JOURNAL OF BACTERIOLOGY, Apr. 1982, p. 245–250
0021-9193/82/040245-06$02.00/0

Vol. 150, No. 1

Ornithine Metabolism in the Genus *Rochalimaea*

EMILIO WEISS,* HERMOISE K. MAMAY, AND GREGORY A. DASCH
Naval Medical Research Institute, Bethesda, Maryland 20814

Received 31 August 1981/Accepted 24 November 1981

Ornithine metabolism was studied in two strains of the trench fever rickettsia *Rochalimaea quintana*, Fuller and Guadalupe, and in the vole agent, a strain of *Rochalimaea* but not necessarily of *Rochalimaea quintana*. The metabolic activity of intact cells and cell-free extracts was measured by monitoring the evolution of $^{14}CO_2$ from [1-^{14}C]ornithine. Low levels of activity were obtained with all three strains, but requirements for the demonstration of this activity differed. With the cells of the Fuller and Guadalupe strains, the decarboxylation of ornithine was almost completely dependent on added pyruvate or succinate, presumably as sources of energy for transport. This enhancement was not prevented by the presence of chloramphenicol. The activity of the vole agent, on the other hand, required the complete medium. This activity was prevented by chloramphenicol added at the same time as the medium but not by chloramphenicol added after 1 h of incubation. In cell-free extracts, the demonstration of ornithine decarboxylase activity in the vole agent required prior induction with medium containing ornithine, whereas in the other two strains, the activity was constitutive. The activities of the extracts of the Fuller strain and the vole agent differed also in pH optimum, which was somewhat lower for the vole agent, and in the added pyridoxal phosphate requirement, which was greater for the Fuller strain. Comparable experiments with *Rickettsia typhi* and *Rickettsia prowazekii* failed to reveal evidence of ornithine metabolism.

Rochalimaea quintana, the rickettsial agent of trench fever, can be grown in bacteriological media of moderate complexity (18), whereas the typhus rickettsiae, *Rickettsia prowazekii* and *Rickettsia typhi*, are obligate intracellular parasites. Despite this difference in nutritional requirements and an 8% difference in DNA base ratio (*Rochalimaea*, 38 mol% guanine plus cytosine; typhus rickettsiae, 30 mol%) (16), Myers and Wisseman (13) have shown that the DNAs of the trench fever and typhus rickettsiae hybridize to a 25 to 33% extent. These surprising results suggest that the two groups of bacteria may have previously unrecognized phenotypic similarities and that *Rochalimaea quintana* well deserves to be studied as a possible axenic model for some of the properties of *Rickettsia* spp.

Our interest in *Rochalimaea quintana* was further stimulated by the recent finding that the vole agent (1) resembles the Fuller strain (18) of *Rochalimaea quintana* in cultural, biochemical, and morphological properties (11, 22). Subsequent work (14, 20) has shown that the DNAs of the vole agent and *Rochalimaea quintana* hybridize to a 31 to 42% extent and that the two strains share antigenic components. The migration patterns of the total solubilized proteins in polyacrylamide gels are also similar, though not identical; this has also been found to be the case in comparisons of *Rickettsia prowazekii* with *Rickettsia typhi*. If the vole agent is regarded as a strain of *Rochalimaea*, not necessarily *Rochalimaea quintana*, the ecological niche of *Rochalimaea* spp. parallels that of the typhus group of *Rickettsia* spp. *Rickettsia prowazekii* is primarily a parasite of humans, as is *Rochalimaea quintana*, whereas *Rickesettia typhi* and the vole agent are parasites of small rodents. When two related genera of bacteria are compared, the availability of more than one species of each genus provides a useful gauge of evolutionary divergence.

This study was prompted by the finding (20) that three strains of *Rochalimaea* catabolize ornithine. This activity has some unusual features that are worth investigating, even though there is as yet no evidence that it occurs in *Rickettsia* spp. We report here that ornithine decarboxylase (ODC) activity was an inducible activity in the vole agent and was constitutive in the other two strains of *Rochalimaea*. However, the constitutive nature of the enzyme was not readily detected in intact cells because they required a source of energy for the transport of ornithine.

MATERIALS AND METHODS

Bacterial strains: cultivation and preparation for metabolic experiments. The passage histories of the Fuller strain of *Rochalimaea quintana* and the vole agent have been previously described (11, 22). The Guadalupe strain (17) was isolated in Mexico City from the blood of a volunteer infected by scarification with a saline suspension of body lice that had been collected in a public dormitory. The strain was isolated and passaged on blood agar, and a seed from the 10th passage was kindly sent to us by the late J. W. Vinson, Harvard University, Cambridge, Mass. In our laboratory, all three strains were reisolated from individual colonies, and seeds were prepared from the second passage in broth medium. The medium (20) consisted of Hanks balanced salt solution (without glucose or calcium chloride) with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (final pH of Hanks solution-HEPES, 7.2), 0.8% Casamino Acids (Difco Laboratories, Detroit, Mich.), 0.1% yeast extract (Difco), 2 mM succinate, 10% fetal calf serum, and 4.5 mM sodium bicarbonate. The cells were grown in 120- or 600-ml bottles containing 30 or 300 ml of medium, depending on the volumes needed, in an incubator-shaker (100 to 150 rotations per min) at 35°C. The cells were harvested at about the end of logarithmic growth. For the preparation of seeds, the cells were sedimented by centrifugation (10,400 × *g* for 30 min), resuspended in 10% of the volume of medium, distributed in glass-sealed ampoules, frozen in a dry ice-alcohol mixture, and stored at −70°C. For metabolic experiments, the cells were sedimented as described above, washed twice in moderately large volumes of Hanks solution-HEPES, and resuspended in the needed volume of the same diluent or, for the preparation of extracts, in very small volumes of distilled water. The cells were disrupted by sonication (Sonifier Cell Disruptor model W185; Heat System Ultrasonics, Inc., Plainview, N.Y.) one 1-ml volume at a time, for one or two 10-s cycles at a setting of 6. To the sonicated cells an equal volume of double-strength TED buffer (final concentrations: Tris buffer [pH 7.5], 50 mM; EDTA, 0.1 mM; dithiothreitol, 5 mM) was added, and the mixture was centrifuged at 17,300 × *g* for 15 min. A small sediment was discarded, and the supernatant was either tested immediately or, more frequently, after storage at −70°C. Soluble and membrane fractions were prepared from cells disrupted and treated as described previously (22).

The strongly ODC-positive strain of *Enterobacter cloacae* used as a control in these studies was isolated from an environmental source by David M. Rollins, Medical Microbiology Branch, Naval Medical Research Institute. It was grown in brain heart infusion broth but otherwise was treated as the strains of *Rochalimaea* were.

The Wilmington strain of *Rickettsia typhi* and the Breinl strain of *Rickettsia prowazekii* were grown, purified, and disrupted as described previously (4).

Metabolic experiments. L-[1-^{14}C]ornithine (specific activity, 55.5 mCi/mmol) and L-[*U*-^{14}C]ornithine (specific activity, 318 mCi/mmol) were obtained from New England Nuclear Corp., Boston, Mass. L-[*U*-^{14}C]glutamic acid (specific activity, 290 mCi/mmol) was obtained from Radiochemical Centre, Amersham, England. These compounds were diluted with their respective unlabeled counterparts. Formation of CO_2 from these substrates was determined as described previously (22, 23), except that 16- by 100-mm tubes were used instead of 25-ml flasks. Unless otherwise indicated, each tube contained, in a total volume of 0.4 ml, 0.2 ml of cell suspension or diluted enzyme preparation, 0.1 ml of the variable to be tested, and 0.1 ml of ornithine (0.4 or 0.04 μmol with 0.05 μCi of [1-^{14}C]- or [*U*-^{14}C]ornithine, respectively). Hanks solution-HEPES was the diluent for all reagents used in the tests of the cells; TED buffer was used for the tests of the extracts. The tubes were closed with rubber stoppers from which plastic cups containing filter paper wicks were suspended. The tubes were incubated at 35°C in a water bath for the indicated time plus a 2-min period for thermoequilibration. Hyamine hydroxide (0.15 ml; New England Nuclear Corp.) was added to the cups after incubation of the cells but before incubation in experiments with extracts. The tube containing cells but not those containing extracts were rotated gently during incubation. All reactions were stopped by the injection of 0.1 ml of 25% trichloroacetic acid to the reaction mixtures. After an additional 0.5 to 1.0 h of incubation to allow absorption of CO_2 to Hyamine, the cups and their wicks were transferred to vials to which were added 1.5 ml of methanol and 7.5 ml of Econofluor (New England Nuclear Corp.), and the radioactivity was measured in a liquid scintillation spectrometer. The amount of CO_2 formed was determined from the counts per minute (minus the small counts per minute of the control tubes [without cells]) and the final specific activity of the substrate. The amount was expressed as nanomoles of CO_2 per milligram of protein. All tests were done in triplicate, and variation among triplicate determinations was usually <10%.

Miscellaneous procedures. All cultures were checked for freedom from contamination with other bacteria by microscopic examination of Gram- and Gimenez-stained smears and cultivation on blood agar and thioglycolate broth. Protein was determined by the method of Lowry et al. (9); bovine plasma gamma globulin (Bio-Rad Laboratories, Richmond, Calif.) was used as the standard.

RESULTS

Ornithine metabolism by *Rochalimaea* cells. The levels of ornithine metabolism by the three strains of *Rochalimaea*, tested in the absence or presence of additional substrates, tested were quite variable. Relatively consistent results were obtained, however, when the cells were washed thoroughly and were harvested while still actively multiplying (optical density at 420 nm <0.5). This was also true of the metabolism of other substrates (E. Weiss and G. A. Dasch, manuscript in preparation). Even under the best conditions, decarboxylation of ornithine proceeded at a slow rate, about two orders of magnitude lower than the rate for *Enterobacter cloacae*, which was tested under identical conditions. This difference may in part be a reflection of the generation times of the organisms: 4 to 6 h for *Rochalimaea* strains and about 20 min for *Enter-*

obacter cloacae. Despite the low level of activity of the *Rochalimaea* strains, it became quite apparent that the decarboxylation of ornithine by the Fuller and Guadalupe strains on the one hand and that by the vole agent on the other were stimulated by different factors.

Decarboxylation of ornithine by the Fuller strain was greatly enhanced by pyruvate, which presumably served as a source of energy for transport (Fig. 1). The optimal concentration of pyruvate for the metabolism of 1 mM ornithine was 1 to 3 mM. At a higher pyruvate concentration (10 mM), ornithine metabolism declined, possibly as a consequence of competition with pyruvate or products of pyruvate metabolism. In subsequent experiments, 2 mM pyruvate was used. With this concentration of pyruvate, decarboxylation of ornithine proceeded at a rate that was approximately linear for at least 2 h (Fig. 2). The low activity that took place in the absence of pyruvate was also linear during this period.

It was shown in other experiments (8, 22; Weiss and Dasch, in preparation) that all three strains of *Rochalimaea* actively metabolize pyruvate and succinate. Glutamine is utilized to a more moderate extent, and glutamate is catabolized by the vole agent, but to a much lesser extent by the other two strains. Table 1 illustrates the effects of these substrates as possible sources of energy for the transport of ornithine. For the Fuller strain, pyruvate and succinate were about equally effective stimulators of ornithine metabolism, whereas glutamine stimulated activity slightly, and glutamate did not stimulate activity at all. Somewhat similar results were obtained for the Guadalupe strain, except that pyruvate was significantly more effective than succinate. In a separate experiment (not shown in Table 1), the results with glutamine and glutamate were identical to those obtained for the Fuller strain.

With the vole agent, none of the four substrates stimulated ornithine metabolism, with the possible exception of a slight stimulation by glutamate and a very slight stimulation by glutamine. When complete medium was added, stimulation of the Fuller and Guadalupe strains was only moderate (somewhat better stimulation of the Fuller strain was obtained in previously reported experiments [20]). A pronounced stimulation was obtained only for the vole agent.

The role of protein synthesis in the above reactions was investigated by the addition of chloramphenicol to the above mixtures (Table 1). Chloramphenicol reduced the enhancement of the reactions with the four substrates to a

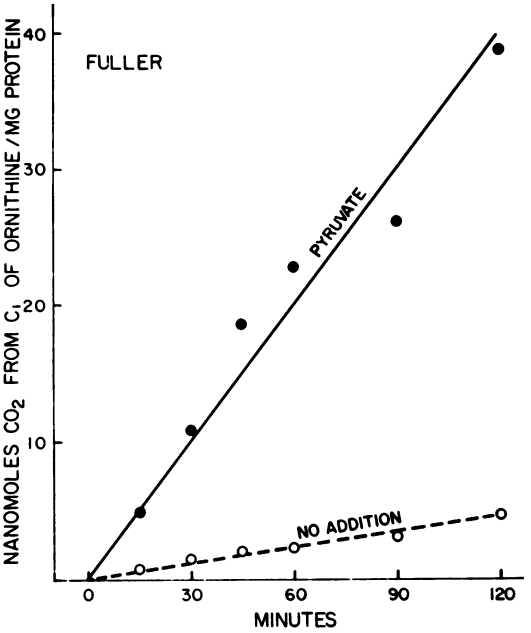

FIG. 2. Kinetics of ornithine metabolism by the Fuller strain at 35°C in the absence or presence of pyruvate. Substrate and cell concentrations are as follows: ornithine, 1 mM; pyruvate, 2 mM; cells, 0.28 mg of protein per tube.

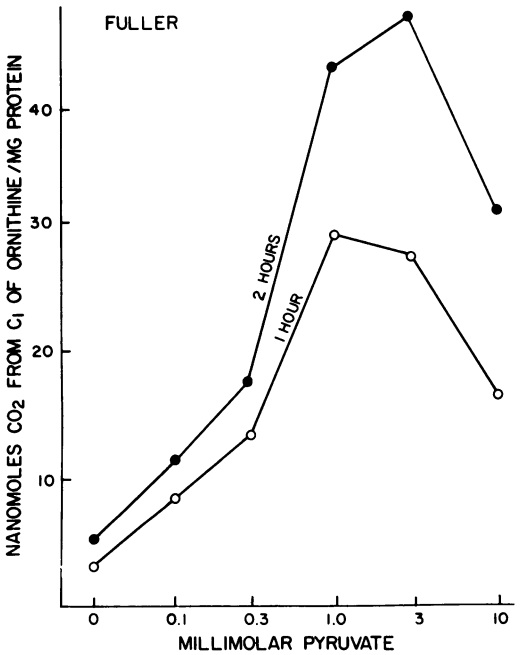

FIG. 1. Ornithine metabolism by the Fuller strain during 1 or 2 h of incubation at 35°C in the absence or presence of various concentrations of pyruvate. Ornithine concentration, 1 mM; cell concentration, 0.22 mg of protein per tube. For other details, see the text.

TABLE 1. Ornithine metabolism by *Rochalimaea* strains: effect of energy source

Energy source[a]	Ornithine metabolism[b] by indicated strain					
	Fuller		Guadalupe		Vole	
	−	+	−	+	−	+
Hanks solution-HEPES	5	3	5	4	2	2
+ Succinate	32	29	19	16	3	3
+ Pyruvate	34	30	31	28	2	2
+ Glutamine	8	6	ND[c]	ND	4	1
+ Glutamate	4	3	ND	ND	5	3
+ 25% Medium	7	5	11	7	12	2

[a] Each energy source was used at 2 mM. The medium is described in the text.

[b] Expressed as nanomoles of CO_2 from C-1 of ornithine per milligram of cell protein formed during 2 h of incubation at 35°C with 1 mM ornithine (400 nmol per tube). The protein contents per tube were as follows: Fuller strain, 0.52 mg; Guadalupe strain, 0.28 mg; vole agent, 0.46 mg. Chloramphenicol content, 0.1 mg/ml when present (+).

[c] ND, Not done.

very limited extent. The only marked reduction of enhancement was obtained in the presence of medium. In the case of the vole agent, chloramphenicol reduced ornithine metabolism to the level seen in cells incubated with no addition to ornithine.

The vole agent, as shown previously (20) and in Table 1, requires chloramphenicol-sensitive ODC induction. Further confirmation of this finding is shown in Table 2. A preparation of vole agent cells was divided into two lots, and both were tested with labeled ornithine for 2 h; one was tested after a 1-h preincubation with unlabeled ornithine, and one was tested without preincubation. The lot tested without preincubation displayed significant ornithine metabolism only in the presence of complete medium, and this activity was prevented by chloramphenicol. The lot preincubated with ornithine displayed a lower level of ornithine metabolism, possibly because of more competition with medium components after preincubation, but this activity was not affected by chloramphenicol. These results suggest that protein synthesis is required for the formation of the enzyme but not thereafter. A previous experiment has shown that for optimal activity, all of the constituents of the medium are required (20).

All of the above experiments were done with ornithine labeled in the C-1 position and were tests of ODC activity. In one experiment with the vole agent, it was shown that added putrescine, the product of ODC activity, was inhibitory. No carbon incorporation was demonstrated in the trichloroacetic acid precipitate in any of these experiments. Carbon incorporation was also not shown to occur when some of the above-described reaction mixtures, some with added *S*-adenosyl-methionine, were used with uniformly labeled ornithine. Thus, there is no indication that under the conditions of these experiments, spermidine was synthesized from putrescine and *S*-adenosyl-methionine. There was also no evidence that CO_2 was produced from carbons other than C-1.

Ornithine metabolism of *Rochalimaea* cell extracts. Cell extracts of the three *Rochalimaea* strains were separated into soluble and membrane fractions and tested for ODC activity. In the presence of 1 mM labeled ornithine and 0.1 mM pyridoxal phosphate, 40 and 21 nmol of CO_2 per mg of protein per h were produced by the soluble fractions of the Fuller and Guadalupe strains, respectively. Only trace amounts were produced by the membrane or soluble fractions when pyridoxal phosphate was omitted or replaced with 1 mM pyruvate. The results for the fractions of the vole agent were negative, although low levels of activity were observed in whole cells and unfractionated extracts. In the experiments described below, the extracts were not separated into soluble and membrane fractions, and the specific activities were lower.

Induced vole agent ODC activity was elicited essentially as described in Table 2. A comparison of crude extract activities derived from noninduced and induced vole agent cells and noninduced Fuller cells at various pHs is shown

TABLE 2. Ornithine metabolism by the vole agent

Addition to Hanks solution-HEPES[a]	Ornithine metabolism[b] by lot[c]:	
	1	2
None	0.4	0.0
Succinate	1.7	0.1
Medium	16.3	9.2
Medium + chloramphenicol[d]	0.7	9.2

[a] Final concentrations: succinate, 2 mM; medium, 25% strength; chloramphenicol, 0.1 mg/ml.

[b] Expressed as nanomoles of CO_2 from C-1 of ornithine per milligram of cell protein (0.76 mg per tube) formed during 2 h of incubation at 35°C with labeled ornithine.

[c] The cells were washed by routine procedure and divided into two lots. To lot 1, all reagents were added without preincubation, including labeled and unlabeled ornithine. To lot 2 labeled ornithine was added after the cells had been incubated for 1 h at 35°C with all of the other reagents, including unlabeled ornithine but excluding chloramphenicol.

[d] Chloramphenicol was added to lot 2 after preincubation with unlabeled ornithine.

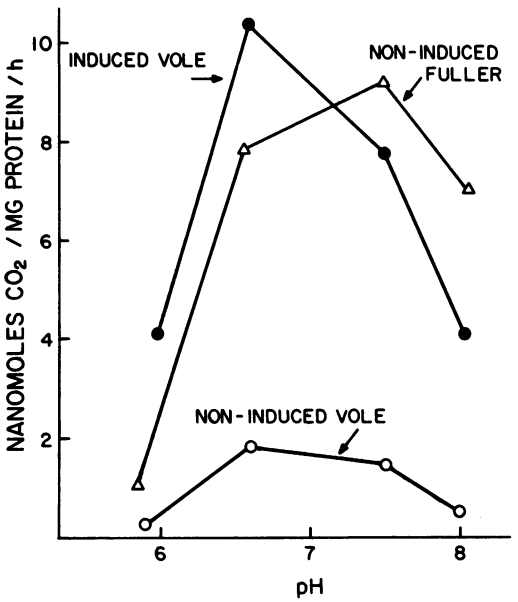

FIG. 3. ODC activity of crude cell extracts of the vole agent and Fuller strain. The extracts were prepared in TED buffer as described in the text. Vole agent cells were induced by incubating 6 ml of partially washed cells with 30 ml of complete medium containing 2 mM added ornithine for 1 h at 35°C. The cells were then washed by routine procedure before extraction. Decarboxylase activity at different pHs was determined as follows: to 0.05 ml of extract were added 0.01 ml of pyridoxal phosphate (final concentration, 0.1 mM) and 0.33 ml of HEPES buffer of the appropriate pH. After a 10-min incubation at 35°C, 0.01 ml of ornithine was added (final concentration, 1 mM). The reaction was carried out at 35°C for 1 h. HEPES buffer, adjusted to the desired pH with 1 M KOH, consisted of the following constituents (final concentrations): 40 mM HEPES, 1 mM EDTA, and 5 mM dithiothreitol. The final pH of each mixture was determined before and after incubation in tubes containing all reagents except [^{14}C]ornithine, and the pHs shown are the means of the two determinations. Protein contents per tube are as follows: vole (noninduced), 0.14 mg; vole (induced), 0.20 and 0.22 mg (the results shown are the means of two tests, each with a different preparation); Fuller (noninduced), 0.31 mg.

in Fig. 3. Some activity was demonstrated in the extract of noninduced cells, but induction greatly increased it. The pH optima of both preparations appeared to be the same, somewhat below neutrality. The ODC activity of the Fuller preparation was entirely comparable to that of the induced vole agent preparation, but the optimum pH was slightly above neutrality. The difference in pH optima, although small, has been highly reproducible.

Other differences were noted between the enzyme activities of the Fuller strain and the vole agent. The activity of the Fuller extract was almost entirely dependent on added pyridoxal phosphate, and a concentration lower than that used in above experiments (0.03 instead of 0.1 mM) was more effective. The activity of the vole agent preparation was enhanced only moderately (20 to 30%) by pyridoxal phosphate, and the enhancement, within limits, was independent of concentration. The reaction was not enhanced by pyridoxal, pyridoxamine, pyruvate, phosphoenolpyruvate, or thiamine pyrophosphate. An extract of *Enterobacter cloacae*, tested under identical conditions, had a pH optimum below neutrality, as did the vole agent, but interacted with pyridoxal phosphate, as did the Fuller preparation.

Ornithine metabolism not demonstrated in *Rickettsia* spp. Two experiments were done with *Rickettsia* spp. *Rickettsia typhi* cells were tested immediately after purification from yolk sacs by the Renografin density gradient procedure (21) for glutamate metabolism and for ornithine metabolism in the absence or presence of glutamate or the medium used for the *Rochalimaea* strains. Glutamate was catabolized with the production of about 1 μmol of CO_2 per mg of protein per h, but ornithine was not metabolized. The soluble fractions of extracts from *Rickettsia prowazekii* and *Rickettsia typhi* were tested for ODC activity in the absence or presence of pyridoxal phosphate. No activity was demonstrated.

DISCUSSION

ODC (E.C. 4.1.1.7) is commonly used for the classification of bacteria (10) and is part of most biochemical, multitest, diagnostic systems. As these tests are usually performed, *Rochalimaea* strains would be classified as ODC negative. However, in one test which does not depend on the growth of bacteria, a weakly positive reaction is occasionally obtained. Lysine decarboxylase activity, on the other hand, is clearly and consistently negative (20).

ODC has several functions in bacteria (2, 6, 12). On the basis of studies of *Escherichia coli*, the constitutive enzymes are regarded as biosynthetic, and the inducible enzymes are regarded as degradative, although this distinction does not hold true in every case. The primary function of the biosynthetic enzyme is the synthesis of putrescine and, eventually, polyamines. The degradative enzymes are produced in response to low pH and may have a function in the control of intracellular CO_2 (5).

The role of ODC in *Rochalimaea* strains is uncertain. On the basis of limited experiments with uniformly labeled ornithine, decarboxylation does not seem to be followed by other reactions. Thus, a role in biosynthesis for ODC in any of the three strains is unlikely. It is apparent, however, that the ODC of the Fuller

and Guadalupe strains on the one hand and the ODC of the vole agent on the other may have different functions. A remote possibility that cannot be discounted is that the Fuller strain decarboxylates ornithine via a glutamate decarboxylase, which has weak affinity for ornithine. Although decarboxylases are generally regarded as enzymes that are highly substrate specific, weak reactions against other substrates have been reported (3, 15). Although glutamate is an important metabolite of rickettsiae (7, 19), early studies of the metabolism of the Fuller strain (8) indicate that glutamate is utilized at a low rate. Recently, we have shown (Weiss and Dasch, in preparation) that the metabolism of glutamate is, like the metabolism of ornithine, greatly enhanced by the addition to the cells of another substrate, such as pyruvate or succinate, or by the addition of glutamine. The extent to which dehydrogenases, transaminases, and decarboxylases participate in the catabolism of glutamate has not yet been investigated. Some of the decarboxylases, such as histidine decarboxylase, require pyruvate instead of pyridoxal phosphate as the cofactor (2). This is clearly not the case for the *Rochalimaea* ODC.

The ODC of the vole agent has some of the attributes of a degradative enzyme: it is inducible and has a somewhat lower pH optimum than that of the Fuller strain. Since the vole agent, in contrast to the Fuller and Guadalupe strains, does not need added CO_2 for growth (Weiss and Dasch, in preparation), the decarboxylases may play a role in the regulation of internal CO_2.

The main purpose of this study was to develop a model for the genus *Rickettsia*, but no evidence that *Rickettsia typhi* or *Rickettsia prowazekii* catabolizes ornithine was obtained. However, it has been established that the two genera share a number of enzymatic activities (8, 22), and other comparisons might be quite fruitful. The results described here point to some of the pitfalls that must be avoided. Superficial similarities in activities of strains or species may obscure important differences that must be taken into account in any comparison of two genera.

ACKNOWLEDGMENTS

We thank Jerry G. Croley for his excellent technical assistance.

This work was supported by the Naval Medical Research and Development Command, Research Work Unit no. MR00001.001.1271.

LITERATURE CITED

1. **Baker, J. A.** 1946. A rickettsial infection in Canadian voles. J. Exp. Med. **84**:37–50.
2. **Boeker, E., and E. E. Snell.** 1972. Amino acid decarboxylases, p. 217–253. *In* P. D. Boyer (ed.), The enzymes. VI. Carboxylation and decarboxylation, 3rd ed. Academic Press, Inc., New York.
3. **Christensen, R. L., and J. C. Schmit.** 1980. Regulation of glutamic acid decarboxylase during *Neurospora crassa* conidial germination. J. Bacteriol. **144**:983–990.
4. **Dasch, G. A., J. R. Samms, and E. Weiss.** 1978. Biochemical characteristics of typhus group rickettsiae with special attention to the *Rickettsia prowazekii* strains isolated from flying squirrels. Infect. Immun. **19**:676–685.
5. **Guirard, B. M., and E. E. Snell.** 1964. Vitamin B_6 function in transamination and decarboxylation reactions, p. 138–199. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry, vol. 15. Group-transfer reactions. Elsevier/North-Holland Publishing Co., Amsterdam.
6. **Guirard, B. M., and E. E. Snell.** 1980. Purification and properties of ornithine decarboxylase from *Lactobacillus* sp. 30a. J. Biol. Chem. **255**:5960–5964.
7. **Hackstadt, T., and J. C. Williams.** 1981. Biochemical stratagem for obligate parasitism of eukaryotic cells by *Coxiella burnetii*. Proc. Natl. Acad. Sci. U.S.A. **78**:3240–3244.
8. **Huang, K.-Y.** 1967. Metabolic activity of the trench fever rickettsia, *Rickettsia quintana*. J. Bacteriol. **93**:853–859.
9. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
10. **MacFaddin, J. F.** 1980. Biochemical tests for identification of medical bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.
11. **Merrell, B. R., E. Weiss, and G. A. Dasch.** 1978. Morphological and cell association characteristics of *Rochalimaea quintana*: comparison of the vole and Fuller strains. J. Bacteriol. **135**:633–640.
12. **Morris, D. R., and R. H. Fillingame.** 1974. Regulations of amino acid decarboxylation. Annu. Rev. Biochem. **43**:303–325.
13. **Myers, W. F., and C. L. Wisseman, Jr.** 1980. Genetic relatedness among the typhus group of rickettsiae. Int. J. Syst. Bacteriol. **30**:143–150.
14. **Myers, W. F., C. L. Wisseman, Jr., P. Fiset, E. V. Oaks, and J. F. Smith.** 1979. Taxonomic relationship of vole agent to *Rochalimaea quintana*. Infect. Immun. **26**:976–983.
15. **Pegg, A. E., and S. McGill.** 1979. Decarboxylation of ornithine and lysine in rat tissues. Biochim. Biophys. Acta **568**:416–427.
16. **Tyeryar, F. J., E. Weiss, D. B. Millar, F. M. Bozeman, and R. A. Ormsbee.** 1973. DNA base composition of rickettsiae. Science **180**:415–417.
17. **Varela, G., J. W. Vinson, and C. Molina-Pasquel.** 1969. Trench fever. II. Propagation of *Rickettsia quintana* on cell-free medium from the blood of two patients. J. Trop. Med. Hyg. **18**:708–712.
18. **Vinson, J. W., and H. S. Fuller.** 1961. Studies on trench fever. I. Propagation of rickettsia-like microorganisms from a patient's blood. Pathol. Microbiol. **24**(Suppl.):152–166.
19. **Weiss, E.** 1973. Growth and physiology of rickettsiae. Bacteriol. Rev. **37**:259–283.
20. **Weiss, E.** 1981. Biochemistry and metabolism of rickettsiae: current trends, p. 387–400. *In* W. Burgdorfer and R. L. Anacker (ed.), Rickettsiae and rickettsial diseases. Academic Press, Inc., New York.
21. **Weiss, E., J. C. Coolbaugh, and J. C. Williams.** 1975. Separation of viable *Rickettsia typhi* from yolk sac and L cell host components by Renografin density gradient centrifugation. Appl. Microbiol. **30**:456–463.
22. **Weiss, E., G. A. Dasch, D. R. Woodman, and J. C. Williams.** 1978. Vole agent identified as a strain of the trench fever rickettsia, *Rochalimaea quintana*. Infect. Immun. **19**:1013–1020.
23. **Weiss, E., H. B. Rees, Jr., and J. R. Hayes.** 1967. Metabolic activity of purified suspensions of *Rickettsia rickettsi*. Nature (London) **213**:1020–1022.