FIXATION OF $C^{14}O_2$ INTO NUCLEIC ACID CONSTITUENTS BY BRUCELLA ABORTUS[1]

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In a variety of animal tissues, the synthesis of nucleic acid constituents can take place from elementary precursors; among these, carbon dioxide and glycine have been demonstrated to be incorporated into purines of many higher animals as well as yeast (Buchanan, 1951). Relatively little direct evidence is available on the precursors of nucleic acid constituents in bacteria. Sutton *et al.* (1951) reported the incorporation of glycine into purines of *Aerobacter aerogenes*, and recently Bolton *et al.* (1952) have shown that carbon dioxide is a precursor of the nucleic acid purines and pyrimidines of *Escherichia coli*.

The synthesis of nucleic acid constituents in *Brucella abortus* is of particular interest because many strains of the organism require added carbon dioxide for growth, and glycine appears to be a major product of CO_2 fixation in such strains (Marr and Wilson, 1951). However, neither glycine nor any compound or complex mixture yet tested will replace added CO_2 for growth of *B. abortus*.

We have investigated the fixation of $C^{14}O_2$ into nucleic acid constituents by *B. abortus* to examine further the biochemical basis of the need for CO_2 by members of this species.

EXPERIMENTAL METHODS

Brucella abortus, strain 6232, was grown on tryptose agar slants at 37 C in an atmosphere of ten per cent CO_2 in air. Cells were harvested at 15 to 17 hours, washed, and resuspended in the test media. Growing cells were exposed to $C^{14}O_2$ in 125 ml Warburg vessels with siamese side arms for generation of $C^{14}O_2$ from $BaC^{14}O_3$. Standard manometric equipment was used with the bath temperature at 34 C. At the end of an experiment, cells were killed and bound $C^{14}O_2$ released by addition of 6 N H_2SO_4 to a final concentration of about 0.1 N. The $C^{14}O_2$ was recovered by flushing it into excess 2 N NaOH with N_2. The cells were recovered then by centrifugation, washed with distilled water, and lyophilized. The dried cells were extracted with hot 80 per cent ethanol and the residue re-extracted with 5 per cent trichloroacetic acid at 90 C for 30 minutes (Schneider, 1945) to remove cellular nucleic acids. Fractions were diluted and counted for total activity. The trichloroacetic acid extract was boiled momentarily to remove most of the volatile acid and then reduced to dryness *in vacuo* at 60 C. Purines and pyrimidines were released by hydrolysis with aqueous perchloric acid (Marshak and Vogel, 1951). Both the trichloroacetic acid extract and centrifuged hydrolyzate were counted for radioactivity and analyzed for purine-pyrimidine content by measuring extinction at 260 mμ in a Beckman model D.U. spectrophotometer. No loss of radioactivity or ultraviolet absorbing material occurred on hydrolysis. Purines and pyrimidines then were separated chromatographically on columns of "dowex 50" by the method of Cohn (1949) as modified by Wall (1953) (figure 1). Aliquots of fractions were counted for radioactivity and examined spectrophotometrically at 260 mμ. Fractions containing the separated bases were concentrated repeatedly *in vacuo* to remove the HCl and chromatographed on paper using a variety of solvents (Vischer and Chargaff, 1948); spots were identified with a "mineralight" lamp (Goeller and Sherry, 1950). The isolated constituents were considered pure based on their chromatographic analysis and ultraviolet absorption spectra. The bases were counted for radioactivity at "infinite thinness", and their concentration was estimated by determining extinction at the absorption maximum for each base.

Uracil was degraded by the method of Heinrich and Wilson (1950). Guanine was degraded by the procedure of Abrams *et al.* (1948). Prod-

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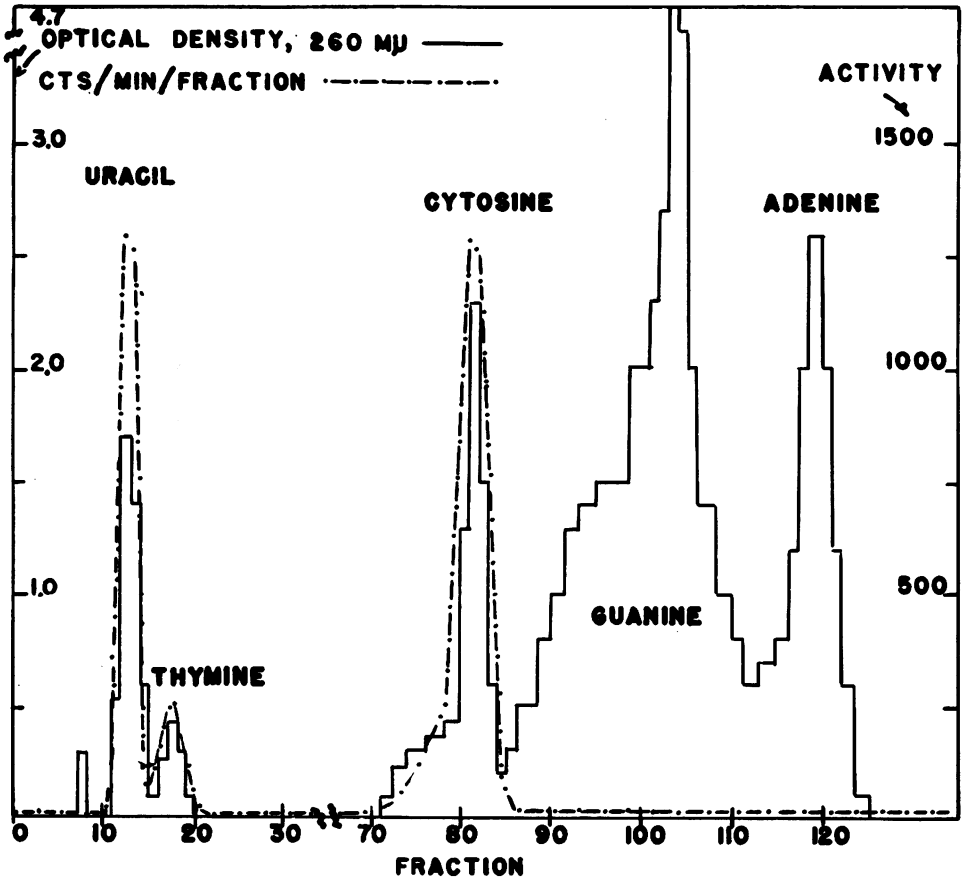

Figure 1. Chromatographic separation of purines and pyrimidines from the cells of *Brucella abortus* exposed to $C^{14}O_2$ in Albimi broth. Column; "dowex 50", 200 to 400 mesh, H-ion form, 50 cm by 1.5 cm; flow rate 35 ml per hour. Irrigated with HCl; 1.5 N (100 ml), 2.5 N (200 ml), 4.0 N (100 ml), 6.0 N (150 ml). Fractions, 5.0 ml volume. Radioactivity measurements are uncorrected for self absorption of the samples.

ucts of the degradations were counted as $BaCO_3$. All radioactivity measurements were made with a flowing-gas Geiger counter, and appropriate corrections for self absorption and background were made.

RESULTS

Fixation of $C^{14}O_2$. The distribution of C^{14} in cells of *B. abortus* exposed to $C^{14}O_2$ in brucella Albimi broth is given in table 1. In agreement with the findings of Marr and Wilson (1951) most of the radioactivity was found in the protein fraction of the cells. The activity in the nucleic acid purines and pyrimidines from the trichloroacetic acid extract of *B. abortus* is given in table 2.

The absence of C^{14} in purines is in direct contrast with results obtained with other organisms and suggested that either a different pathway of purine biosynthesis was operative in *B. abortus* or that CO_2 fixation into purines of *B. abortus* was quantitatively insignificant under these conditions. Uracil was combined with inactive carrier and degraded chemically. Data in table 3 show that radioactivity was found in the ureide carbon atom only.

It appeared possible that some constituent of the complex medium was suppressing CO_2 fixation into purines. Exogenous purines and pyrimidines have been shown to inhibit CO_2 fixation into nucleic acid constituents by *E. coli* (Bolton *et al.*, 1952). Consequently, the fixation of $C^{14}O_2$ into the nucleic acid constituents of cells

TABLE 1

*Distribution of C^{14} in the cells of Brucella abortus, strain 6232, exposed to $C^{14}O_2$ in Albimi broth**

FRACTION†	TOTAL ACTIVITY
	counts/min
Ethanol extract	6,450
Trichloroacetic acid extract	34,350
Residue	231,400

* Growing cells exposed to (0.1 atm) $C^{14}O_2$ in Albimi brucella broth 2 hours at 34 C. Activity of $C^{14}O_2$; 1×10^8 cts/min/mg C.

† 80 mg dried cells extracted.

TABLE 2

Specific activities of nucleic acid purines and pyrimidines from Brucella abortus exposed to $C^{14}O_2$ in Albimi broth

COMPOUND*	COUNTS/ MIN/μMOLE†
Uracil	3,820
Thymine	1,692
Cytosine	2,200
Guanine	0
Adenine	0

* Center cuts of ultraviolet absorption peaks.

† Determined spectrophotometrically and counted at "infinite thinness".

TABLE 3

Location of radioactivity in the uracil molecule from Brucella abortus exposed to $C^{14}O_2$

COMPOUND ISOLATED	COUNTS/ MIN/MG C†
CO_2	0
Urea (C no. 2)	1,080
Oxalate	0

Carrier uracil was added; data include carrier carbon.

† All compounds counted as $BaCO_3$.

growing in a synthetic medium was investigated. The data in table 4 show that cells growing in a synthetic medium also fix $C^{14}O_2$ principally into pyrimidines; however, there is slight incorporation of C^{14} into purines under these conditions.

Since glycine-$1C^{14}$ is formed rapidly from $C^{14}O_2$ by growing cells of *B. abortus* in either complex or synthetic media (Marr and Wilson, 1951) and the carboxyl carbon of glycine is incorporated into carbon atom number four of purines in other organisms (Buchanan, 1951), guanine from cells grown in the synthetic medium was degraded in a manner permitting specific isolation of carbon atom four. Twenty per cent of the radioactivity in guanine could be recovered in carbon atom four.

TABLE 4

Activities of purines and pyrimidines from cells exposed to $C^{14}O_2$ in synthetic medium

COMPOUND	COUNTS/ MIN/μMOLE
Uracil	1,576
Thymine	1,024
Cytosine	1,216
Adenine	96
Guanine	471

Growing cells exposed to $C^{14}O_2$ 6 hours in the medium of Gerhardt and Wilson (1948) as modified by Marr and Wilson (1951).

DISCUSSION

In *B. abortus* there is quantitative digression from the pattern of purine synthesis established in other organisms. This is attested to by the finding that carbon dioxide, which is rapidly incorporated into nucleic acid purines of all other organisms which have been studied previously, is utilized only slowly for purine biosynthesis by *B. abortus*.

Our results show that the major products of carbon dioxide fixation in the nucleic acids of *B. abortus* are pyrimidines. Fixation of $C^{14}O_2$ into purines of *B. abortus* is quantitatively insignificant. It is indeed remarkable that an organism which requires added carbon dioxide for growth is slow in catalyzing the fixation of CO_2 into purines (Buchanan, 1951), which is a biochemical reaction characteristic of a wide variety of animal tissues and lower organisms.

Uracil biosynthesis in *Brucella* appears to be similar to the systems which have been investigated in mammals (Heinrich and Wilson, 1950) in that carbon dioxide is incorporated into the ureide carbon atom. These findings suggest that uracil biosynthesis may occur via cyclization of a precursor which contains a carbamyl group formed from CO_2. In this connection it is interesting to note that evidence for a urea cycle in

B. abortus has been presented recently by Cameron *et al.* (1952) who found the accumulation of citrulline by resting cells incubated in the presence of glutamate.

Since all of the radioactivity in guanine could not be recovered in carbon atom number four, there appears to be several pathways for the entry of CO_2 into the purine in contrast with other organisms which fix CO_2 into carbon atom number six only. Carboxyl labeled glycine synthesized by the organism from $C^{14}O_2$ enters the purine, labeling it in position four, but another synthetic path must account for the other 80 per cent of the radioactivity in the molecule. Neither pathway leads to a very rapid incorporation of $C^{14}O_2$.

Although these findings provide no direct explanation of the requirement for added CO_2 by *B. abortus*, they indicate that pyrimidine biosynthesis from CO_2 may be more obligatory in added CO_2 requiring *Brucella* than it is in other heterotrophic organisms, such as *E. coli* (Bolton *et al.*, 1952) which preferentially uses exogenous pyrimidines for nucleic acid synthesis. Cells of *B. abortus*, strain 6232, growing in complex media in an atmosphere of CO_2 are given a choice of pyrimidine precursors, yet they continue to synthesize pyrimidines from carbon dioxide.

SUMMARY

The major products of CO_2 fixation in nucleic acids of *Brucella abortus*, strain 6232, are pyrimidines.

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