The discrepancy becomes all the more apparent in considering the fact that a reasonable value for the efficiency of most biological mechanisms is about 20 to 30 percent.

This analysis renders untenable the Franck-Mayer hypothesis for the maintenance of the osmotic gradient. However, it does not rigorously exclude an osmotic gradient hypothesis for the transport of water if (i) the water-transporting cells have a metabolic rate more than 1000 times greater than the maximal value reported for mammalian tissues, (ii) a gradient-maintaining mechanism can be found that would be capable of funneling energy from a high fraction of the total cellular mass to a few milligrams of water-transporting mass, (iii) there were cells of inordinate thickness ($\Delta X > 6$ cm), or (iv) there existed in cells small solute particles of high osmotic activity with a much smaller diffusion constant than that recorded for the largest protein molecule. The analysis will be presented in detail elsewhere (*3*).

References and Notes

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A Study of Leucine Biosynthesis in *Torulopsis utilis*

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As part of a study of biosynthetic mechanisms, the formation of the branched chain amino acids, valine, isoleucine, and leucine in *Torulopsis utilis* is under investigation using isotope tracer methodology. Data are presented in this paper (*1*) that indicate that the leucine carbon chain consists of an acetyl group attached to the isobutyryl moiety of valine. In previous studies (*2*), results of experiments were reported in which *T. utilis* was grown on glucose as the principal carbon source in the presence of variously C^{14}-labeled acetates and lactates. Valines and isoleucines were isolated from the yeast cells, they were degraded by chemical procedures, and the individual carbon atoms were assayed for radioactivity. Based on these findings, mechanisms for the biosynthesis of these amino acids have been proposed (*2*).

Using a degradation procedure similar to that employed for valine and isoleucine, C^{14}-distribution patterns were obtained for the leucines isolated from the same experiments; these are given in Table 1. Acetate carboxyl carbon was exclusively and abundantly present in the carboxyl carbon of leucine, and the acetate methyl carbon appeared overwhelmingly in the leucine α carbon. Lactate carbon 1 was incorporated to a negligible extent, hence, degradation was not conducted. Large amounts of lactate carbons 2 and 3 also appeared in the respective carboxyl and α carbons of leucine, a result anticipated on the basis of the ready conversion of lactate carbons 2 and 3 to acetate. However, lactate carbon 2 was also incorporated readily and equally in leucine carbons 3 and 4, and lactate carbon 3 also appeared in leucine carbons 5,5′. The similarity in the distribution of all three lactate carbons in leucine carbons 3 to 5,5′ to that observed previously in carbons 2 to 4,4′ of valine (*2*), shown at the right of the table, leaves little doubt of the common origin of the isobutyryl moieties of both amino acids. Gilvarg and Bloch (*3*) and Ehrensvard *et al.* (*4*) also found that acetate carboxyl and methyl carbons were incorporated into leucine biosynthesized by yeast. Adelberg (*5*) reported learning, in a private communication from Ehrensvaard, that yeast grown on acetate as the sole carbon source yielded valine and leucine with the same isotope distribution in their isobutyryl moieties. Abelson, (*6*) recently showed that pyruvate, α-ketoisovalerate and L-valine all lowered the specific activities of leucines synthesized by *Escherichia coli* from uniformly labeled glucose. He suggested that these substances are intermediates of leucine biosynthesis, and further suggested that α-ketoisovalerate combines with acetate to yield the

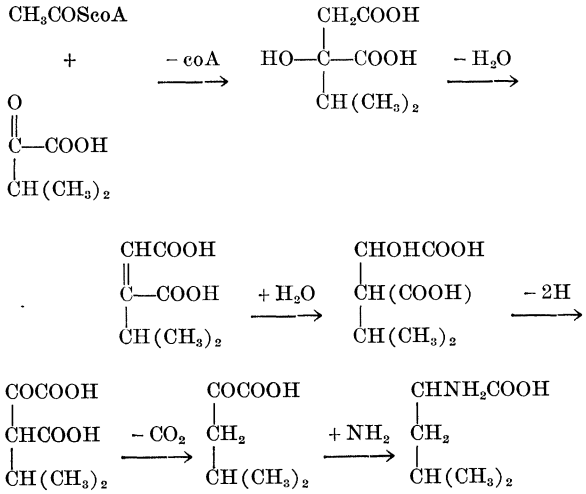
Table 1. Pattern of C^{14} distribution in leucine and valine. Values are based on standard dosage of 100 μc administered.

Substrate	Specific activity of leucine	Percentage of total activity in leucine carbon					Specific activity of valine	Percentage of total activity in valine carbon			
		5,5′ $(CH_3)_2$	4 CH	3 CH_2	2 $CHNH_2$	1 COOH		4,4′ $(CH_3)_2$	3 CH	2 $CHNH_2$	1 COOH
Acetate-1-C^{14}	14,040	0	0	0	1	99					
Acetate-2-C^{14}	21,380	3	1	2	89	2					
Lactate-1-C^{14}	156*						12,080	0	0	1	99
Lactate-2-C^{14}	25,630	2	32	33	1	31	20,670	1	47	49	3
Lactate-3-C^{14}	21,540	59	1	2	37	1	14,730	91	4	4	1

* This leucine sample was not degraded.

keto analog of leucine. The present results confirm and extend this postulation.

By analogy with the condensation of oxalacetate with acetate to yield citric acid, the following detailed mechanism is proposed for the synthesis of the leucine carbon chain.



α-Ketoisovalerate is presumed to condense with the methyl carbon of acetyl coA to yield α-hydroxy-α-isopropylsuccinic acid. By the same series of reactions undergone by citric acid to yield α-ketoglutaric acid, this hydroxy acid would be converted to α-keto-γ-methylvaleric acid, which, by transamination with glutamic acid (*7*), would yield leucine. The possible participation of these hypothetical intermediates in leucine biosynthesis is now under study (*8*). A similar reaction sequence was suggested by us to account for the synthesis of α-aminoadipic acid in connection with the biosynthesis of lysine (*9*).

References and Notes

* Postdoctoral fellow of the National Institutes of Health, U.S. Public Health Service.

1. This work was done under contract with the U.S. Atomic Energy Commission, contract No. AT(30-1)777, and was aided in part by grants from the National Cancer Institute of the U.S. Public Health Service and the American Cancer Society.
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8. Reed *et al.* [*J. Am. Chem. Soc.* **76**, 5574 (1954)], in experiments similar to ours but with a different degradation procedure, found essentially the same distribution of acetate carboxyl and pyruvate-2-carbon in leucine as we report here with acetate carboxyl and lactate-2-carbon. These investigators postulated a mechanism involving successive condensations of acetate methyls with the carbonyl carbon of α-ketoglutarate in such fashion that acetyl methyls ultimately become the methyls of leucine. Our data with acetate-2-C^{14} in the table clearly show this does not occur.
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22 November 1954.

Segregation of Sex Factors in a Diploid Line of *Ustilago zeae* Induced by Alpha Radiation

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During investigations of the effect of naturally radioactive heavy metals on microorganisms, a phenomenon that appears to be somatic segregation and recombination has been induced repeatedly in solopathogenic, diploid lines of *Ustilago zeae,* the common corn smut fungus, by alpha radiation. This report (*1*) on the solopathogenic line 410qq compares the segregation and recombination of the factors for sexual compatibility that occurred during meiosis in chlamydospore germination with that which was induced by the irradiation of vegetative cells.

U. zeae is normally heterothallic, and galls and chlamydospores (zygotes) are produced when corn is inoculated with pairs of compatible haploid lines (*2, 3*). Solopathogens were described by Christensen (*4, 5*) and Eddins (*6*) as monosporidial lines that by themselves produced galls and chlamydospores in inoculated corn. The cultural, sexual, and pathogenic segregants obtained during germination of the chlamydospores produced by these lines (*4, 5*) are identical to those recovered from chlamydospores of a cross of haploid lines. Since cells of solopathogens are uninucleate (*5*), they are considered to be diploid, although cytological proof of the presence of $2n$ chromosomes has not been obtained.

The solopathogenic character of monosporidial lines of *U. zeae* is not always stable. Some of the solopathogenic lines characterized by Christensen (*5*) and Stakman *et al.* (*7*) were found to be avirulent in later tests. Chilton (*8*) isolated a variant from a solopathogen that was compatible with certain haploid lines but nonpathogenic when inoculated alone in corn. The solopathogenic line 410qq used in this study was isolated in 1943 by Stakman *et al.* (unpublished) from a cross of lines 10A4 × 17D4, and the character of solopathogenicity has remained unchanged in stock cultures since that time. However, variants that were compatible with 10A4 but not with 17D4 were isolated by Gattani (*9*) from line 410qq grown on mediums containing uranyl nitrate. During the current investigation, 349 monosporidial isolates from suspensions of cells of line 410qq that were not exposed to alpha radiation were tested as controls, and all were solopathogenic.

Vegetative sporidia of the diploid line 410qq that were exposed to alpha radiation were harvested from young monosporidial cultures grown in potato-dextrose-broth (PDB) by shake culture. In the original trials, washed sporidia were exposed to alpha radiation by suspension in a solution of 1 μc/ml of polonium-210 after the method of Rowell *et al.* (*10*). For later exposures, however, the alpha radiation emitted from a 10-mc source of polonium-210 plated