METABOLISM OF PENTOSES BY CLOSTRIDIA

II. The Fermentation of C^{14}-Labeled Pentoses by *Clostridium perfringens*, *Clostridium beijerinckii*, and *Clostridium butylicum*[1]

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In an attempt to elucidate the pathway of glucose fermentation in *Clostridium perfringens* by the use of C^{14}-labeled substrates, Paege *et al.* (1956) obtained results which were inconclusive. In their investigation, the distribution of tracer in the products of glucose fermentation would have been consistent with the hypothesis that glucose is fermented exclusively through the Embden-Meyerhof-Parnas (EMP) pathway, if it were not for the specific activity data. The fermentation of C^{14}-labeled glucose by the EMP pathway would be expected to give rise to acetic acid and ethanol with identical specific activities. In the case of *C. perfringens*, however, the fermentation of glucose-1-C^{14}, glucose-2-C^{14}, and glucose-6-C^{14} gave rise to acetic acid which had a specific activity twice as great as that of the ethanol.

A study of the fermentation of pentose by *C. perfringens* was undertaken with two objectives in mind. First, it was felt that such a study would contribute to the scanty knowledge available concerning the metabolism of carbohydrates in the genus *Clostridium*. Second, it was hoped that light would be cast on the anomalous data obtained from the fermentation of C^{14}-labeled glucose. In the previous paper (Cynkin and Delwiche, 1958), evidence was presented for ribokinase and phosphopentoisomerase activity in cell free extracts of *C. perfringens*. In addition, the conversion of ribose-5-phosphate to hexose monophosphate was demonstrated, suggesting the presence of the transketolase-transaldolase sequence of reactions.

In the present investigation, the distribution of tracer in the products of the fermentation of C^{14}-labeled pentoses by resting cells of *Clostridium perfringens*, *Clostridium beijerinckii*, and *Clostridium butylicum* indicates that these organisms metabolize D-xylose and D-ribose by means of the transketolase-transaldolase sequence followed by the EMP pathway. Results obtained from the fermentation of glucose-2-C^{14} by *C. perfringens* are not in agreement with the results of Paege *et al.* (1956).

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MATERIALS AND METHODS

Clostridium perfringens strain BP6K, *Clostridium beijerinckii*, and *Clostridium butylicum* were obtained from the Indiana University collection through the courtesy of Dr. L. S. McClung. *C. perfringens* was maintained by transfer in heart infusion broth containing D-ribose. *C. beijerinckii* and *C. butylicum* were maintained in medium A (Bard and Gunsalus, 1950) containing 1 per cent yeast extract (Difco), 1 per cent tryptone (Difco), and 0.5 per cent K_2HPO_4, to which was added D-xylose, sterilized by filtration, in a final concentration of 1 per cent. For the preparation of resting-cell suspensions, *C. perfringens* was grown in medium A, containing D-ribose, for 5 hr at 37 C. *C. beijerinckii* and *C. butylicum* were grown in medium A, containing D-xylose, for 12 hr at 37 C. The cultures were harvested by centrifugation, washed once, and resuspended in distilled water.

Fermentations were carried out in 150 ml Warburg vessels at 37 C. The gas phase was nitrogen. Separation and degradation of the fermentation products was carried out in the following manner. Carbon dioxide was absorbed by 10 per cent NaOH. The resulting $Na_2C^{14}O_3$ was converted to barium carbonate for assay of radioactivity. The Warburg vessel contents were centrifuged to remove the cells. The supernatant liquid was made alkaline to phenol red with sodium hydroxide and distilled to remove ethanol. The residue was adjusted to pH 1 with H_2SO_4 and steam-distilled to remove acetic

and butyric acids. The acids were separated in the earlier experiments by the celite method of Beuding and Yale (1951). In later experiments, higher yields were obtained using a modification of the celite method of Phares *et al.* (1952). The location of tracer in acetic acid was determined by the following procedures: (1) The method of Phares (1951) which yielded the carboxyl carbon, and (2) the total oxidation methods of Osburn and Werkman (1932) or of Van Slyke *et al.* (1951). The difference between the activity of the total oxidation and that of the carboxyl carbon was taken as the activity of the methyl carbon. Ethanol was converted to acetic acid (Gibbs *et al.*, 1954) and degraded as such. Butyric acid was totally oxidized by the methods mentioned above. All C^{14} samples were assayed for activity as barium carbonate in a methane flow beta proportional counter.

RESULTS AND DISCUSSION

In the previous paper (Cynkin and Delwiche, 1958), cell free extracts of *Clostridium perfringens* were shown to convert ribose-5-phosphate to hexose monophosphate. If this conversion were due to the operation of the transketolase-transaldolase sequence of reactions (Gunsalus *et al.*, 1955), then it would be predicted that ribose-1-C^{14} would be metabolized by intact cells as is shown in figure 1. The dissimilation of the resulting hexoses and trioses through the EMP pathway would give rise to $C^{14}O_2$ possessing a specific activity one-fifth that of the original tracer carbon.

The above prediction would appear to be confirmed by the data listed in table 1. That is, the data are consistent with the hypothesis that, in *C. perfringens*, *C. beijerinckii*, and *C. butylicum*, pentoses are metabolized by means of the trans-

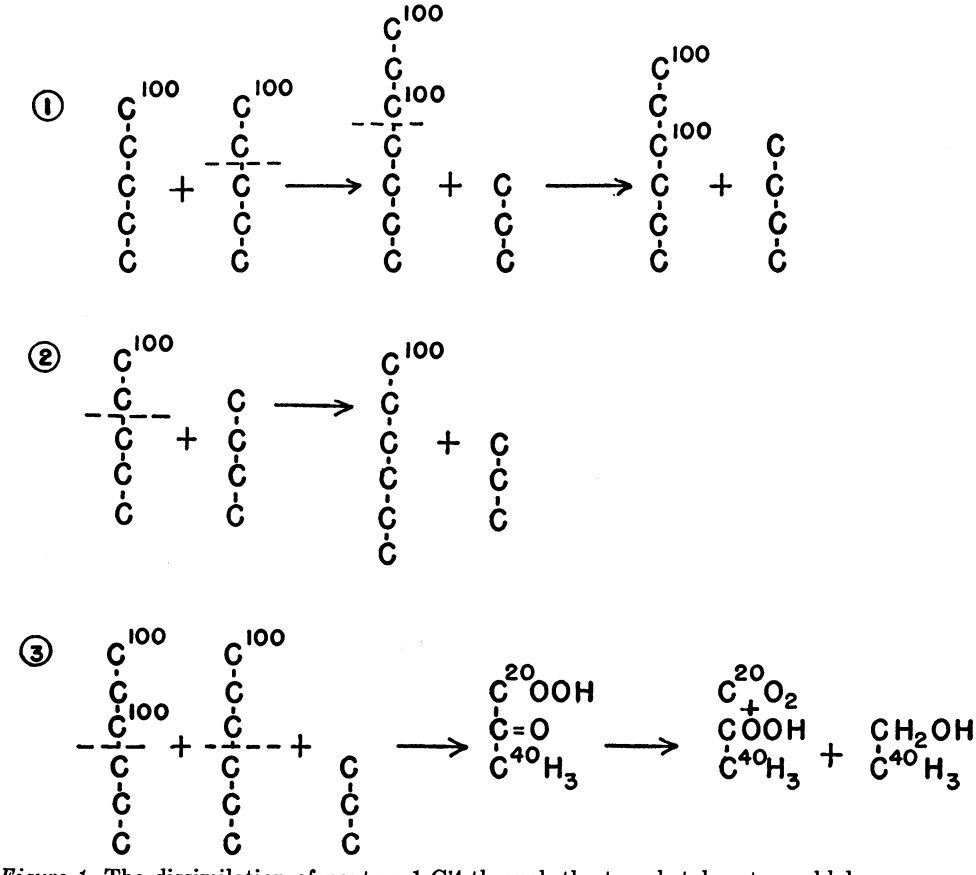

Figure 1. The dissimilation of pentose-1-C^{14} through the transketolase-transaldolase sequence and the EMP pathway. In the diagram the aldehyde carbon of pentose is assigned an arbitrary specific activity of 100.

TABLE 1

Formation of carbon dioxide from pentose-1-C^{14} by Clostridia

	Specific Activity, mμc/mg C	
	Pentose carbon atom 1	CO_2
Clostridium perfringens BP6K	59.0	11.8
Clostridium beijerinckii	144.0	24.9
Clostridium butylicum	144.0	25.1

In the experiments with *C. perfringens*, each Warburg vessel contained 120 μmoles of D-ribose. In the experiments with *C. beijerinckii* and *C. butylicum*, 100 μmoles of D-xylose were present in each vessel. In addition to the sugar solutions, each Warburg vessel contained 3.3 ml 0.067 M phosphate buffer, pH 6.5, and CO_2-free water to bring the final liquid volume to 6.0 ml. The center well contained 0.3 ml 2.5 N CO_2-free NaOH. The reaction was begun by tipping in 1.2 ml of cell suspension. The gas phase was nitrogen. The temperature was 37 C. After gas evolution had ceased, 0.1 ml 6 N H_2SO_4 was tipped in from the second sidearm. Carbon dioxide was collected as described under Materials and Methods.

Data expressed as mμc/mg C = millimicrocuries per milligram carbon.

ketolase-transaldolase sequence and the EMP pathway.

Since the available evidence indicates that hexose monophosphate is an intermediate in pentose catabolism in these clostridia (Cynkin and Gibbs, 1957), it might be expected that the ethanol and acetic acid resulting from pentose dissimilation would bear the same relationship to each other as if they came from glucose. Thus, upon consideration of the studies of Paege *et al.* (1956) on the metabolism of C^{14}-labeled glucose by *C. perfringens*, it would be expected that the fermentation of ribose-1-C^{14} by this organism would yield ethanol with a specific activity one-half that of the acetic acid. It can be seen from the data in table 2 that this expectation was not fulfilled. Indeed, the ethanol possessed a specific activity somewhat higher than that of acetic acid. The specific activity of butyric acid was about the same as that of the ethanol, suggesting that the two compounds possessed a common precursor.

It now was deemed necessary to repeat, at least in part, the experiments of Paege and his

TABLE 2

Distribution of tracer in products of fermentation of ribose-1-C^{14} by Clostridium perfringens

	Specific Activity, mμc/mg C	
	Expt 1	Expt 2
Tracer carbon of ribose	14.8	59.0
Carbon dioxide	2.8	11.1
Ethanol CH_3—	4.9	15.1
—CH_2OH	0.1	0.4
Acetic acid CH_3—	4.0	11.6
—COOH	—	—
Butyric acid (total oxidation)	2.3	7.4

In each experiment the main compartment of each of two Warburg vessels contained 3.0 ml 0.10 M phosphate buffer, pH 6.7, 500 μmoles D-ribose, and 2 drops of a phenol red solution. The reaction was begun by tipping in 2 ml of cell suspension from one sidearm. The other sidearm contained 0.3 ml 2.5 N CO_2-free NaOH. The gas phase was nitrogen. The temperature was 37 C. As acid was produced, resulting in a change in the indicator color, the pH was prevented from dropping by the injection of 0.1 N CO_2-free NaOH through a rubber cap attached to an opening on the side of each Warburg vessel.

Separation and degradation of the fermentation products are described under Materials and Methods.

Data expressed as mμc/mg C = millimicrocuries per milligram carbon.

TABLE 3

Distribution of tracer in products formed from fermentation of glucose-2-C^{14} by Clostridium perfringens

	Specific Activity, mμc/mg C	
	Expt 1	Expt 2
Carbon dioxide	0.0	0.0
Ethanol CH_3—	0.0	0.9
—CH_2OH	8.2	6.9
Acetic acid CH_3—	0.0	0.0
—COOH	8.0	8.7
Butyric acid (total oxidation)	2.7	2.5

The procedure was essentially the same as that described in table 2, except that in each experiment, each of two Warburg vessels contained 400 μmoles of D-glucose.

Data expressed as mμc/mg C = millimicrocuries per milligram carbon.

co-workers. Resting cells of *C. perfringens* were permitted to ferment glucose-2-C^{14}, and the products were analyzed for radioactivity. The results are shown in table 3. It can be seen that the ratio of ethanol specific activity to acetate specific activity approaches unity. These data are not in agreement with the data of Paege *et al.* (1956). They are consistent with the exclusive operation of the EMP pathway in the dissimilation of glucose.

The discrepancy between the data presented here and the data of Paege and co-workers cannot easily be explained. The experimental conditions and degradation methods used were essentially the same. One difference between the two groups of experiments was that, in the present investigation, *C. perfringens* was adapted to and maintained on D-ribose, whereas the culture used by the Paege group had never been exposed to pentoses. However, it is difficult to rationalize a correlation of the discrepancy between the two sets of data with the difference in cultural conditions.

SUMMARY

Evidence obtained from the fermentation of C^{14}-labeled pentoses indicates that *Clostridium beijerinckii*, *Clostridium butylicum*, and *Clostridium perfringens* dissimilate pentoses by means of the transketolase-transaldolase sequence, followed by the Embden-Meyerhof-Parnas pathway. The fermentation of ribose-1-C^{14} by *C. perfringens* gave rise to ethanol and acetic acid possessing similar specific activities. The fermentation of glucose-2-C^{14} by *C. perfringens* likewise gave rise to similarly labeled ethanol and acetic acid, a result which is consistent with the exclusive operation of the Embden-Meyerhof-Parnas pathway in the dissimilation of glucose.

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