3. The cholesteryl ion, first formed, rearranges to form the *i*-cholesteryl ion, but (a) the rearrangement does not reach equilibrium, or (b) the rearrangement does effectively reach equilibrium but the proportion of the two ions is changed by a change in the medium.

It is not possible to eliminate possibilities (a) or (b) on the basis of the data presented here. However, if rearrangement of the cholesteryl ion can be thought of as an intramolecular reaction between an ion (the carbonium ion at $C_3$) and a neutral molecule (the double bond at $C_5$—$C_6$), alternative (b) can be eliminated, because there is no primary salt effect in such a reaction.

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## Degradation of Glucose-1-$C^{14}$ and a Possible New Step in the Mechanism of Fermentation[1]


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The availability of glucose-1-$C^{14}$ [3] has permitted the verification of a scheme of glucose degradation applied to sugars formed in photosynthesis to determine the distribution of isotopic carbon within the sugar (*1*). As a result of the present investigation, there appears to be a second, though minor, pathway of fermentation by the test organism, *Lactobacillus casei* ε.

The degradation procedure is depicted in Fig. 1 and involves the following sequence of reactions: the fermentation of glucose to lactic acid; the oxidation of lactic acid to carbon dioxide (carbons three and four of the original glucose) and acetic acid; the pyrolysis of barium acetate to acetone and barium carbonate (carbons two and five); and the formation of iodoform (carbons one and six) from the acetone. The various steps in the process have been tested with (a) methyl- and carboxyl-labeled acetate, (b) α-labeled lactate,[4] and (c) glucose-1-$C^{14}$.

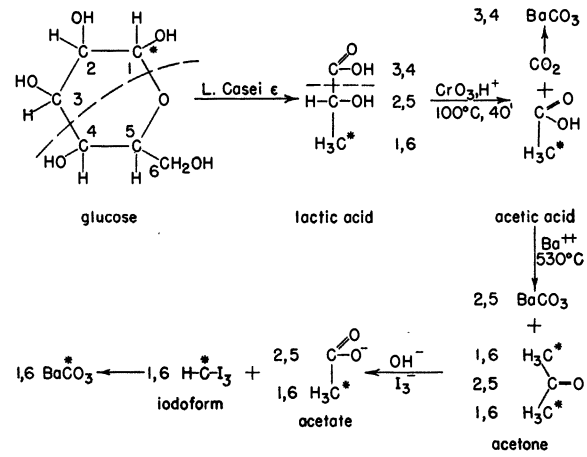


FIG. 1.

Both methyl- and carboxyl-labeled acetates have been pyrolyzed under a variety of conditions, a number of which can be so unfavorable (e.g., that at 450° C with flowing argon), as to result in the appearance of more than 8% of the activity of methyl-labeled acetate in the purified residual barium carbonate. Under similar conditions, with carboxyl-labeled acetate, less than ½ of 1% of the activity is in the iodoform. Using flowing argon, at a temperature of 530° C for 10 min, and liberating the carbon dioxide from the residual carbonate with lactic acid, only 0.86% of the activity of the methyl-labeled acetate is found in the carbonate.

Electrolysis of sodium acetate (*2*), the products of which are carbon dioxide and ethane, resulted in 1% of the radioactivity of the methyl group in the carbon dioxide from methyl-labeled acetate.

The chromium trioxide oxidation of α-labeled lactate (*7*) purified and recrystallized as the zinc salt, resulted in 4.3% of the activity in the carbon dioxide evolved. This activity arises primarily from oxidation of compounds other than the acetic acid itself, as less than 1% of the theoretical barium carbonate arises from acetic acid under identical conditions.

The degradation of lactate from the bacterial fermentation, similarly isolated and purified as the zinc salt, resulted in 9.3 ± 0.1% of the activity in the barium carbonate, rather than the empirical 4.3 ± 0.1. The difference of 5% must be ascribed to the activity of carbons three and four. In fermentation the carboxyl group of the lactic acid is presumed to arise solely from carbons three and four of glucose, whereas in ordinary chemical

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[3] The glucose used by the senior author was synthesized by John C. Sowden (*6*); that used in the California laboratory was synthesized by H. Mahler according to Sowden's general method (*4*).

[4] The acetates were synthesized by Dr. B. Tolbert, and the lactic acid by Dr. R. Lemmon (both at the University of California Radiation Laboratory).

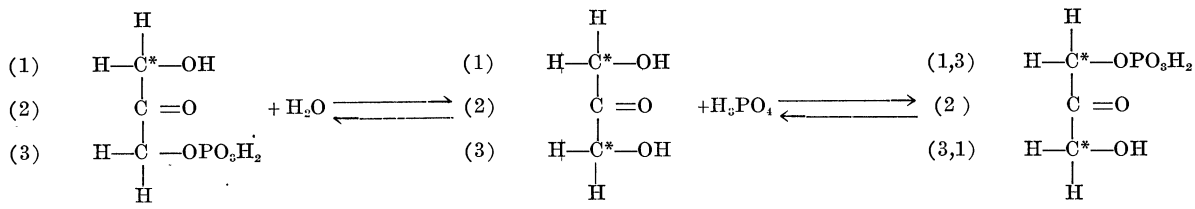

FIG. 2.

alkaline rearrangements resulting in lactic acid (*5*) the carboxyls may stem from carbons one and six. This is certainly so in the case of gluconic acid, where 1.3 moles of lactic acid per mole of gluconic acid can be obtained.

These experiments are in general accord with the recent note by Koshland and Westheimer (*3*) on the fermentation of glucose-1-$C^{14}$ by yeast. In yeast fermentation, the carbon dioxide may be derived from a variety of intermediary products of metabolism. Furthermore, the ability of yeast to fix carbon dioxide is pronounced. In view of these and the rather large counting deviations in their experiments, a quantitative interpretation of their results is not possible.

At least three apparent explanations of our results are suggested, the latter two of which represent deviations from the normal fermentation mechanism: (a) that metabolic carbon dioxide (from the radioglucose) is reversibly reincorporated in lactic acid, in which case the activity would reside primarily in the carboxyl group, (b) that there is a fermentation mechanism in which carbons one and six rather than three and four are oxidized, (c) during the course of the normal fermentation mechanism the triose phosphates are in (partial) equilibrium with the free trioses, as in Fig. 2. This would, of course, result in activity appearing in both carbons one and three of lactic acid, to an extent depending on the amount of approach to an equilibrium. We have not investigated the enzyme systems involved and are thus not able to choose between the second and third explanations. The first explanation appears difficult to reconcile with the fact that the fermentation is conducted in M/10 bicarbonate (pH 6.8), with subsequent dilution of carbon dioxide formed by metabolism. Furthermore, control experiments with inactive glucose but with radiocarbonate ($2.8 \times 10^6$ disintegrations per min in 5 ml of reaction mixture) resulted in approximately 0.02% of the activity in the lactic acid formed. There still remains the possibility, albeit dubious, that the metabolic carbon dioxide is preferentially used at the site of formation, prior to the diffusion and subsequent dilution effects of the solution's inactive bicarbonate.

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# Diurnal Responsiveness of *Erysiphe graminis* to Nutrients


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Because it can be grown readily on barley in the greenhouse throughout the year, because the conidia germinate well on various culture media, and because germ tube growth is sharply increased by a number of nutrient chemicals, the fungus *Erysiphe graminis* D.C. appears well suited to studies of growth *in vitro* of a powdery mildew. No member of the Erysiphaceae has been cultured on a nonliving substrate, and all are commonly regarded as obligate parasites.

Previous studies of *Erysiphe graminis* have indicated no diurnal cycle in the germinability of the conidia or in the division of the generative cell such as is exhibited by *Erysiphe polygoni* (*1*). On an agar substrate, germ tube growth of *E. graminis* is increased by crude animal and plant extracts, especially a solution of hen's egg yolk; by iron sulfate, sucrose (*2*), disodium α-tocopherol phosphate, and Tween 60 (*4*). More recently, a medium consisting of 0.8% agar, 0.6% fructose, and 0.003% animal lecithin has been used. Using this medium, there has been great variation in germ tube length in different trials; such variation being associated, apparently, with the time of day the trials were started.

To check on the effect of time, plates of plain agar and lecithin-fructose agar, prepared simultaneously and stored at 0° C, were seeded with conidia of *E. graminis* from greenhouse plants at various times throughout the day and incubated at 16° C in the dark, and the germ tube growth was measured 5 days after seeding. Results of the first trial, started on February 11, 1949, are presented in Fig. 1, and indicate that germ tube growth on nutrient agar was greatest from seedings made about 12 M., and poorest from seedings made at 8:30 A.M. The germ tubes of the 12:30 P.M. seeding were 85 μ greater or 175% greater than the germ tubes of the 8:30 A.M. seeding. Germ