COMPARATIVE METABOLIC STUDIES ON THE GENUS BRUCELLA

I. EVIDENCE OF A UREA CYCLE FROM GLUTAMIC ACID METABOLISM

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During investigations to ascertain the mechanism involved in the differential bacteriostatic effect of dyes on the growth of the three species of *Brucella*, it became apparent that relevant data could be obtained by studying the comparative metabolism of glutamic acid by the three species. This was initiated using resting cell techniques and determining the metabolites by paper chromatography. The appearance of ornithine and arginine as metabolites from *Brucella abortus* and *Brucella melitensis*, but not from *Brucella suis*, suggested a urea cycle in the first two species. This paper is a report on these investigations.

MATERIALS AND METHODS

A smooth strain of each species of *Brucella* was grown for 48 hours on tryptose agar. The growth was washed off with \( \text{m/15} \) phosphate buffer with a pH of 7.0. The cells were washed three times and resuspended in buffer to a concentration such that a 1 to 20 dilution gave a reading of 5 per cent light transmission at a wavelength of 600 m\( \lambda \) on a Coleman Junior model spectrophotometer. One milliliter of concentrated cells contained one milligram of nitrogen as determined by a micro-Kjeldahl technique. Substrates were in a concentration of 10 mg per ml of the buffer. The desired amount was placed in flasks, and the volume was adjusted to 3.0 ml with buffer. One milliliter of concentrated cells was added then and the flasks placed in a water bath at 37 C. The flasks were removed at various intervals and the reaction stopped with sodium azide. Cells were centrifuged down, the supernatant decanted into shell vials, and stored under refrigeration. Conventional paper chromatography techniques were used to identify amino acids. Butanol-acetic solvent was used and ninhydrin employed as a color reagent.

RESULTS

The action of each species of *Brucella* on glutamic acid is shown in figure 1. Five milligrams of glutamic acid were used in this series and the reaction stopped at 18, 24, and 48 hours. The lower spots, not identified on this figure, were found later to be ornithine and arginine. It will be observed that these metabolites were not detected when *B. suis* was used. At 18 hours both alanine and ornithine were produced by *B. abortus*. These amino acids also appeared at 18 hours with *B. melitensis* but were more pronounced at 24 hours. Citrulline was detected occasionally with *B. abortus* and *B. melitensis*. The difference in the two species apparently lies in the rate of metabolism only. By 48 hours glutamic acid had been entirely metabolized by *B. abortus* and *B. melitensis* with arginine also
appearing as a metabolite. Under these conditions, *B. suis* was not active in its metabolism of glutamic acid.

In view of the possibility of a urea cycle from glutamic acid by *B. abortus* and *B. melitensis*, it seemed advisable to use each component amino acid of the cycle as a single substrate. Inasmuch as *B. suis* under these conditions did not metabolize glutamic acid and since *B. melitensis* apparently followed the same pattern as *B. abortus*, only the latter species was used in the study. When ornithine was used as a single substrate, alanine and a trace of glutamic acid appeared as metabolites in 24 hours. These were not apparent after 48 hours' incubation. At 72 hours no metabolites were apparent. When aspartic acid was used as a single substrate, ornithine and a trace of glutamic acid were apparent in 24 hours. At 48 hours 1 and 5 mg of the substrate were metabolized and neither substrate nor metabolite could be detected. Alanine and ornithine, on the other hand, were apparent at the higher concentrations. No additional changes were apparent after 72 hours' incubation.

Arginine was used as a substrate because the completion of the cycle was dependent upon arginase activity. Figure 2 shows the metabolism of varying

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**Figure 1.** Chromatogram showing metabolites from glutamic acid by three species of *Brucella* at varying time intervals. The spot with the lower Rf value was identified as ornithine and that immediately above (as in 32) as arginine. Number 18 represents *suis* cells + 5 mg glutamic acid 24 hours.
amounts of this substrate by *B. abortus* at 24 and 72 hours' incubation. Metabolites were ornithine and alanine. At 72 hours only traces of the original arginine substrate remained, and the metabolite ornithine was sufficiently metabolized in the smaller amounts that none could be detected.

![Figure 2](http://jb.asm.org/)

**Figure 2.** Chromatograms showing metabolites from arginine by *Brucella abortus* following 24 and 72 hours' incubation. 1, 21 = cell and buffer. 2, 22 = substrate 10 mg and buffer. Nos. 3 to 10 represent arginine in amounts of 1, 2, 5, 8, 10, 12, 15, 20 mg, respectively. 23 to 30 represent corresponding amounts at 72 hours.

**DISCUSSION**

The presence of ornithine, arginine, and citrulline as metabolites from the utilization of glutamic acid by *B. abortus* and *B. melitensis* but not by *B. suis* suggested that a urea cycle was involved in the metabolic activity of these two species.

Since *Brucella* organisms have a relatively high concentration of urease as compared to other microorganisms (Sanders and Warner, 1951), it is reasonable to assume that this enzyme is available because of the occurrence of urea as a metabolite. The work of Borsook and Dubnoff (1941) showed that, in mammals, the conversion of citrulline to arginine, under aerobic conditions, is facilitated
by glutamic and aspartic acids. Ratner and Pappas (1949) later showed that glutamic acid had a dual purpose in this system in that it served as a substrate for oxidation to maintain the adenosine triphosphate concentration and also provided a source of aspartic acid. Altenbern and Housewright (1951), working with smooth \textit{B. abortus}, strain 19, showed that this strain possessed both the aspartic-glutamic and glutamic-alanine transaminases. This is undoubtedly one of the mechanisms involved here, accounting for the appearance of alanine and providing the necessary aspartic acid. Bonner (1946) and Srb and Horowitz (1944), working with biochemical mutants of \textit{Neurospora} and \textit{Penicillium}, elucidated a cycle going from glutamic acid through ornithine and citrulline to arginine.

Under the experimental condition of the work herein reported aspartic acid did not appear as a metabolic product of glutamic acid although it is a necessary amino acid in the cycle. Possible explanations are that aspartic acid was not present in concentrations great enough to be detected by chromatograms, or it was used in the cycle as rapidly as it was produced. When used as a single substrate with \textit{B. abortus} it was rapidly metabolized to ornithine as well as to alanine. Since aspartic acid is considered a specific nitrogen donor in this system, since the transaminase system was actively producing alanine, and since the cycle was proceeding to ornithine and citrulline, presumptive evidence indicates that aspartic acid must have been produced.

The conversion of arginine to ornithine when used as a single substrate warrants the conclusion that arginase is present in \textit{B. abortus} and that a urea cycle is involved in the metabolism of that species. As far as can be determined this has not been reported previously in a bacterial species.

**SUMMARY**

Washed cells from the three species of \textit{Brucella} were incubated with glutamic acid, and the metabolites identified by paper chromatography. The results suggested a urea cycle in \textit{Brucella abortus} and \textit{Brucella melitensis}. Incubation with ornithine, arginine, and aspartic acid as single substrates confirmed this observation.

**REFERENCES**


