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Metabolic Characterization of *Brucella* Strains that Show Conflicting Identity by Biochemical and Serological Methods*

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Each of 87 strains of brucellae examined for its utilization of amino acid and carbohydrate substrates displayed a metabolic pattern that characterized it as to its species identity, irrespective of its serological and biochemical characters. Strains that displayed the metabolic pattern of Br. abortus *were lysed by* Brucella *bacteriophage type* abortus *strain 3. Strains that displayed the metabolic pattern of* Br. melitensis *were not lysed by this phage.*

On this basis of identification, it is shown that there occur strains of Br. abortus *that do not produce hydrogen sulfide, do not require carbon dioxide and are thionin-resistant, thereby duplicating the features generally regarded as characteristic of* Br. melitensis. *They can be identified by their metabolism and phage susceptibility. The distribution of* Br. abortus *and* Br. melitensis *antigens, as measured by agglutination with monospecific antisera, is also not always related to other species-identifying characteristics. Therefore, neither the serological method nor the biochemical method can be considered a reliable guide to the identification of* Brucella *species.*

Brucella organisms are ordinarily identified as members of a species by the conventional biochemical methods of Huddleson (1929) and, to a lesser extent, by the serological test of agglutination with monospecific antisera (Wilson & Miles, 1932). Investigators who have attempted to correlate the serological identity of organisms with the results of typing by the biochemical method have reached contradictory conclusions. Veazie & Meyer (1936), after comparing the results of the two methods in the typing of over 400 strains, concluded that serological identification was an indispensable aid to proper classification. Wilson (1933), Castañeda et al. (1942), and Brim et al. (1950) have also reported favourably upon the use of agglutinin absorption for typing purposes.

Conversely, Kabler & MacLanahan (1936) reported that 9 of 41 strains failed to fit into any of the species and concluded that this test was of no value for identification purposes. Spink (1956) stated that he had abandoned the use of serological procedures because of difficulties encountered in the preparation of satisfactory antisera, although others (Jones, 1958) have not had this difficulty.

Other investigators, rather than ignore the results of one or the other procedures, report identity according to both sets of tests. As a consequence, there are numerous references in the literature to *Brucella* strains that actually are not classified as to species, but are described in terms of their biochemical and serological behaviour. Organisms that have not been classified specifically are referred to as *Br. melitensis* biochemically, *Br. abortus* serologically (Taylor et al., 1932; Wilson, 1933; Veazie & Meyer, 1936; Renoux, 1952; Cruickshank, 1954; Pickett & Nelson, 1955); *Br. melitensis* biochemically, but with more *Br. abortus* antigen than usual (Stableforth, 1959); *Br. melitensis* biochemically, but with equal amounts of *Br. melitensis* and *Br. abortus* antigens (Gargani et al., 1957); *Br. melitensis* that requires carbon dioxide (Stableforth, 1959); and *Br. abortus* biochemically, *Br. melitensis* serologic-

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ally (Wilson, 1933; Cruickshank, 1954; Pickett & Nelson, 1955).

Prior to the introduction of the manometric method of species identification, there was no method of establishing the comparative value of the conventional biochemical techniques and of the serological test for identification of these organisms. By the use of manometric methods, Meyer & Cameron (1961a) have recently presented evidence that it is possible to identify correctly the organisms in the genus *Brucella* according to the oxidative metabolic patterns formed by their utilization of amino acid and carbohydrate substrates. These patterns were found to be consistent and definitive for each species, inclusive of their biotypes (Meyer & Cameron, 1961b) and of variants that displayed biochemical features anomalous of a species (Meyer, 1961a). Additionally, it was found (Meyer, 1961b) that all strains that were classified metabolically as *Br. abortus* were susceptible to *Brucella* bacteriophage, type *abortus*, strain 3, and that all strains that displayed a metabolic pattern characteristic of another species were not lysed by this phage at the routine test dilution.

To determine whether the distribution of *Br. abortus* and *Br. melitensis* antigens is, in fact, related to other species-identifying characteristics, the oxidative metabolic patterns and susceptibility to lysis by *Brucella* bacteriophage, type *abortus*, strain 3, were determined on 87 strains of *Brucella* that displayed the characteristics of one species by the conventional biochemical methods and of another species by agglutination with monospecific antisera. The results of this study are reported herein.

MATERIALS AND METHODS

All manometric procedures and materials used in this study were identical with those described previously by Meyer & Cameron (1961a, 1961b). The assessment of phage susceptibility was performed as described by Morgan et al. (1960) and Meyer (1961b). The concentration of phage particles used for the routine test dilution was 2.0×10^6 plaque-forming units per ml. Monospecific antisera were prepared and used as reported by Jones (1958). The conventional determinative methods were carried out as recommended by the Joint FAO/WHO Expert Committee on Brucellosis (1953, 1958).

RESULTS AND DISCUSSION

The accompanying table shows 87 strains of brucellae that have been segregated into eight groups according to the combination of biochemical and serological characteristics that they possess.

Group 1-4 represent strains of *Brucella* which unquestionably possess the biochemical characteristics of *Br. abortus*, display the metabolic pattern that is singular for this species, and are susceptible to lysis by a strain of phage that lyses only *Br. abortus*, even though the *Br. melitensis* antigen predominates as the one measurable by agglutination with monospecifically absorbed antisera.

Groups 5 and 6 contain strains that have been described as *Br. melitensis* biochemically and *Br. abortus* serologically. These can be divided into two categories. By the conventional determinative methods, the organisms in group 5 display the characteristics generally regarded as identifying for the species *Br. melitensis*. However, each of these strains agglutinated only with *Br. abortus* antisera, displayed the metabolic pattern of *Br. abortus*, and was lysed by the *Br. abortus* bacteriophage. These organisms are evidently strains of *Br. abortus* that do not produce hydrogen sulfide. In relation to the present classification scheme of species with biotypic variants (Huddleson, 1957), they should be considered as a biotype of *Br. abortus*. They differ from those described by Bevan (1930) as non-carbon-dioxide-requiring, thionin-resistant strains (commonly referred to as Rhodesian *abortus*) only by the single characteristic of not producing hydrogen sulfide. By the conventional tests, these strains duplicate exactly the characteristics of *Br. melitensis*. However, they can be identified accurately by their oxidative metabolism and by their phage susceptibility.

The strains in group 6 have also been described as *Br. melitensis* biochemically and *Br. abortus* serologically. These organisms differ from those in group 5 in that they display the metabolic pattern of *Br. melitensis* and are not lysed by the phage that lyses *Br. abortus*. Even though the predominant antigen that is measurable by present techniques is the *Br. abortus* antigen, these organisms possess all the other species-identifying characteristics of *Br. melitensis* and it is suggested that they be classified as such.

Group 7 contains 18 strains described as *Br. melitensis* biochemically but containing both antigens. Organisms of this description can also be divided into two categories—those that are metabolically *Br. melitensis* and not susceptible to the phage (group 7), and those that are metabolically *Br. abortus* and are phage-susceptible (group 8; 2 strains). They differ from their counterparts in

OXIDATIVE METABOLISM AND SUSCEPTIBILITY TO *BRUCELLA* BACTERIOPHAGE, TYPE *ABORTUS*, STRAIN 3, OF 87 *BRUCELLA* STRAINS THAT SHOW CONFLICTING IDENTITY BY BIOCHEMICAL AND SEROLOGICAL METHODS

Description of strains	Number of strains	Results of conventional biochemical tests	Oxidative metabolic pattern [a]	Susceptibility to phage	Species identity
GROUP 1: *Br. abortus*, type 1 biochemically, *Br. melitensis* serologically	31	Require CO_2 for isolation. Produce H_2S for 4 days. Grow on basis fuchsin. Do not grow on thionin.	All strains in groups 1, 2, 3, 4 and 5 oxidize: L-alanine (40-241), L-glutamic acid (72-490), L-asparagine (64-301), L-arabinose (45-161), D-galactose (50-296), D-ribose (132-446). They do not oxidize: DL-ornithine, DL-citrulline, L-arginine or L-lysine.	All strains in groups 1-5 were lysed.	*Brucella abortus*
GROUP 2: *Br. abortus*, type II biochemically, *Br. melitensis* serologically.	2	Require CO_2 for isolation. Produce H_2S for 4 days. No growth on dyes. Require serum for growth.			
GROUP 3: *Br. abortus*, type III biochemically, *Br. melitensis* serologically.	1	Require CO_2 for isolation. Produce H_2S for 4 days. Grow on both dyes.			
GROUP 4: *Br. abortus*, type III biochemically, agglutinate monospecific antisera of both species.	2	Same as group 3.			
GROUP 5: *Br. melitensis* biochemically. *Br. abortus* serologically.	11	Do not require CO_2. Do not produce H_2S. Grow on both dyes.			
GROUP 6: *Br. melitensis* biochemically, *Br. abortus* serologically.	20	Same as group 5.	None of these strains oxidizes: L-arabinose, D-galactose, D-ribose, DL-ornithine, DL-citrulline, L-arginine, L-lysine.	None of these strains was lysed.	*Brucella melitensis*
GROUP 7: *Br. melitensis* biochemically, agglutinate monospecific antisera of both species.	18	Same as group 5.	Same as group 6.	None of these strains was lysed.	*Brucella melitensis*
GROUP 8: Same description as group 7.	2	Same as group 5.	Same as groups 1-5.	Both strains were lysed.	*Brucella abortus*

[a] Figures in parentheses show the ranges in rates observed for all strains in groups 1-5. For the validity of the rate ranges, see Meyer & Cameron (1961a, 1961b).

groups 5 and 6 only in the quantitative distribution of measurable antigens.

From the data presented in the table, it can be seen that each of the 87 *Brucella* strains displayed a pattern of oxidative metabolism that was identical both qualitatively and quantitatively with the pattern of substrate utilization previously established as definitive and characteristic for the species of *Br. abortus* or *Br. melitensis*, and that the susceptibility to lysis by *Brucella* bacteriophage, type *abortus*, strain 3, was correlated consistently with the metabolic pattern. Therefore, irrespective of the predominant antigen or antigens measurable by agglutination with monospecific antisera, or of their biochemical characters, each of these strains could be accurately classified into an existing species and biotype.

It has been known since the original report by Wilson & Miles (1932) that *Br. abortus* and *Br. melitensis* contained qualitatively similar antigens which varied in quantitative distribution. The evidence presented herein shows that the quantitative antigen distribution varies not only from species to species, but also within a species, and that this antigen distribution is frequently unrelated to other species characteristics.

Wilson & Miles (1932) also reported that the species of *Br. suis* was intermediate between the other two species in its antigenic structure, but that it could not be distinguished from *Br. abortus* when monospecific antisera were used for identifying cultures. However, *Br. suis* has continuously been regarded as a species distinct from *Br. abortus*. It is now known that the two species differ both quantitatively and qualitatively in their metabolism and phage susceptibility as well as in their growth patterns on basic fuchsin and thionin, and that they show decidedly different animal host preferences. It therefore seems unjustifiable taxonomically to use the serological test of Wilson & Miles (1932) for species differentiation when it will not distinguish antigenically such otherwise definable organisms as *Br. suis* and *Br. abortus*.

CONCLUSION

The foregoing has demonstrated that there occur strains of *Br. abortus* that do not produce hydrogen sulfide, do not require carbon dioxide and are thionin-resistant, thereby duplicating the features generally regarded as characteristic of *Br. melitensis*. They can be identified by their metabolism and phage susceptibility. The distribution of *Br. abortus* and *Br. melitensis* antigens, as measured by agglutination with monospecific antisera, is also not always related to other species-identifying characteristics. Therefore, neither the serological method nor the biochemical method can be considered a reliable guide to the identification of *Brucella* species.

RÉSUMÉ

L'agglutination par antisérums à spécificité univoque est un mode sérologique d'identification des organismes faisant partie du genre *Brucella*. Elle constitue souvent même une méthode utilisée pour décider de l'appartenance des souches étudiées à une espèce donnée. Or, les chercheurs qui se sont attachés à déterminer la valeur de la sérologie comme procédé de typage sont parvenus à des conclusions contradictoires. Cependant, avec l'application à l'identification des *Brucella* des techniques manométriques, on est désormais en possession d'une méthode qui permet de faire une discrimination qualitative parmi les espèces et de mesurer avec précision des différences quantitatives. Il peut être affirmé que les méthodes tant biochimiques que sérologiques sont d'un grand intérêt pratique pour la différenciation des espèces appartenant à ce genre. Au total, 87 souches *Brucella* qui faisaient partie chimiquement d'une espèce et sérologiquement d'une autre espèce ont été examinées au point de vue des caractéristiques de leur métabolisme d'oxydation et de leur sensibilité au bactériophage de *Brucella*, type *abortus*, souche 3. Dans bien des cas, il est apparu que l'identité sérologique des souches *Brucella* ne correspondait pas à celle donnée par leur métabolisme ou leur sensibilité au bactériophage; il en est souvent de même des caractéristiques biochimiques. Ainsi, à titre d'exemple, il existe des souches de *B. abortus* qui ne produisent pas d'hydrogène sulfuré, prolifèrent en l'absence d'anhydride carbonique, et sont résistantes à la thionine — toutes caractéristiques qui sont, en général, reconnues à *B. melitensis*. En conclusion, les procédés sérologiques ou biochimiques ne permettent pas d'identifier l'espèce en toute certitude à l'intérieur du genre *Brucella*.

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