STUDIES ON THE FORMATION OF FORMATE, GLYCINE, SERINE, PYRUVATE AND ACETATE FROM PURINES BY *CLOSTRIDIUM ACIDI-URICI*[1]

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Clostridium acidi-urici and *Clostridium cylindrosporum* derive their entire carbon, nitrogen, and energy supplies by the degradation of these purines: uric acid, guanine, xanthine, and hypoxanthine. End products of the degradation are ammonia, carbon dioxide, and acetate (Barker, 1938; Barker and Beck, 1941; 1942). The pathways by which these compounds are formed are not entirely known, but some intermediates have been characterized (Rabinowitz and Barker, 1953; Rabinowitz and Pricer, 1954; 1955; Radin and Barker, 1953; Bradshaw and Beck, 1953; Sagers and Beck, 1955*a*, *b*).

In earlier tracer studies, Karlsson and Barker (1949), using whole cells of *C. acidi-urici*, showed that the methyl carbon of acetate was derived mainly from the number 8 carbon of uric acid, while the carboxyl carbon of acetate was formed mainly from the number 5 carbon of uric acid. The 2, 4, and 6 carbons of the purine were converted to carbon dioxide.

The observation that glycine and formate appear in the reaction mixtures during purine breakdown by preparations of *C. acidi-urici* (Radin and Barker, 1953; Bradshaw and Beck; 1953; Sagers and Beck, 1955*a*, *b*), and the further observation that serine is converted to pyruvate by this organism (Radin and Barker, 1953) suggests that glycine and formate might be condensed to form serine, which would then be converted to pyruvate. Pyruvate upon oxidation could yield acetate and carbon dioxide, the carbon-containing end products of the purine degradation.

This paper presents results which show that the isotopic distribution in the degradation of various C^{14}-labeled substrates by intact cells of *C. acidi-urici* is that predicted by the following reaction sequence:

$$\text{Purine} \rightarrow \text{formate} + \text{glycine} \rightarrow \text{serine} \rightarrow \text{pyruvate} \rightarrow \text{acetate} + CO_2.$$

METHODS AND MATERIALS

Cell suspensions of *C. acidi-urici* were prepared from 24-hr cultures grown on a medium of the following composition, in g per L: uric acid, 2.0; yeast extract, 0.5; K_2HPO_4, 0.5; and magnesium sulfate, 0.2. Sufficient sodium hydrosulfite was added to the medium and to cell suspensions to render them oxygen-free. Cells were harvested by centrifugation and, after two washings, were suspended in 0.07 M phosphate buffer at pH 7.3–7.5. Suspensions were standardized to contain from 0.9 to 1.1 mg nitrogen per ml.

Radioactivity was determined by use of a Tracerlab Windowless Flow Counter, the performance of which was checked frequently with a standard C^{14} sample. Barium carbonate or other radioactive material was usually mounted on filter paper discs which were secured to brass planchets for easy handling and accurate weighing. In some instances liquid samples were evaporated to dryness in steel planchets. Corrections were made for background counts and self-absorption.

Quantitative determination of products. Ammonia and *volatile* acids were determined as previously outlined (Barker and Beck, 1941). The distillate, following steam distillation of acidified reaction mixtures, was evaporated to dryness at pH 9 and formic acid determined according to the method of Perlin (1954). In tracer studies the carbon dioxide produced from the oxidation of formate with lead tetraacetate was collected as barium carbonate and the radioactivity determined.

When it was desirable to remove formate

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from the volatile acid mixtures, the material was distilled from mercuric oxide, according to the method of Friedemann (1938). Traces of pyruvate also are destroyed by this procedure.

Carbon dioxide was determined by use of either the Warburg or the van Slyke manometric apparatus.

To determine the radioactivity of CO_2, the acidified mixture was swept with CO_2-free air and the carbon dioxide collected in 0.2 N barium hydroxide. The barium carbonate obtained was collected on a filter paper disc and washed thoroughly with freshly boiled distilled water and with ethanol, 95 per cent. The samples were dried in a vacuum desiccator.

To remove "contaminating" radioactive carbon dioxide from a mixture, CO_2-free air was bubbled for 30 min through the material, which had been brought to pH 3 by adding acid.

Pyruvate was estimated by the method of Friedemann and Haugen (1943). For radioactivity determinations it was isolated as the 2,4-dinitrophenylhydrazone. The procedure of Kavamme and Hellman (1954) was used when necessary to avoid contamination with radioactive acetate, which is strongly adsorbed by the hydrazone. However, advantage was usually taken of the lower steam volatility of pyruvate as compared to acetate and formate. The latter acids were removed quantitatively from the reaction mixtures by steam distillation, leaving about 40 per cent of the pyruvate behind and available for study. Other radioactive compounds used or formed in these investigations are not adsorbed by the 2,4-dinitrophenylhydrazone.

The *radioactivity* of individual carbon atoms of *pyruvate* and *acetate* was estimated as follows. The carboxyl group of pyruvate was removed by oxidation with ceric sulfate (Krebs and Johnson, 1937). The acetate in the residue, or other acetate, was recovered by steam distillation and degraded in a stepwise manner using sodium azide (Phares, 1951). Each of the carbons is thus oxidized to carbon dioxide, and recovered as barium carbonate. In preliminary studies, acetate was degraded by pyrolysis of the barium salt, the methyl group of acetate being recovered as iodoform. Since this volatile compound was found to interfere with the operation of the gas flow counter, this method was abandoned and the azide method adopted.

Glycine was isolated either by ascending paper chromatography, using a phenol-water solvent system as described by Block *et al.* (1952), or by Dowex column chromatography, as outlined by Moore and Stein (1948; 1951). Glycine was estimated quantitatively by the ninhydrin reaction.

Spectrophotometric analyses were performed with a Beckman Model DU spectrophotometer.

RESULTS

Preliminary experiments on the metabolism of glycine and formate. Inasmuch as glycine and formate had been shown to accumulate during purine degradation by cell-free preparations of of *C. acidi-urici* (Radin and Barker, 1953; Bradshaw and Beck, 1953; Sagers and Beck, 1955*a*, *b*), but not by whole cells, the most logical conclusion is that whole cells are able to metabolize free glycine and formate. Manometric experiments were carried out in which glycine and formate together were incubated anaerobically with cell suspensions. Neither carbon dioxide nor ammonia was produced. Addition of folic acid or leucovorin to the mixtures had no effect. These results indicate that formate and glycine are not decomposed under the conditions employed. Radin and Barker (1953) showed that with a suitable hydrogen carrier system, either glycine or formate supplied separately is oxidized by whole cells.

Inhibition studies. It was noted that during the normal catabolism of either xanthine or uric acid by whole cells, very small amounts of pyruvate appeared in the reaction mixture. This was the first indication that this compound occurred as a reaction product when a purine was the only substrate. Degradation of 40 μmoles of either xanthine or uric acid produced pyruvate up to 2–3 μmoles. The pyruvate was detectable after 5–10 min of reaction time, but did not increase beyond the above value during a 2-hr incubation. This small amount of pyruvate was suggestive of its possible intermediary role in purine degradation.

Various inhibitors or trapping agents were used in order to accumulate larger amounts of pyruvate or other intermediate products. Compounds used were sodium bisulfite, sodium salicylate, sodium arsenite, sulfathiazole, penicillin, and streptomycin. Of these compounds, only arsenite and sulfathiazole proved of interest. Table 1 shows the results of experiments in which whole cells were preincubated with arsenite or sulfathiazole for 20 min prior to the addition of xanthine. It

TABLE 1

The effect of inhibitors on the accumulation of glycine and formic acid from xanthine by cell suspensions of Clostridium acidi-urici

Tube No.	Inhibitor	Substrate	Products			
		Xanthine	NH_3	Glycine	Formate	Pyruvate
		μmole	*μmole*		*μmole*	*μmole*
1	None	40	138	None	5.4	2.2
2	Arsenite 0.00025 M	40	95	++	—*	Trace
3	Arsenite 0.0005 M	40	74	++	11.7	None
4	Arsenite 0.001 M	40	34	+	—	None
5	Arsenite 0.0005 M	None	5.4	None	None	None
6	None	40	135	None	5.4	2.4
7	Sulfathiazole 0.0004 M	40	132	+	—	Trace
8	Sulfathiazole 0.002 M	40	129	++	19.1	None
9	Sulfathiazole 0.006 M	40	92	++	—	None
10	Sulfathiazole 0.006 M	None	5.8	None	None	None

Cells were preincubated with the inhibitors for 20 min prior to addition of the substrate. Incubated 35 C, for 60–90 min in 0.05 M phosphate buffer (pH 7.3) in evacuated Thunberg tubes; 2 ml cell suspension. Total volume 8 ml.

* Not determined.

will be noted that in the absence of inhibitor, small amounts of pyruvate occur as a product but, when inhibitor is present, no pyruvate occurs. Glycine occurs in the presence of arsenite or sulfathiazole, but not in their absence. As the concentration of arsenite is increased there is an apparent over-all inhibition which results in almost complete inactivity of the cells. Spectrophotometric analysis showed that in tubes 1, 2, 6, 7, and 8 all xanthine had disappeared, whereas in tubes 3 and 4 large quantities, and in tube 9 a small amount of xanthine remained. The ammonia yields, however, indicated that some of the nitrogen was still in a form other than ammonia, and in each of these instances paper chromatography showed glycine to be present. Analysis of the reaction mixtures showed that some formic acid appeared in all flasks, but that considerably more formate was present in the tubes containing inhibitor than in those without inhibitor. The observation that sulfathiazole produces some degree of inhibition of purine degradation by *C. acidi-urici* is of interest, especially in the light of the accumulation of glycine and formate, apparently, at the expense of pyruvate. The inhibition of pteroylglutamic acid derivatives by sulfonamides possibly may be involved. In the normal degradation process, formate may be carried by a derivative of pteroylglutamic acid to glycine to form a 3 carbon compound, i. e., serine, which is then converted to pyruvate. Inhibition of such condensation may cause the accumulation of formate and glycine and prevent the formation of pyruvate. It is of interest to observe that sulfathiazole has an inhibitory effect on resting cells. This

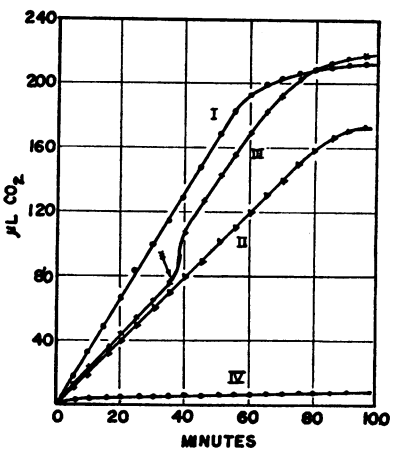

Figure 1. Reversal of sulfathiazole inhibition of xanthine degradation by *para*-aminobenzoic acid. Warburg flasks contained intact cells in 0.1 M phosphate buffer (pH 7.3) and additions as follows: flask 1, 10 μmoles xanthine; flask 2, 10 μmoles xanthine and 10 μmoles sulfathiazole; flask 3, same as flask 2, with 20 μmoles *para*-aminobenzoic acid added at the time indicated by the arrow; and flask 4, 20 μmoles *para*-aminobenzoic acid.

TABLE 2

Ammonia formation from serine by cell suspensions of Clostridium acidi-urici

Isomer	Serine Supplied	Ammonia Recovered	Per Cent of Serine N
	μmoles	*μmoles*	
D-L	40.0	19.4	48.5
	40.0	19.4	48.5
L	40.0	38.7	96.8
	40.0	38.3	95.9

Incubated for 70 min at 35 C in 0.05 M phosphate buffer (pH 7.3) in evacuated Thunberg tubes; 2 ml cell suspension. Total volume 6 ml.

TABLE 3

Formation of glycine and formate from uric acid by cell suspensions of Clostridium acidi-urici

Specific Activity of C^{14} Supplied*		Specific Activity of Products Recovered		Per Cent of Available C^{14}
c/m/μmole		*c/m/μmole*		
C_3	53.6	Formate	21.4	40
C_4C_5	1.4	Glycine	1.1	79

10 ml 0.02 M uric acid-2,8-C^{14} (108,750 c/m/mole), 10 ml 0.05 M sulfathiazole, 10 ml cell suspension. Incubated for 3 hr, 35 C, in 0.05 M phosphate buffer (pH 7.5) in evacuated flasks. Total volume 35 ml. Cells preincubated with sulfathiazole for 20 min.

* Distribution of labeling in c/m/μmole C_2, 53.6; C_3, 53.6; C_4, 0.2; C_5, 1.1; C_6, 0.2.

inhibitor is generally effective only on cells which are actively synthesizing their folic acid-like constituents from *p*-aminobenzoic acid. However, figure 1 shows that with resting cells of *C. acidi-urici*, *p*-aminobenzoic acid partially reverses sulfathiazole inhibition.

L-serine as the active isomer in deamination. Radin and Barker (1953) showed that when whole cells of *C. acidi-urici* act upon DL-serine, a quantity of ammonia corresponding to approximately half of the total serine nitrogen is recovered; a quantity of pyruvate corresponding roughly to half of the total amount of serine could also be recovered. Preliminary experiments using cell suspensions in the present studies gave similar results. Further experiments were carried out using L-serine. The results in table 2 show that L-serine is the isomer which is deaminated and converted to pyruvate by *C. acidi-urici*. The pyruvate which is formed is not metabolized by whole cells to any appreciable extent (Radin and Barker, 1953).

Origin of glycine and formate from purines. Inasmuch as formate and glycine appeared to be intermediates in the degradation of uric acid by *C. acidi-urici*, it was desirable to determine from which portions of the purine molecule these compounds originated. To accomplish this, uric acid-2,8-C^{14} was employed. The radioactivity of the purine was distributed largely between the 2 and 8 carbons, with some small amount located in the 4, 5, and 6 carbon atoms, as shown in table 3.

The distribution of labeling in the products of uric acid degradation indicates that formate must have been derived from either the 2 or 8 carbons of uric acid. Since the 2 carbon is converted to CO_2 (Rabinowitz and Barker, 1953), the formate must have originated from the 8 carbon. The specific activity of glycine is approximately that of the 4 and 5 carbons of uric acid. Karlsson and Barker (1949) have shown that the 2 and 6 carbons of uric acid are converted to carbon dioxide. The dilution in activity of the formate may result from exchange between formate and unlabeled carbon dioxide.

The formation of pyruvate. The foregoing observations together with the postulation that formate and glycine condense to produce serine suggested that the use of double substrate composed of L-serine and a labeled purine would yield labeled pyruvate. The probable reactions involved are outlined in figure 2. The use of this procedure is based on the following assumptions: (1) serine and pyruvate are normally occurring intermediates in the degradation of purines by this organism; (2) serine supplied as a substrate along with a purine will equilibrate with the serine formed from the purine; and (3) when excess serine is supplied, correspondingly large quantities of pyruvate will accumulate, the pyruvate having been derived in part from the added serine, and in part from the purine molecule. Using purines labeled in various positions, studying the positions of labeling in the isolated pyruvate should show the manner in which 4, 5, and 8 carbons of purines are converted to pyruvate and ultimately to acetate and carbon dioxide.

Labeling in products formed by action of intact cells on uric acid-2,8-C^{14} in presence of unlabeled L-serine is presented in table 4, ex-

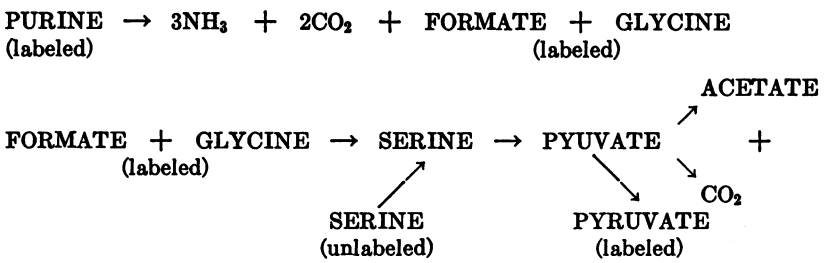

Figure 2. Probable reactions showing mixing of pyruvate originating from purines and from L-serine.

TABLE 4

Distribution of labeling in products of uric acid degradation by Clostridium acidi-urici

Experiment No.	Substrates	c/m/μmoles	Specific activity of Products (c/m/μmole)					
			CO_2	Pyruvate		Acetate		
				Total	Carboxyl	Total	Methyl	Carboxyl
1	Uric acid-2,8-C^{14}	108.7	20.0	25.1	3.24	10.9	—*	0.374
2	Uric acid-2,8-C^{14}	108.7	20.2	—*	—*	27.8	26.1	0.249
3	Glycine-2-C^{14}	8,640	93	851	60.1	543	66.2	363.5

Incubated for 90 min, 35 C, in 0.05 M phosphate buffer (pH 7.5) in evacuated reaction flask.

Experiment 1: 10 ml 0.02 M uric acid-2,8-C^{14}, 10 ml 0.02 M L-serine, 10 ml cell suspension. Total volume 35 ml. Experiment 2: 10 ml 0.02 M uric acid-2,8-C^{14}, 10 ml cell suspension. No serine. Total volume 25 ml. Experiment 3: 10 ml 0.02 M glycine-2-C^{14}, 10 ml 0.02 M uric acid, 10 ml 0.02 M L-serine, 10 ml cell suspension. Total volume 45 ml.

Combined acetate from degradation of purine and decarboxylation of pyruvate.

* Not determined.

periment 1. The CO_2 produced during the fermentation had a high specific activity. Dilution of the activity of CO_2 formed from the 2 carbon by unlabeled CO_2 from the 4 and 6 carbons should have resulted in an over-all specific activity of approximately 17.0 c/m/μmole. The slightly higher activity obtained may have resulted from the conversion of some of the the 8 carbon to CO_2, as reported by Karlsson and Barker (1949). The activity of the pyruvate phenylhydrazone appeared to be about 46 per cent of the activity of the 8 carbon of uric acid. However, it was found that the phenylhydrazone adsorbed some of the labeled acetate produced in the mixture, thus giving an excessively high observed activity for the pyruvate phenylhydrazone.

In these reactions it was assumed that if the serine, supplied in equimolar amounts with the labeled uric acid, were to equilibrate with the serine formed from the purine, the maximum activity that could appear in the pyruvate would be one-half of that of the 8 carbon. This seemed to be accomplished in experiment 1, so far as the phenylhydrazone was concerned, but the combined acetates from the decarboxylation of the pyruvate and from the reaction mixture showed an activity of only about one-fifth the activity of the 8 carbon. Furthermore, the reaction sequence required that the pyruvate and acetate possess the same specific activities, since pyruvate is decarboxylated to form acetate. The only difference would be in the activity contained in the carboxyl group of pyruvate. Interestingly, the carboxyl group of pyruvate did contain some labeling. This is a phenomenon which was not immediately explicable by the reaction sequence proposed.

The major portion of the radioactivity of acetate appeared in the methyl group. From the above observations it was deduced that the major portion of the labeling in the pyruvate occurred also in the methyl group.

Experiment 2 helped to clarify the problem of dilution of activities encountered during degradation of uric acid-2,8-C^{14}. In this experiment no

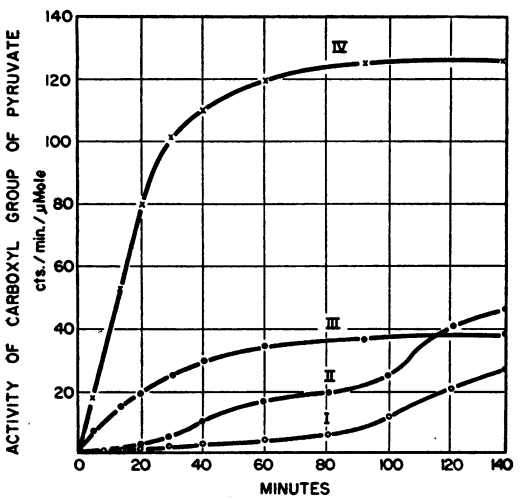

Figure 3. Exchange of radioactive bicarbonate with the carboxyl group of pyruvate. Each tube contained 60 μmoles sodium pyruvate and 150 μmoles sodium bicarbonate in 10 ml H_2O. Initial specific activity of sodium bicarbonate was 226 c/m/μmole. Tubes 1 and 2 contained intact cells; tubes 3 and 4, cell-free preparations. Cells used for tubes 1 and 3 were preincubated with pyruvate for 2 hr at 37 C.

serine was supplied with the purine. The specific activity of the carbon dioxide from the fermentation mixture was essentially the same as in experiment 1. However, the acetate recovered showed more than twice the activity of the acetate in experiment 1. Nevertheless, the activity of the acetate (in which the major portion of labeling occurred in the methyl group) was still only half of the activity available from the 8 carbon of uric acid. This was evidence for the dilution of the 8 carbon of uric acid, possibly with unlabeled bicarbonate through some exchange mechanism. Thus, in experiment 1, if the 8, or "formate" carbon of uric acid were first diluted by half, through some unknown mechanism, and then the products formed therefrom were further diluted by half through the addition of equimolar amounts of serine, the amount of labeling in the final products would be approximately one-fourth of the amount originally available in the 8 carbon.

In experiment 3, glycine-2-C^{14} was added to the reaction mixture containing unlabeled uric acid and unlabeled serine. The radioactive glycine was expected to equilibrate with the glycine derived from the purine molecule, so that some of the labeled glycine would be incorporated into the serine and pyruvate formed. The results show that some of the glycine is incorporated, and that the α carbon of glycine becomes the carboxyl carbon of acetate. This evidence supports the hypothesis that the 8 carbon of uric acid is converted to an active form of a "1 carbon fragment" which is condensed with glycine to form a 3 carbon compound. The 1 carbon compound becomes the β carbon of the condensation product and later the methyl carbon of acetate which is formed by decarboxylation of the 3 carbon intermediate.

The appearance of radioactivity in carbon dioxide formed during the reactions indicates that the methylene carbon of glycine is oxidized to CO_2 as shown by Karlsson and Barker (1949).

It is of interest that when the sole source of radioactivity is the α carbon of glycine, some labeling appears in both the carboxyl group of pyruvate and in the methyl group of acetate, as well as in the carbon dioxide from the reaction mixture. This phenomenon, which may be explained by exchange reactions between carbon dioxide and either pyruvate or the 1 carbon compound, was further investigated.

Exchange of bicarbonate with the carboxyl group of pyruvate. A mixture containing unlabeled xanthine, unlabeled serine, and radioactive bicarbonate was incubated with whole cells, the result being that pyruvate which accumulated contained a considerable amount of activity in the carboxyl group (Sagers and Beck, 1955*a*). In further investigations, whole cells were incubated with unlabeled pyruvate and radioactive bicarbonate (specific activity, 226 c/m/μmole) as the only substrates. It was found that labeled CO_2 was incorporated into the carboxyl group of the pyruvate. The results of these experiments are shown in figure 3. The peculiar shape of curve II may be the result of increased permeability of the cells to the substrates or of increasing concentration of the exchange enzyme. In an attempt to elucidate this problem some cells were preincubated with pyruvate for 2 hr at 35 C prior to the addition of labeled bicarbonate. Curve I represents the activity of the cells under these conditions. Strangely, the amount of labeling appearing in the carboxyl group of pyruvate was less using preincubated cells, as compared with the non-preincubated cells.

The ability of cell-free enzymes of *C. acidi-*

urici to carry out the exchange reaction is also shown in figure 3. Cells were ground with powdered alumina, and the cell free materials extracted in 0.05 M phosphate buffer at pH 7.3. Equilibrium between pyruvate carboxyl and bicarbonate carbons would yield a specific activity of 113 c/m/μmole which was reached in curve IV in 80 min. The activities of the exchange enzyme system obtained from cells which had not been preincubated with pyruvate (IV) and from those which had been preincubated in the presence of pyruvate for 2 hours prior to grinding (III) are presented. The marked difference in activity of the two enzyme preparations shows that preincubation of the cells with pyruvate decreases, rather than increases, the ability of the enzymes to carry out the exchange. Reasons for this are not known at present.

Exchange of bicarbonate with labeled formate. The high dilution in activity of the products derived from the number 8 carbon of uric acid-2,8-C^{14}, and the occurrence of labeling in the methyl group of acetate when glycine-2-C^{14} is metabolized in the presence of unlabeled uric acid suggested that the number 8 "formate" carbon of uric acid equilibrated at least to some extent with carbon dioxide. To investigate this matter further, a series of flasks containing equimolar amount of formate-C^{14} and unlabeled bicarbonate were inoculated with equal quantities of whole cells. The mixtures were incubated for varying lengths of time, as shown in figure 4. At the end of the reactions, the total amount of CO_2, its specific activity, and the activity of the residual formate were determined. The specific activity of the carbon dioxide increased rapidly with time to approximately half of the initial specific activity of the formate. The total amount of CO_2 remained constant, and the activity of the residual formate was approximately half of the initial activity. In reaction vessels containing labeled formate and cells, but no bicarbonate, it was found that virtually no oxidation of formate occurred. In formate-bicarbonate controls, in which no cells were present, no detectable exchange occurred during the 4-hr period of observation. The foregoing experiments show that exchange of labeled bicarbonate with formate or the carboxyl group of pyruvate occurs under the conditions described.

Experiments with radioactive guanine. In table 5 are shown the results of experiments employing guanine. In experiment 1, guanine-8-C^{14} and

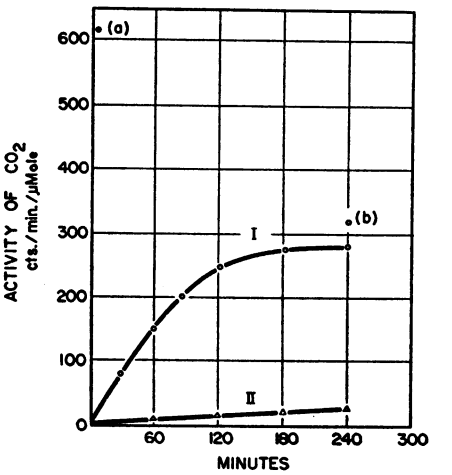

Figure 4. Exchange of radioactive formate with bicarbonate. Each tube contained intact cells in 0.05 M phosphate buffer (pH 7.3) and 60 μmoles formate-C^{14}, 620 c/m/μmole. Total volume, 10 ml; 60 μmoles sodium bicarbonate added at zero time to tube 1 and, as carrier, at 240 min to tube 2. Points (a) and (b) are the initial and final formate specific activities, respectively.

unlabeled serine were the substrates. The carbon dioxide produced showed considerable activity. This may be accounted for by the equilibration of formate and carbon dioxide as noted above. The major portion of the C^{14} in the pyruvate appears in the β carbon. This is consistent with the idea that the 8 carbon of the purine is converted to an active form of formate, which then condenses with the glycine portion comprising the 4 and 5 carbons and nitrogen 7. This would place the 8 carbon in the β position of serine and pyruvate as shown. Activity in the carboxyl group of pyruvate may be accounted for by the exchange between carbon dioxide and the pyruvate carboxyl group. The activity in the α carbon is not predicted by the proposed mechanism but may be explained from the results found in experiment 3 of table 4.

In experiment 2, guanine-4-C^{14} was catabolized as in experiment 1. In this case the greater portion of the activity in the pyruvate appeared in the carboxyl group. This is in agreement with the idea that the pyruvate is formed from the 4, 5, and 8 carbons, and is decarboxylated to form acetate and labeled carbon dioxide. Inasmuch as carbons 2 and 6 of the purine molecule are also converted to carbon dioxide, the labeled CO_2 from the over-all reaction would show at least a 3-fold dilution.

TABLE 5

Distribution of C^{14} in products of guanine-C^{14} degradation by Clostridium acidi-urici

Experiment No.	Radioactive Substrate	Specific Activity	Specific Activity of Products (c/m/μmole)				
			CO_2	Pyruvate			
				Total	β-C	α-C	Carboxyl
		c/m/μmole					
1	Guanine 8-C^{14}	414	123	117	86	16.8	13.3
2	Guanine 4-C^{14}	36	8.3	9.1	1.3	0	8.4
3	Glycine-2-C^{14} + Guanine	640	14.5	78.2	6.9	53.8	16.1
4	Formate-C^{14} + Guanine	580	83.8	34.9	23.5	5.5	14.7

300 μmoles of each substrate indicated, and 300 μmoles of unlabeled L-serine were used in each experiment. Incubated for 90 min, 35 C in 0.05 M phosphate buffer (pH 7.5) in evacuated reaction flasks. Total volume, 35 ml.

In experiment 3, unlabeled guanine was catabolized in the presence of unlabeled serine and glycine-2-C^{14}. In this case it was postulated that the radioactive glycine would equilibrate with the glycine formed from the purine molecule prior to the formation of serine, and some of the labeled glycine would be incorporated into the serine and pyruvate. The results show that C^{14} appears mainly in the α carbon of pyruvate. However, as shown above, labeled carbon dioxide appears in the reaction mixture indicating that a small amount of α carbon of glycine undergoes oxidation to CO_2.

In experiment 4, unlabeled guanine was catabolized in the presence of unlabeled serine and formate-C^{14}. The carbon dioxide from the over-all reaction showed a high activity resulting from the rapid equilibration of formate and CO_2. Only a small amount of formate is incorporated into pyruvate, but the activity of the β carbon indicates that formate is incorporated into this position. Again, the activity of the carboxyl group of pyruvate may be accounted for by the exchange of labeled CO_2 with the carboxyl carbon.

When unlabeled purine was degraded in the presence of unlabeled serine and labeled acetate, no activity was found in the pyruvate.

DISCUSSION

The exchange of carbon dioxide with formate and with the carboxyl group of pyruvate are reactions which may be of considerable significance in explaining the apparent fixation of carbon dioxide in some systems. The exchange of bicarbonate with the pyruvate carboxyl carbon was reported by Strecker and Ochoa (1954) to occur with enzyme preparations of *Escherichia coli*. These workers also reviewed the occurrence of a similar exchange in other organisms. Sagers and Beck (1955*a*) demonstrated the exchange of radioactive bicarbonate with the carboxyl group of pyruvate by *C. acidi-urici*.

The mechanism by which this exchange occurs is not known. It might be the reversal of a clastic split, which occurs in several other organisms; however this type or reaction has not as yet been shown to occur in *C. acidi-urici*. This reaction was shown to be reversible by Utter, Lipmann and Werkman (1945). However, in experiments with *C. acidi-urici* in which labeled acetate was added to a reaction mixture containing unlabeled purine and unlabeled serine, no radioactivity whatsoever was detected in the recovered pyruvate. This would indicate that the acetate must be supplied in an active form, or that the system in which acetate and carbon dioxide (or formate) are condensed to form pyruvate is not reversible under the conditions of these experiments. Reversal is not ruled out, however, under other conditions.

The exchange of formate with bicarbonate by *C. acidi-urici* may be a cyclic oxidation-reduction process in which one molecule of formate is oxidized to one molecule of carbon dioxide. The hydrogens from the formate are then transferred to another molecule of carbon dioxide to reduce it to formate. When equimolar amounts of labeled formate and unlabeled bicarbonate are supplied, this process would continue without net change in the quantities of the compounds and would reach an equilibrium value at half of the initial activity of the formate, as observed in these experiments.

The results of the present tracer studies on pyruvate formation are in agreement with earlier tracer experiments by Karlsson and Barker (1949). These workers determined the position of labeling in acetate formed during the fermentation of radioactive uric acid by *C. acidi-urici*. Their results indicated that the 8 carbon of uric acid is incorporated into the methyl group of acetate, and that some carbon dioxide is also formed from this carbon. When uric acid-5-C^{14} was the substrate, the greater part of the activity appeared in the carboxyl group of acetate, with some labeling appearing in the methyl group. When glycine-1-C^{14} was employed practically no activity appeared in the acetate, but most of the activity was recovered as carbon dioxide. With glycine-2-C^{14}, activity appeared mainly in the carboxyl group of acetate. These workers also observed that when large quantities of labeled sodium bicarbonate were supplied to the cells as the sole source of radioactivity during uric acid degradation, some labeled acetate was formed in which the labeling was equally distributed between the methyl and carboxyl carbons.

Previous investigations of purine degradation by *C. acidi-urici*, together with the results of the present investigation, substantiate the proposed sequence of reactions. The purine molecule is degraded in a stepwise manner to yield glycine and a "formate" component, derived from the 4, 5, and 8 carbons, and the 7 nitrogen. It is to be emphasized that the postulated glycine and formate intermediates may not occur as free compounds during the normal degradation process, but rather they may occur as "activated" forms of these compounds. These active intermediates may then condense to form serine, which is deaminated to form pyruvate. The 8 carbon of the purine is incorporated into the β position of the pyruvate, and the 5 and 4 carbons become respectively the α and carboxyl carbons of pyruvate. Upon decarboxylation, the 4 carbon is converted to carbon dioxide, leaving the 5 and 8 carbons as the carboxyl and methyl groups of acetate.

The failure to incorporate a large fraction of formate C^{14} into pyruvate indicates a very slow rate of equilibration between free formate and formate produced from purine degradation, and suggests that the degradative formate is in an active state. It is of interest that formaldehyde and glycine are rapidly converted to pyruvate by extracts of *C. acidi-urici* and tetrahydrofolic acid is required for this reaction (Beck, 1955). This agrees with the observations of Kisliuk and Sakami (1955) in which tetrahydrofolic acid is required for serine biosynthesis. Results on the degradation of formiminoglycine (Sagers *et al.* 1956) by cell-free extracts of *C. acidi-urici* show that tetrahydrofolic acid is necessary for this reaction. Whole cells and cell-free systems fail to activate free formate or even to produce rapid equilibration between free and "active formate."

Although serine has not been isolated directly from the purine molecule, the indirect evidence for its intermediary role, as shown in the experiments described, and the known conversion of glycine and formate to serine by several types of organisms, is rather suggestive.

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SUMMARY

The possible reactions by which the formate and glycine intermediates in purine degradation by *Clostridium acidi-urici* are converted to acetate, carbon dioxide, and ammonia have been investigated. The use of radioactive tracers has served as a means of elucidating the steps by which the reactions proceed.

Arsenite and sulfathiazole inhibition of purine degradation causes the accumulation of glycine and formate. These compounds are shown to originate from the 4, 5, and 8 carbons, and 7 nitrogen of the purine molecule.

Trace amounts of pyruvate normally appear as a product of purine catabolism by this organism. The use of excess serine made it possible to accumulate large amounts of pyruvate for study.

Evidence is presented which supports the hypothesis that the formate and glycine intermediates are condensed to form serine. This compound is then quantitatively deaminated and converted to pyruvate. The 8 carbon of the purine molecule is incorporated into the β position of pyruvate, whereas the 5 and 4 carbons are incorporated into the α and carboxyl carbons of pyruvate. Pyruvate is probably oxidatively decarboxylated to form acetate and carbon dioxide.

Evidence for the enzymatic exchange of bicarbonate with the carboxyl group of pyruvate and with formate is presented.

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