++
B. abortus strain 2308	+	+	+	−	−	−	++
B. suis strain 148A	−	−	−	+	+	+	++++
B. melitensis strain Silva	+	+	−	+	+	−	−

* Micrograms dye per ml of media. − = bacteriostasis; + = growth.

TABLE 2

Comparative oxidative rates ($Q_{O_2}(N)$) on amino acid and carbohydrate substrates by species in the genus Brucella

Substrates	*B. abortus* strain 2308	*B. suis* strain 148A	*B. melitensis* strain S90	*B. neotomae* strain:						
				5K 33	7E 164	6H 8488	5E 1266	5G 239	5E 1169	6D 152
L-Arginine	18	106	0	0	0	5	0	4	10	8
D-Asparagine	32	0	70	86	94	101	84	78	83	78
L-Asparagine	182	20	85	91	92	98	84	116	70	90
L-Aspartic acid	60	20	160	70	10	10	40	13	60	25
DL-Citrulline	30	140	30	0	0	0	0	7	11	8
L-Glutamic acid	300	50	200	469	206	170	255	180	205	203
DL-Ornithine	30	200	20	0	0	8	0	0	20	20
Adonitol	46	40	45	112	339	152	192	113	162	147
L-Arabinose	55	357	0	363	286	194	276	218	267	252
D-Galactose	112	239	0	365	360	274	379	371	398	387

Endogenous rates subtracted.

colonies were obtained that would grow when transferred to the respective dye medium. Such colonies, although growing on either basic fuchsin or thionin, exhibited no difference in their metabolic pattern from the original strain that failed to grow on either dye media.

DISCUSSION

Stoenner and Lackman (1957) have shown that actively proliferating cells of *B. neotomae* display growth and biochemical characteristics which distinguish these organisms from the three recognized species of *Brucella* and all subclassifications (types) within the species (Huddleson 1957). The bacteriostatic effect of the dyes upon *B. neotomae* is identical to that of *B. abortus*, type II. However, all three types of *B. abortus* require increased carbon dioxide tension for initial isolation. According to Huddleson (1957), this characteristic, rather than the dyes, apparently is sufficient to identify the species since growth on dyes is different in each type. Because it does not depend on CO_2 for initial isolation, the new organism would not appear to belong in the species *B. abortus*. *B. neotomae* does not share common characteristics with *B. melitensis*.

The former is vigorous in its production of hydrogen sulfide, and since it behaves the opposite of *B. melitensis* on basic fuchsin and thionin, it is decidedly not in this species. On the basis of the above characteristics, classification into the *B. suis* species could be arguable. The outstanding characteristic it shares with *B. suis* is the excellence of its hydrogen sulfide production. However, none of three types of *B. suis* share its inability to grow in the presence of thionin.

The oxidative metabolic pattern of *B. neotomae* also distinguishes this organism from the other species. Although the biochemical features of proliferating cells, such as dye tolerance and H_2S production, are more closely similar to *B. suis*, the metabolic pattern on amino acid substrates clearly separates it from this species. The pattern of *B. suis* is dissimilar to both *B. abortus* and *B. melitensis* and distinctively defines this species. *B. suis* will oxidize L-arginine, DL-citrulline, DL-ornithine, and L-lysine. It will not oxidize D-asparagine, and shows consistently low oxidative rates on L-asparagine, L-aspartic and L-glutamic acids, and L-proline. These data are in agreement with those previously reported by Meyer and Cameron (1958). *B. neotomae*, on the basis of amino acid metabolism, is apparently not a strain of *B. suis* that displays atypical sensitivity to thionin. In its amino acid metabolism it resembles *B. melitensis* only in utilizing the D- and L-isomers of asparagine at comparable rates. It differs, however, from that species in the oxidation of L-aspartic acid.

In carbohydrate metabolism significant differences also were observed. These were apparent on adonitol, where the rates were considerably higher in *B. neotomae* than in any of the other species; on L-arabinose where *B. suis* and *B. neotomae* rates were similar; on D-galactose where again there was a strong similarity between those same organisms. In carbohydrate metabolism the proposed species resembles *B. suis*. On the other hand, in amino acid metabolism it is distinctly different from that species. Numerous reports are available on "atypical" strains of *Brucella*, based chiefly on H_2S production, CO_2 dependency, and dye tolerance. Variations, through temporary modifications or mutation and selection may occur in the latter characteristic. From the results reported herein, a change in dye tolerance does not affect the oxidative metabolic pattern, which apparently is a relatively stable characteristic.

Whether or not a new species is warranted in the case of the organism isolated from the wood rat may be debatable. It can be said, however, that it differs significantly in oxidative metabolism from the three typical species of *Brucella* and there would seem to be considerable justification for naming it a separate species.

SUMMARY

The species *Brucella neotomae* was isolated from the wood rat and identified as a new species by Stoenner and Lackman on the basis of conventional methods for speciating the genus *Brucella*. These observations were substantiated by manometric techniques in that the oxidative metabolic pattern of the proposed new species is not identical to the patterns of any of the three recognized species.

REFERENCES

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

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

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

MEYER, M. E. AND CAMERON, H. S. 1958 Species metabolic patterns in the genus *Brucella*. Am. J. Vet. Research, **19**, 754–758.

STOENNER, H. G. AND LACKMAN, D. B. 1957 A new species of *Brucella* isolated from the desert wood rat, *Neotoma lepida* Thomas. Am. J. Vet. Research, **18**, 947–951.

UMBREIT, W. W., BURRIS, R. H., AND STAUFFER, J. F. 1945. *Manometric techniques and related methods for the study of tissue metabolism.* Burgess Publishing Co., Minneapolis.

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