Metabolic Activity of the Trench Fever *Rickettsia, Rickettsia quintana*

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A study of the metabolic activity of *Rickettsia quintana* was carried out by conventional Warburg and radioisotope techniques with intact cells harvested while growing in the fluid counterpart of the medium of Vinson and Fuller. Like other rickettsiae, *R. quintana* did not utilize glucose, but did metabolize glutamate and glutamine. Unlike typhus rickettsiae, *R. quintana* did not require a diluent high in K^+ for metabolic activity, and it utilized glutamine more efficiently than glutamate. In typical experiments, this microorganism produced 1.6 to 2.0 μ moles of CO_2 from glutamine per mg of rickettsial protein per hr at 37 C, while consuming 1.5 to 1.7 μ moles of O_2 . *R. quintana* also utilized, in descending order, succinate, α -ketoglutarate, glutamate, pyruvate, and citrate; the first-named substrate was utilized more rapidly than glutamine. *R. quintana*, like typhus rickettsiae, has a glutamate-oxaloacetate transaminase because aspartate was isolated, by means of thin-layer chromatography, as one of the end products of the utilization of glutamine. When the microorganisms were incubated with glutamine- ^{14}C and unlabeled intermediates of the citric acid cycle, labeled dicarboxylic acids of the cycle were recovered. Labeled tricarboxylic acids, however, were not recovered, possibly because of cellular impermeability to the corresponding unlabeled intermediates. In the case of *cis*-aconitate, it was shown that this substrate interfered with the uptake of glutamine. These observations are believed to provide convincing evidence that glutamine is utilized through the citric acid cycle and that *R. quintana*, with the differences noted, resembles other rickettsiae.

Rickettsiae have often been set apart from other bacteria as the most typical of the nonviral obligate intracellular parasites. The demonstration by Bovarnick and Snyder (3) of respiration in typhus rickettsiae led, however, to the discovery of a wide variety of metabolic reactions similar to those occurring in bacteria. It has become clear that most pathogenic rickettsiae have a similar metabolic pattern; i.e., they utilize glutamate via the citric acid cycle and fail to catabolize glucose (1, 3, 11, 12, 15, 19, 20).

Rickettsia quintana received relatively little attention in the past because of the lack of a suitable technique for its cultivation. Recently, this microorganism has been cultivated in a cell-free medium (18) and has been characterized serologically and morphologically (7, 17, 18). This study represents an attempt at further characterization of *R. quintana*. If its metabolism can be shown to be similar to that of other rickettsiae, *R. quintana*, which can be cultivated with relative ease, may serve as a model for the study and understanding of other rickettsiae. Moreover,

because *R. quintana* is an extracellular parasite in vitro as well as in vivo (7, 18, 21), as opposed to all other pathogenic rickettsiae which are strictly intracellular, such study may yield information on the evolutionary progression from extracellular to intracellular parasitism.

MATERIALS AND METHODS

Organism. The Fuller strain of *R. quintana* in its ninth passage on blood-agar was obtained from J. W. Vinson. In this laboratory, it was passed twice on blood-agar. The third passage, in which it was grown on agar and in a fluid medium consisting of 4% washed and lysed horse erythrocytes and 6% inactivated horse serum in Brain Heart Infusion (BHI, Difco), provided the material for all metabolic experiments.

Pools of rickettsiae were grown in 32-oz (ca. 900-ml) prescription bottles containing Vinson's agar at 37 C in a gas phase of 5% CO_2 in air for 3 days. The microorganisms were harvested in SP (0.2 M sucrose, 0.02 M potassium phosphate, pH 7.0) containing 0.6% bovine plasma albumin, distributed in appropriate portions, and stored at $-70^{\circ}C$ until used. The day

before the metabolic experiment, flasks containing 100 ml of fluid medium were inoculated with approximately 2×10^{10} viable cells. Sterile 0.9 M NaHCO₃ was added to the medium to obtain a final concentration of 14.5×10^{-3} M to meet the CO₂ requirement of *R. quintana*. The flasks were incubated in a water-bath shaker (New Brunswick Scientific Co., New Brunswick, N.J.) at 37 C for 24 hr. Two flasks were prepared for an experiment of average size.

The fluid medium was removed by centrifugation ($5,000 \times g$ for 30 min) at the end of the period of incubation, and the microorganisms were purified by the following procedure. The cells were washed twice with SP, and the pellet was suspended in KN (0.07 M NaCl, 0.07 M KCl, 0.01 M potassium phosphate, pH 7.0). Aggregated microorganisms were separated by treatment with a homogenizer (Omnimixer, Ivan Sorvall, Inc., Norwalk, Conn.) operated twice for 30 sec each time at approximately 10,000 rev/min, with a 3-min interval between operations. The suspension was then subjected to centrifugation, first at low speed ($200 \times g$ for 10 min) to remove remaining clumps of microorganisms and then at high speed ($5,000 \times g$ for 30 min). The final suspension was made in KN, or in K36 (0.1 M KCl, 0.015 M NaCl, 0.05 M potassium phosphate, pH 7.0). The harvests or the washed suspensions were checked for bacterial contamination by microscopic examination of smears and inoculation of thioglycolate broth and blood-agar plates. Viable counts were done on blood-agar plates, and protein content was determined by the method of Lowry et al. (8).

Metabolic experiments. Reagents were obtained from commercial sources (Fisher Scientific Co., Pittsburgh, Pa.; Calbiochem, Los Angeles, Calif.). They were dissolved in KN, and the pH was adjusted to 7.0 with KOH. Compounds labeled with radioactive carbon were also obtained from commercial sources (Calbiochem; New England Nuclear Corp., Boston, Mass.). They were diluted to the desired specific activity and molarity with their respective unlabeled compounds. The conventional Warburg respirometer with flasks of a total capacity of about 22 ml was used in all except the last few experiments. The microorganisms, to which were added MgCl₂ (0.1 mM), MnCl₂ (0.02 mM), and substrate, were placed in the central compartment. When O₂ consumption or respiratory quotient was to be determined, substrates were placed in the side arm and were tipped in after allowing 15 min for thermal equilibration. The flasks were then incubated at 37 C for 2 hr in a gas phase of air. CO₂ was trapped with 0.2 ml of 40% KOH for the measurement of O₂ uptake, or of Hyamine (Packard Instrument Co., Inc., Downers Grove, Ill.) for the determination of CO₂ production; both were placed in the center well. All determinations were carried out in duplicate or triplicate. Control preparations consisted of complete reaction mixture either without microorganisms or with boiled microorganisms. Erlenmeyer flasks of 25-ml capacity were used in the last few experiments. The flasks were topped with rubber caps which supported small cups containing 0.2 ml of Hyamine in a position 1 to 2 cm above the level of the reaction mixture (6). The flasks were

incubated in a metabolic shaker. At the end of the period of incubation, when conventional Warburg flasks were used, Hyamine was diluted in methanol, 2 ml of which were placed in counting vials to which was added a scintillation mixture consisting of 0.6% 2,5-diphenyloxazole (PPO, Packard Instrument Co.) and 0.01% 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP, Packard Instrument Co.) in toluene. When Erlenmeyer flasks were used, the plastic cups were placed into the counting vial and processed as above. The results, unless otherwise indicated, are expressed in terms of millimicromoles or micromoles of O₂ taken up or CO₂ produced per milligram of rickettsial protein. It was calculated from the disintegrations per minute (dpm, counts per min divided by efficiency of counting), the specific activity of the substrates, and the number of carbon atoms labeled. The dpm of the control groups (complete reaction mixture without microorganisms or with boiled microorganisms) usually amounted to approximately 50 dpm, a small fraction of the total. They were subtracted from those of test groups. For the determination of glutamine uptake, 0.5-ml samples were taken from the flasks at desired intervals and were immediately washed on membrane filters (pore size, 0.3 μ ; Millipore Filter Corp., Bedford, Mass.). The membranes were allowed to dry in air. They were then placed in the counting vials, to which 15 ml of Bray's (4) scintillation mixture were added, and the radioactivity was determined as above.

In experiments requiring chromatographic separation of the reaction mixture, relatively high concentrations of microorganisms were used. The reaction was stopped by adding 2.5 M perchloric acid, and the precipitate was removed by centrifugation. Excess acid in the supernatant fluid was eliminated as potassium salt by adding 2.5 M KHCO₃, followed by centrifugation. Volumes of 2.5 μ liters of the supernatant fluid were separated on plates of cellulose powder according to the technique of Myers and Huang (10). Radioactivities of the isolated compounds were determined by the method of Snyder and Stephens (16) and are expressed as the dpm obtained.

RESULTS

Properties of rickettsiae harvested from fluid medium. During the first part of this study, metabolic experiments were conducted with *R. quintana* harvested from blood-agar. Cells thus obtained proved unsatisfactory, because considerable variation in metabolic activity was observed from experiment to experiment. Therefore, an attempt was made to harvest the microorganisms in their logarithmic phase of growth in a fluid medium. The number of viable cells increased approximately eightfold in 24 hr (Fig. 1) in this medium (see Materials and Methods). This increase in viability paralleled an increase in metabolic activity, as measured by testing samples of the culture for ¹⁴CO₂ production from added randomly labeled glutamine-¹⁴C. However, in an experiment not shown in Fig. 1, after 24 hr of in-

FIG. 1. Growth and glutamine metabolism of *Rickettsia quintana*. *R. quintana* was grown in fluid medium, and portions were removed at intervals and tested for number of viable cells (●) and for glutamine metabolism (○). The procedure described in Materials and Methods was followed, except that the microorganisms were not washed.

cubation this agreement was lost, owing to gradual loss of metabolic activity. By proper timing, it became possible, however, to harvest the microorganisms while this agreement still existed. The results were highly reproducible with this type of preparation.

The final suspension, after washing, appeared homogeneous. Each Warburg flask usually received 1.2 to 2.5 mg of cell protein, with a viable cell count ranging from 1.2×10^{10} to 3.4×10^{10} . Some extraneous material could still be discovered microscopically, but the culture medium which routinely served as a control in metabolic experiments did not contribute measurable enzyme activity.

Factors affecting metabolic activity. *R. quintana* failed to produce CO_2 from randomly labeled glucose- ^{14}C in three experiments. Glutamate was utilized, but less efficiently than glutamine; therefore, glutamine was used in most subsequent experiments.

Table 1 shows the influence of the composition of diluents on the utilization of glutamine. The diluent containing equivalent K^+ and Na^+ (KN) was as satisfactory as the one with K^+ . High Na^+ seemed to reduce CO_2 slightly, and sucrose appeared to enhance it, but the differences among these diluents were of doubtful significance. Consequently, KN was used as the diluent of choice.

The concentration of glutamine also affected the rate of glutamine utilization (Fig. 2). Within the range of concentrations tested (from 1.0 to 30 mM), CO_2 production increased gradually

from 0.3 to 0.9 μmole per hr per mg of protein. Most of subsequent experiments were done with 30 mM substrates.

Respiration. *R. quintana* respired at a rate of 1.0 μliter of O_2 per hr per mg of protein in the absence of added substrate. Glutamine and succinate stimulated O_2 uptake to 18 and 34 μliters , respectively. Fumarate, malate, and *cis*-aconitate failed to elicit O_2 uptake. The respiratory quotient for glutamine, determined in a series of three experiments, was 1.1 to 1.2, suggesting that part of the CO_2 evolution from glutamine was anaerobic. In three subsequent experiments under anaerobic conditions, CO_2 was indeed produced

TABLE 1. Glutamine utilization by *Rickettsia quintana* in different diluents

Principal components of diluent	CO_2 production (μmoles per hr per mg of protein) ^a			Mean
K, Na ^b	0.62,	0.55,	0.57	0.58
K ^c	0.58,	0.57,	0.57	0.57
Na ^d	0.49,	0.48,	0.48	0.48
Sucrose ^e	0.67,	0.65,	0.65	0.66

^a Values represent triplicate specimens.

^b KCl, 0.07 M; NaCl, 0.07 M; K phosphate buffer, 0.01 M; pH 7.0 (KN).

^c KCl, 0.12 M; NaCl, 0.02 M; K phosphate buffer, 0.01 M; pH 7.0.

^d NaCl, 0.14 M; K phosphate buffer, 0.01 M; pH 7.0.

^e Sucrose, 0.24 M; NaCl, 0.02 M; K phosphate buffer, 0.01 M; pH 7.0.

FIG. 2. Effect of the concentration of glutamine on its utilization by *Rickettsia quintana*. Each flask contained in a total volume of 2.0 ml: washed cells (1.3 mg of protein), KN diluent, Mn^{++} , Mg^{++} , and glutamine in the amounts shown and containing 40 μ c of randomly labeled glutamine- ^{14}C .

from glutamine at a rate 25% of that under aerobic conditions. This anaerobic pathway of glutamine utilization was not studied in detail.

Pathway of glutamine utilization: utilization of substrates of intermediary metabolism. Table 2 shows that glutamate, α -ketoglutarate, succinate, citrate, and pyruvate were utilized by *R. quintana* to various extents. Glutamate was utilized at a rate only 13 to 17% that of glutamine. Experiments with glutamate labeled in position 1, 3, 4,

TABLE 2. Substrate utilization by *Rickettsia quintana*

Expt no.	Substrate	Carbon position labeled	CO ₂ production (μ moles per hr per mg of protein) ^a
1	Glutamate	All	0.2
	Glutamine	All	1.2
2	Glutamate	All	0.5
	Glutamate	1	0.1
	Glutamate	3,4	0.2
	Glutamate	5	0.02
3	Glutamine	All	1.5
	Pyruvate	1	0.1
	Pyruvate	3	0.02
4	Glutamine	All	1.2
	Succinate	1,4	3.0
5	Glutamine	All	2.1
	α -Ketoglutarate	5	0.4
	Citrate	1,5	0.02

^a Mean of triplicate experiment.

and 5 indicated that CO₂ was produced from all carbon atoms. (Carbon 2 is identical to carbon 5 after the first decarboxylation.) Pyruvate was also utilized. Assuming that the amount of CO₂ produced from carbon position 2 was about the same as that produced from carbon 3, it can be calculated that pyruvate was utilized at a rate one-eighth to one-tenth that of glutamine. Succinate was utilized at a rate considerably higher than glutamine. CO₂ produced from carbons 1 and 4 of succinate was more than twice as high as that evolved from all carbons of glutamine. α -Ketoglutarate also seemed to be a useful substrate, although only the CO₂ produced from carbon 5 was measured. CO₂ production from carbons 1 and 5 of citrate was 1% of that obtained from glutamine.

As shown in Table 3, arsenite and malonate strongly inhibited CO₂ production from glutamine. No attempt was made, however, to detect the accumulation of α -ketoglutarate and succinate after inhibition.

Effects of added unlabeled substrates on glutamine utilization. Glutamate, α -ketoglutarate, and pyruvate inhibited 60% and malate inhibited 85% of CO₂ production from glutamine (Table 4). Oxaloacetate at high concentrations (ratio of glutamine to substrate = 1:25 and 1:100) was strongly inhibitory; at a lower concentration (ratio = 1:10), it enhanced the utilization of glutamine. Succinate and fumarate did not affect CO₂ production. As expected from the cellular impermeability to tricarboxylic acids, citrate and isocitrate did not show any effect on glutamine metabolism. However, *cis*-aconitate, in contrast to citrate, strongly inhibited glutamine utilization.

Recovery of labeled intermediates. The reaction mixtures of experiment 6 were chromatographed, and the results are presented in Table 5. Radioactivity increased markedly in the spots identified by carrier glutamate, α -ketoglutarate, and succinate when the homologous substrate was added to glutamine- ^{14}C in reaction mixture. Labeled

TABLE 3. Effect of metabolic inhibitors on glutamine^a utilization by *Rickettsia quintana*

Inhibitor	Concn	CO ₂ production (μ moles per hr per mg of protein) ^b	Per cent of control
	<i>M</i>		
None		0.68	100
Arsenite	1.7×10^{-3}	0.06	9
Malonate	2.5×10^{-2}	0.04	7

^a Glutamine concentration was 0.0065 M.

^b Means of triplicate experiment.

fumarate and malate were also isolated when either unlabeled α -ketoglutarate or succinate was added, and succinate was labeled by adding unlabeled α -ketoglutarate.

TABLE 4. Effect of added citric acid cycle intermediates and other substrates on the utilization of glutamine^a by *Rickettsia quintana*

Expt no.	Added unlabeled substrate	Ratio of glutamine to added substrate	Amt (mumoles) of glutamine utilized per hr per mg of protein ^b	Per cent of control
6	None		221	
	Glutamate	1:10	91	41
	α -Ketoglutarate	1:10	80	36
	Succinate	1:10	195	88
7	None		90	
	Oxaloacetate	1:100	0	0
	Oxaloacetate	1:25	27	30
	Oxaloacetate	1:10	117	130
	Pyruvate	1:10	40	44
8	None		150	
	Citrate	1:25	147	98
	Citrate	1:10	151	101
9	None		159	
	Fumarate	1:10	162	102
	Malate	1:10	30	18
	<i>Cis</i> -aconitate	1:10	0.3	0.2

^a Glutamine, 0.00125 M, containing 1 μ C of randomly labeled glutamine-¹⁴C.

^b Mean of triplicate experiment.

TABLE 5. Pathway of glutamine utilization by *Rickettsia quintana*^a

Compound isolated and assayed	Added unlabeled substrate			
	None	Glutamate	α -Ketoglutarate	Succinate
Glutamate.....	261 ^b	453	378	236
α -Ketoglutarate	5	5	164	15
Succinate.....	75	82	144	552
Fumarate.....	50	56	139	194
Malate.....	52	56	260	302
Oxaloacetate...	8	8	10	8
Citrate.....	8	5	15	15
<i>Cis</i> -aconitate...	3	6	8	9
Isocitrate.....	4	7	7	13

^a Cells were incubated for 2 hr at 37 C with 0.00125 M randomly labeled glutamine-¹⁴C and 0.0125 M substrate indicated.

^b Values indicate the mean disintegrations per minute of each compound in 2.5 μ liters of triplicate specimens.

FIG. 3. Inhibitory effect of *cis*-aconitate on the uptake of glutamine by *Rickettsia quintana*. Cells were incubated with randomly labeled glutamine-¹⁴C in the presence (○) or absence (●) of *cis*-aconitate. Samples of 0.5 ml of the reaction mixture were taken at intervals and immediately filtered and washed with KN. Each point represents the mean of triplicate determinations.

In other experiments not shown on Table 5, no increase in the ¹⁴C label of intermediates was obtained by the addition of unlabeled fumarate, malate, *cis*-aconitate, or isocitrate.

Effect of *cis*-aconitate on glutamine uptake. An experiment was carried out to study the strong inhibitory action of *cis*-aconitate on ¹⁴CO₂ production from glutamine-¹⁴C. As shown in Fig. 3, the uptake and accumulation of labeled glutamine, which increased linearly with time in the absence of *cis*-aconitate, were prevented by its presence.

Transaminase activity. Since evidence of a glutamate-oxaloacetate transaminase was obtained in other rickettsiae (1), an attempt was made to demonstrate the accumulation of aspartate as a by-product of the glutamine utilization in *R. quintana*. Since succinate is vigorously utilized by *R. quintana*, it was assumed that it would provide a good source of oxaloacetate. Thus, cells were incubated with succinate-1,4-¹⁴C, with or without unlabeled glutamine, serving as the donor of the amino group. The results, expressed as the means of triplicate tests, are presented in Fig. 4. The aspartate spots showed radioactivities of 18 and 120 dpm in the absence and presence of added glutamine, respectively. This difference is believed to be highly significant.

DISCUSSION

Like other rickettsiae, *R. quintana* failed to utilize glucose. The mechanism of such failure is

FIG. 4. Recovery of labeled aspartate by chromatography. Cells (0.8 mg of protein) were incubated for 2 hr with 0.006 M succinate-1,4- ^{14}C (3 μC) with or without 0.03 M unlabeled glutamine, and the reaction mixtures were processed for chromatography. Each figure represents average disintegrations per minute of spots identified by the indicated unlabeled compounds in triplicate determinations. Upper figure: with glutamine; lower figure: without glutamine. Background counts were subtracted. Solvent 1: ether-formic acid-water, 7:2:1. Solvent 2: phenol-water-formic acid, 75:25:1. Absorbent: cellulose powder.

still unknown. Paretsky et al. (13) showed that extracts of *Coxiella burnetii* phosphorylated glucose. Ormsbee and Weiss (12), however, failed to obtain any evidence of CO_2 production from glucose by intact *C. burnetii* cells with techniques similar to those presented here. Thus, it is possible that the inability of some rickettsiae to utilize glucose is due to impermeability rather than to lack of enzymes.

The results show that in *R. quintana* glutamine and succinate were utilized most actively. These substrates appear to be metabolized via the citric acid cycle, and most likely represent a chief energy source. The evidence for the presence of a citric acid cycle includes the following: (i) the utilization of glutamate, α -ketoglutarate, succinate, and citrate; (ii) the reduction of $^{14}\text{CO}_2$ production from glutamine- ^{14}C when unlabeled glutamate, α -ketoglutarate, and oxaloacetate are added; (iii) the recovery under the above conditions of labeled glutamate, α -ketoglutarate, succinate, fumarate, and malate; and (iv) the recovery of labeled aspartate from the reaction mixture containing succinate- ^{14}C and glutamine, which also indicates that a glutamate-oxaloacetate transaminase is present.

Except for slight utilization of citrate, no evidence was obtained of the involvement of the tricarboxylic acid portion of the cycle. The same is true of other rickettsiae (1, 11, 20). Since all this work was carried out with intact cells, however, such failure cannot be taken as evidence against the operation of this portion of the cycle.

When unlabeled intermediates were added to the reaction mixture with glutamine as the ^{14}C -labeled substrate, different types of effects on $^{14}\text{CO}_2$ production were observed. In the case of glutamate, α -ketoglutarate, and pyruvate, appreciable amounts of the substrates were apparently taken up, and the specific activities of the corresponding labeled compounds derived from glutamine- ^{14}C were diluted. The net result of this dilution was the reduction of $^{14}\text{CO}_2$ production and the accumulation of the labeled homologous compounds within the cells. Oxaloacetate produced a somewhat more complex effect. At high concentrations, it greatly reduced, whereas at lower concentration it enhanced, $^{14}\text{CO}_2$ production. In the former condition, the effect was probably the same as discussed for glutamate. In the latter condition, the role of oxaloacetate as the acceptor of the amino group apparently predominated; thus, the deamination of glutamate, as well as the utilization of glutamine, was accelerated. Succinate did not reduce $^{14}\text{CO}_2$ production to a great extent, since it was rapidly converted to oxaloacetate and produced a net result similar to that observed with the lower concentration of oxaloacetate. The failure of fumarate, citrate, and isocitrate to affect $^{14}\text{CO}_2$ production was attributed to cellular impermeability to these compounds. Such impermeability in rickettsiae was suggested by Bonvarnick and Miller (1) and by Wisseman et al. (20), who failed to demonstrate the oxidation of tricarboxylic acids by intact cells of typhus rickettsiae. Paretsky et al. (14) and Consigli and Paretsky (5), however, showed that disrupted cells of *C. burnetii*, unlike intact cells, formed citrate and oxidized isocitrate under certain conditions. *Cis*-aconitate and malate greatly reduced $^{14}\text{CO}_2$ production from glutamine, but none of the above explanations can be invoked. It was shown with *cis*-aconitate that the mechanism is an interference with glutamine uptake. Whether this phenomenon was due to a steric hindrance or a biochemical effect is not clear, but this type of interference by one substrate with the uptake of another may well affect cell growth on a given medium. *Rickettsia quintana* must carry out deamination or transamination as a necessary step for glutamine utilization via the citric acid cycle. The recovery of labeled aspartate clearly shows that *R. quintana*, like typhus rickettsiae (1), possesses a glutamate-oxaloacetate transaminase.

This microorganism may also possess a glutamic acid dehydrogenase, but no attempt was made to determine its activity. The presence of such an enzyme has been clearly demonstrated in other rickettsiae (1, 14).

In summary, in *R. quintana* glutamine is deamidated to glutamate, which by the action of the transaminase gives off the amino group to oxaloacetate and enters the citric acid cycle. Such metabolic reactions of *R. quintana*, along with its failure to catabolize glucose, are qualitatively indistinguishable from those occurring in other rickettsiae.

It is tempting to seek from these results clues for the understanding of the progression of rickettsiae from extracellular to intracellular parasitism. It has been shown that typhus rickettsiae require high K^+ for stability and metabolic activity (2). The finding that *R. quintana* does not require a high K^+/Na^+ ratio may indicate that this microorganism is less adapted to the intracellular ionic environment than are typhus rickettsiae. Moulder (9) pointed out that the adaptation of a microorganism to intracellular parasitism requires the acquisition of permeability to large and more highly charged molecules. This study showed that *R. quintana* utilizes glutamine more actively than glutamate, which, although more highly charged, is the preferred substrate of the typhus rickettsiae. Thus, it appears that the extracellular growth of *R. quintana* and the intracellular growth of typhus rickettsiae, both in vitro and in vivo, are reflected in subtle biochemical differences.

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LITERATURE CITED

- BOVARNICK, M. R., AND J. C. MILLER. 1950. Oxidation and transamination of glutamate by typhus rickettsiae. *J. Biol. Chem.* **184**:661-676.
- BOVARNICK, M. R., J. C. MILLER, AND J. C. SNYDER. 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. *J. Bacteriol.* **59**:509-522.
- BOVARNICK, M. R., AND J. C. SNYDER. 1949. Respiration of typhus rickettsiae. *J. Exptl. Med.* **89**:561-565.
- BRAY, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.* **1**:279-285.
- CONSIGLI, R. A., AND D. PARETSKY. 1962. Oxidation of glucose-6-phosphate and isocitrate by *Coxiella burnetii*. *J. Bacteriol.* **83**:206-207.
- FAIN, J. N., R. O. SCOW, AND S. S. CHERNICK. 1963. Effects of glucocorticoids on metabolism of adipose tissues in vitro. *J. Biol. Chem.* **238**:54-58.
- ITO, S., AND J. W. VINSON. 1965. Fine structure of *Rickettsia quintana* cultivated in vitro and in the louse. *J. Bacteriol.* **89**:481-495.
- 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.
- MOULDER, J. W. 1962. The biochemistry of intracellular parasitism, p. 43-83. Univ. of Chicago Press, Chicago.
- MYERS, W. F., AND K. HUANG. 1966. Separation of intermediates of the citric acid cycle and related compounds by thin-layer chromatography. *Anal. Biochem.* **17**:210-213.
- ORMSBEE, R. A., AND M. G. PEACOCK. 1964. Metabolic activity in *Coxiella burnetii*. *J. Bacteriol.* **88**:1205-1210.
- ORMSBEE, R. A., AND E. WEISS. 1963. Trachoma agent: glucose utilization by purified suspensions. *Science* **142**:1077-1078.
- PARETSKY, D., R. A. CONSIGLI, AND C. M. DOWNS. 1962. Studies on the physiology of rickettsiae. III. Glucose phosphorylation and hexokinase activity in *Coxiella burnetii*. *J. Bacteriol.* **83**:538-543.
- PARETSKY, D., C. M. DOWNS, R. A. CONSIGLI, AND B. K. JOYCE. 1958. Studies on the physiology of rickettsiae. I. Some enzyme systems of *Coxiella burnetii*. *J. Infect. Diseases* **103**:7-11.
- PRICE, W. H. 1953. The epidemiology of Rocky Mountain spotted fever. I. The characterization of strain virulence of *Rickettsia rickettsii*. *Am. J. Hyg.* **58**:248-268.
- SNYDER, F., AND N. STEPHENS. 1962. Quantitative carbon-14 and tritium assay of thin-layer chromatography plates. *Anal. Biochem.* **4**:128-131.
- VINSON, J. W. 1964. Etiology of trench fever in Mexico. *Ind. Trop. Health* **5**:109-114.
- 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. Suppl.* **24**:152-166.
- WEISS, E., H. B. REES, JR., AND J. R. HAYES. 1966. Metabolic activity of purified suspensions of the Rocky Mountain spotted fever rickettsia. *Nature*, in press.
- WISSEMAN, C. L., JR., F. E. HAHN, E. B. JACKSON, F. M. BOZEMAN, AND J. E. SMADEL. 1952. Metabolic studies of rickettsiae. II. Studies on the pathway of glutamate oxidation by purified suspensions of *Rickettsia mooseri*. *J. Immunol.* **68**:251-264.
- WOLBACH, S. B., J. L. TODD, AND F. W. PALFREY. 1922. The etiology and pathology of typhus. Harvard Univ. Press, Cambridge, Mass.