bin. The solutions of activated fibrinogen were prepared from fibrin clots dissolved in urea. The Kerr coefficients were obtained, and development and decay curves resulting from square pulses were analyzed following Benoit.[6] The decay curves provided rotary diffusion constants in agreement with those previously obtained from flow birefringence[7] and corresponding to rotation of the long axis[8] of about 600 Å. The development curves showed that at *p*H 6 the orientation was entirely attributable to induced polarization. However, at higher *p*H (7 to 10) there were contributions from permanent dipole moment. In this range, the calculated dipole moment[9] of activated fibrinogen was found to exceed that of the unactivated molecule by not more than 110 *D*.

Regardless of the value of net charge, the change in dipole moment $\Delta\mu$ accompanying an alteration of charge by Δz units at a distance x from the center of symmetry of the molecule[11] is $x\epsilon\Delta z$, where ϵ is the electronic charge. Since the loss of peptides upon activation involves[2,12-14] a Δz of 10 to 14, it follows that x is not greater than 1 to 2 Å. This represents a distance measured along the long axis, of course; components of dipole moment parallel to the short axis would not be detected in these experiments. We conclude that the site of attack by thrombin is on one side, equidistant from the ends of the fibrinogen molecule.[15]

Further details will be reported subsequently.

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RECEIVED AUGUST 9, 1954

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BIOSYNTHESIS OF LEUCINE IN BAKERS' YEAST[1]

Sir:

Recent isotopic studies of the biosynthesis of leucine in micro-organisms have indicated that in yeast,[2] $CH_3C^{14}OOH$ gave rise to leucine with approximately half of its activity residing in the carboxyl carbon. In similar experiments in *Escherichia coli*[3] and *Rhodospirillum rubrum*[4], over 70% of the activity in leucine was located in the carboxyl carbon. $C^{14}O_2$ was found to be incorporated to only a limited extent into leucine in *Rhodospirillum*.[4]

In this laboratory the test organism, *Saccharomyces cerevisiae*, was obtained by isolation from a cake of Fleischmann's bakers' yeast, stock cultures being carried on malt–agar slants. To these samples, previously grown on glucose, was administered 20 mM. each of $CH_3C^{14}OCOOH$ or $CH_3C^{14}OOH$ with a specific activity of 18.5×10^5 c.p.m. per mM., as the sole carbon source in a salts–$(NH_4)_2SO_4$ medium. In the pyruvate experiment all the labeled substrate was utilized aerobically in four hours. With acetate as substrate, 39% was utilized under similar conditions. Details of these fermentations have been given elsewhere.[5]

Purity of the leucine samples, isolated from the hydrolysates of the bakers' yeast by means of Dowex-50 column chromatography,[6] was established by paper chromatography, using *sec*-butanol–NH_3 as the solvent system.[7] Various concentrations of the leucine samples were employed, and in no case were other amino acids observed. Degradation studies of this amino acid were carried out in the following manner: (1) combustion for the whole molecule; (2) ninhydrin decarboxylation for

TABLE I

RADIOACTIVITY IN THE CARBON SKELETONS OF GLUTAMIC ACID AND LEUCINE FROM YEAST GROWN ON $CH_3C^{14}OOH$ AND $CH_3C^{14}OCOOH$

Glutamic Acid		Leucine			
Radioactivity					
Carbon atom	Found,[12] %	Carbon atom	Calcd. %	Found %	Found c.p.m.,[b] $\times 10^{-5}$/mM
Acetate substrate					
1 COOH[a]	34	5′CH_3	0	0	
		5 CH_3	0	0	
2 $CHNH_2$	0	4 CH	0	0	
3 CH_2	0	3 CH_2	0	0	
4 CH_2	0	2 $CHNH_2$	0	0	
5 COOH	64	1 COOH	100	100	0.50
Total					0.50
Pyruvate substrate					
1 COOH[a]	26	5′CH_3	0	1	0.04
		5 CH_3	0	1	0.04
2 $CHNH_2$	17	4 CH	23	25	0.72
3 CH_2	19	3 CH_2	26	25	0.71
4 CH_2	0	2 $CHNH_2$	0	0	0.01
5 COOH	39	1 COOH	51	47	1.37
Total					2.89

[a] Carbon 1 of glutamic acid is lost in the proposed scheme of leucine biosynthesis. [b] Specific activity (total) is expressed as counts per minute per millimole of amino acid; the activities of individual carbon atoms are counts per minute per millimole of carbon.

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the carboxyl group; (3) deamination with HNO_2 and oxidation of the hydroxy acid formed with CrO_3 and acetic acid to yield acetone[8] (carbons 4, 5 and 5′); (4) hypoiodite oxidation of acetone to give iodoform, equivalent to carbon atoms 5 and 5′; (5) Ag_2O oxidation of leucine to yield isovaleric acid,[9] and decarboxylation of the latter by means of the Schmidt reaction[10] to produce 2-methyl-*n*-propylamine, equivalent to carbon atoms 3, 4, 5 and 5′, and CO_2 equivalent to carbon atom 2. All samples were counted as $BaCO_3$ with appropriate corrections applied for background and self-absorption. The degradation data are given in Table I.

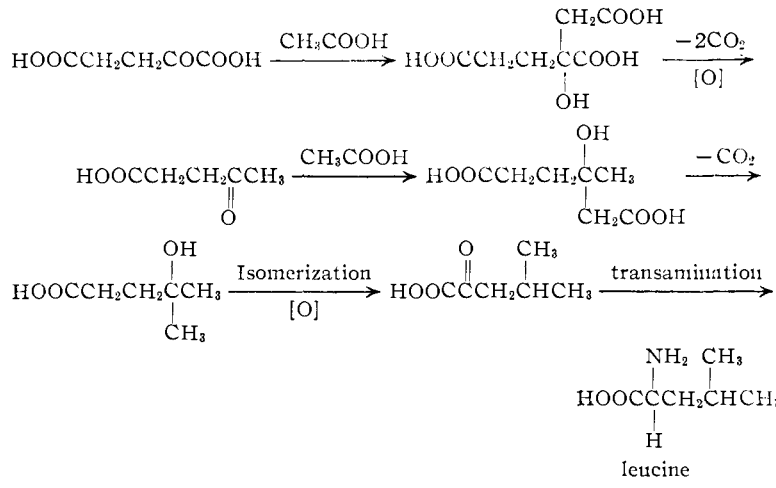

Fig. 1.—Proposed scheme of leucine biosynthesis in yeast.

The finding of exclusive carboxyl labeling in leucine derived from $CH_3C^{14}OOH$ is not exactly in agreement with the observation made in the Torula yeast and *E. coli* experiments.[2,3] However, it is evident that the carboxyl carbon of leucine bears an intimate relationship to the carboxyl of acetate.

The labeling in leucine from the pyruvate yeast sample bore several noteworthy features. Carbons 2, 5 and 5′ contained no isotope, which indicated that their origin might be the pyruvate methyl carbon (since groups referable to pyruvate C-1, such as the carboxyl of alanine, phenylalanine or tyrosine possessed considerable radioactivity). The absence of isotope in carbon 2 was unique among nine amino acids thus far examined in this yeast and indicated that no randomization or other distribution reactions had occurred during leucine biosynthesis which could have introduced C^{14} into this position. Moreover, when $C^{14}O_2$ and non-isotopic pyruvate were employed as substrates, no C^{14} incorporation into this amino acid could be observed.[11] This indicated that no reversible carboxylation reactions were involved in the formation of the leucine skeleton. The equal distribution of C^{14} in carbons 3 and 4, on the other hand, indicated a possible isotope equilibration in the synthesis of this portion of the carbon chain.

Further examination of the leucine skeleton revealed almost exact duplication of the isotope pattern in carbons 1, 2, 3 and 4 with that of carbons 5, 4, 3 and 2 of glutamic acid.[12] It therefore appeared possible that two "acetate" condensations may have occurred onto α-ketoglutarate, to produce the leucine skeleton as depicted in Fig. 1. The condensation pictured with acetate is a familiar type, as in the biosynthesis of citric acid, whereas the suggested shift of the hydroxy group from C-4 to C-2 of the leucine precursor might involve reversible dehydrations similar to that found in the aconitase system.

The calculated percentage distribution of C^{14} in leucine, based on the isotope distribution in glutamic acid[12] is in good agreement with the observed value both in the pyruvate and acetate yeast samples as shown in Table I. This scheme also accounts for the failure to incorporate $C^{14}O_2$ into leucine, since the only labeled carbon (α-carboxyl) in glutamic acid would be expected to be lost in the decarboxylation processes postulated.

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RECEIVED AUGUST 23, 1954

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