THE UTILIZATION OF THE THREE SINGLY-C^{14}-MARKED LACTIC ACIDS BY ESCHERICHIA COLI

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As a preliminary to preparing bacteriophages marked with C^{14}, it was desirable to ascertain which singly-labeled lactic acid, when used as a carbon source, gives the highest incorporation of radioactive carbon in the cells of *Escherichia coli*. The results, while not unexpected, present several interesting features and serve to supplement the much more complete data obtained by Wiame and Doudoroff (1951) with another bacterium under rather different conditions (see the accompanying article).

A synthetic medium containing sodium lactate as the energy source has been used widely in studies of the T-series of *Escherichia coli* bacteriophages (Adams, 1950). Since we have found that our usual glycerol medium gives better yields of T3 bacteriophage when fortified with casein hydrolysate, experiments on the utilization of lactates were conducted in the presence or absence of such a casein hydrolysate supplement, each set containing a control of the opposite type. The medium was prepared as usual (Adams, 1950).

The radioactive lactates were available as zinc salts. These were converted to the free acid by treatment with washed "dowex 50"[1] ion exchange resin in the acid form. Each sample was dissolved in 1 ml water, mixed with 1 ml wet packed resin, and the resin separated using a low speed centrifuge. The resin was washed with two half ml portions of water. The combined solutions in 1.5 ml water were made up to 50 ml with inactive lactate medium, the pH adjusted, and the solution autoclaved as usual. The specific radioactivity of each of the final mixtures was determined (Dauben *et al.*, 1947).

Each culture was aerated with a simple bubbler in a test tube, and the effluent CO_2 was collected in a scrubber of 1 N NaOH using an apparatus which passed the gas in a helical path. The bacteria were harvested at a concentration of 1 to 2 $\times$ 10^9 cells per ml, washed by centrifugation twice with a 1.5 per cent phosphate buffer (pH 7.0) and once with distilled water. This suspension was plated directly for radioactivity (Dauben *et al.*, 1947). The CO_2 in the scrubbers was precipitated as $BaCO_3$ on aluminum discs (Calvin *et al.*, 1949). The radioactivity of the samples was determined either with a proportional counter ("nucleometer") or with thin end window GM tubes, depending on the specific activity. The final supernatant fluid from each sample was tested to show that no significant activity remained.

RESULTS

Table 1 (experiment 1) shows the results for a set of cultures in which lactate was the sole carbon source except for sample 4, in which an amino acid supple-

[1] Dow Chemical Company, Midland, Michigan.

ment was added to a duplicate of sample 2. As expected, most of the utilized carbon from the lactate-1-C^{14} is discharged as CO_2. Some, nonetheless, is retained as bacterial substance. With lactate-2-C^{14} approximately three times as much of the carbon is retained in the bacteria and correspondingly less escapes as CO_2; with the lactate-3-C^{14} the efficiency of utilization is slightly greater still.

Table 1 (experiment 2) shows an experiment in which each of the three lactates was supplemented with casein hydrolysate (the culture with lactate-2-C^{14} was paralleled by a similar unsupplemented culture). The results are similar to those shown in experiment 1, except that in each case less of the radioactive carbon is

TABLE 1

Utilization of the C^{14}-singly-labeled lactic acids by Escherichia coli with and without casein hydrolysate supplement

ORIGINAL MEDIUM		BACT./ML	DISINTEGRATIONS/10^9 BACT./ML	PER CENT C^{14} IN BACTERIA	PER CENT C^{14} IN CO_2
Carbon Source	Disintegrations/ml/min				
Experiment 1					
a. Lactate-1-C^{14}	5.63×10^5	2.6×10^9	2.4×10^3	1.1	6.6
b. Lactate-2-C^{14}	7.58×10^5	2.6×10^9	10.4×10^3	3.7	2.5
c. Lactate-3-C^{14}	4.80×10^5	2.6×10^9	8.0×10^3	4.3	1.3
d. Lactate-2-C^{14} plus casein hydrolysate	9.08×10^5	2.6×10^9	6.9×10^3	1.9	0.50
Experiment 2					
a. Lactate-1-C^{14} plus casein hydrolysate	5.68×10^5	1.5×10^9	2.4×10^3	0.64	5.5
b. Lactate-2-C^{14} plus casein hydrolysate	5.36×10^5	1.8×10^9	5.7×10^3	1.9	0.58
c. Lactate-3-C^{14} plus casein hydrolysate	4.36×10^5	2.1×10^9	3.9×10^3	1.9	0.16
d. Lactate-2-C^{14} unsupplemented	4.48×10^5	1.5×10^9	6.1×10^3	2.1	1.3

used; i.e., the bacteria are supplying some of their requirements at the expense of the inactive casein hydrolysate.

It is interesting to note that in both experiments the total utilization of radioactive carbon is greater for carboxyl-labeled lactate than for the other isomers. Since the same total amount of lactate must be used in each case, this suggests that a portion of the C_2 fragment left after removal of the carboxyl carbon is excreted into the medium[2] (1.5 and 2.0 per cent excreted *vs* 6.2 and 5.7 per cent utilized in experiment 1). The difference in total utilization with and without amino acid supplement shows that the bacteria draw on lactate as a carbon and energy source to a smaller extent when provided with amino acids. Since almost

[2] The sum of *per cent C^{14} in CO_2* and *per cent C^{14} in bacteria* can differ for the three isomers only by C^{14} excreted into the medium. In either experiment subtraction of this total for parts *b* or *c* from part *a* thus gives unused or excreted C^{14}.

the same amount of carboxyl carbon is converted to CO_2 in the presence and absence of the supplement, it seems unlikely that the energy requirement of the bacteria is appreciably lowered by the provision of amino acids, the data suggesting rather that amino acids are used as a carbon and particularly as an energy source in preference to the two-carbon lactate fragment, which is apparently largely excreted[2] (3.6 and 4 per cent *vs* 2.5 and 2.1 per cent utilized in experiment 2). This is not surprising in view of the poor utilization of two carbon compounds as a sole carbon source by *Escherichia coli* (Stephenson, 1949).

It has been presumed that the carbohydrate of the *Escherichia coli* arises through reversal of the glycolytic cycle; i.e., through conversion of lactate to pyruvate, etc. The indication that lactate is used synthetically mostly as a two-carbon-fragment rather than a three-carbon unit cannot, however, be interpreted as militating against this mechanism—as might appear at first consideration—because from the composition of *Escherichia coli* (Taylor, 1946) it can be seen that carbohydrate carbon is but some 10 per cent of the total carbon. The ratio of incorporation of carbon-1 to carbons 2 and 3 thus is still consistent with the hypothesis that carbohydrate is made from the intact three-carbon unit, and that the protein and nucleic acid constituents are synthesized from a smaller unit. The present results do not offer decisive evidence on this problem.

It may be of interest to note, incidentally, that the incorporation of the three lactates into T3 bacteriophage follows a parallel pattern.

SUMMARY

Study of the utilization of the three C^{14}-singly-labeled lactic acids by *Escherichia coli* has shown that carboxyl carbon is largely, but not exclusively, converted to CO_2. The *alpha* and *beta* carbons are mostly utilized in synthetic reactions, but some are converted to CO_2 and an appreciable amount of the C_2 fragment left from decarboxylation of the lactate is excreted into the medium. Casein hydrolysate is apparently used synthetically and used as an energy source in preference to the C_2 fragment.

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