Although the occurrence of exchange reactions independent of and in addition to the reactions that lead to net uptake of amino acids and peptides by bacteria complicates measurement of the kinetics of accumulation with radioactive tracers, previous conclusions that transport of these substances into the cell occurs by an active, energy-dependent process with structural specificity characteristic of enzymic processes remain unchanged.

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Department of Biochemistry, University of California, Berkeley, Calif. (U.S.A.)

FRANKLIN R. LEACH*
ESMOND E. SNELL

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* Present address: Department of Biochemistry, Oklahoma State University, Stillwater, Okla.

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Leucine biosynthesis in higher plants

There is considerable evidence from labelling experiments that in microorganisms leucine arises from the condensation of 2-ketoisovaleric acid (ketovaline) and acetate[1–3]. Support for such a pathway, involving the intermediate formation of α-isopropylmalate, has recently been obtained at the enzyme level in *Salmonella typhimurium* and *Torulopsis utilis*[4,5] and α-isopropylmalate has been isolated from cultures of several microorganisms[6]. In the course of an investigation of the biosynthesis of linamarin in flax, some evidence for the presence of this pathway for leucine biosynthesis was obtained incidentally and the results are briefly reported here.

Flax seeds (*Linum usitatissimum* L. var. *Imperial*) were germinated and grown for 72 h in the dark on gauze moistened with dilute nutrient solution. They were next exposed to artificial light of intensity approx. 2000 ft-candles from an incandescent lamp for 16 h. Uniformly labelled L-[^{14}C]valine was administered by either of the following methods:

(a) 20 seedlings selected for uniformity were placed in a 5-ml beaker containing 5 μmoles L-[^{14}C]valine (1 μC) in 2 ml water. This was administered for 48 h with aeration and continuous illumination.

(b) The stems of 20 seedlings were cut with a sharp razor blade 2 cm below the cotyledon leaves and placed in a 1-ml beaker containing 5 μmoles L-[^{14}C]valine (1 μC) in 0.2 ml water. Successive amounts of 0.1 ml water were added as required during a 7-h absorption period in continuous light.

The plant material was harvested, frozen in liquid nitrogen, ground to a fine powder with a mortar and pestle and extracted with 100 vol. boiling 80% (v/v) aq. ethanol for 5 min. The solvent was evaporated *in vacuo* and the residue extracted with 2 ml 10% (v/v) aq. isopropanol. The extract was clarified by centrifugation if necessary and suitable aliquots were used for paper chromatography.

Similar experiments were also carried out with seedlings of *Sorghum vulgare* var. *Honey Drip*. In some experiments the plant residue remaining after aq. ethanol extraction was refluxed with 20 vol. 6 N HCl for 16 h and the acid removed by repeated evaporation to dryness. The residue was extracted with 2 ml 10% (v/v) aq. isopropanol and suitable aliquots taken for paper chromatography.

Paper chromatography in one dimension on Whatman No. 1 paper was carried out in each of the following solvent systems: *n*-butanol–pyridine–water (60:40:30, v/v), methylethyl ketone–acetone–water (30:10:6, v/v), butanol–acetic acid–water (120:30:50, v/v) and phenol–water (80:20, v/v). The distribution of radioactivity was determined using a chromatogram strip scanner (Nuclear Chicago Model R 1000). With ethanol extracts from flax seedlings the major radioactive peaks corresponded with linamarin and L-valine markers in all solvents; a smaller peak was also seen corresponding with L-leucine marker. With ethanol extracts from sorghum seedlings two peaks were seen corresponding to L-valine and L-leucine markers. Chromatograms of the acid hydrolysates from both flax and sorghum seedlings showed the presence of two radioactive peaks corresponding to L-valine and L-leucine markers in all solvent systems.

The identity of the suspected [^{14}C]leucine in flax seedlings was definitely established by the preparation of the naphthalene-β-sulphonyl (nasyl) derivative[7]. Partial purification was first accomplished by successive paper chromatography and elution using butanol–pyridine–water and methylethyl ketone–acetone–water. L-Leucine (50 mg) was added and the nasyl derivative prepared and dried. A suitable sample was dissolved in a known volume of ethanol and aliquots were evaporated on planchettes and counted at infinite thinness in a gas-flow counter (Nuclear-Chicago Model 181B). The counting procedure was repeated through 3 recrystallisations with the same specific activity (290 counts/min/mg derivative) being observed throughout.

Table I shows the percentages of ^{14}C found in free and bound leucine in an experiment with sorghum seedlings. The low ratio of 2.7 for the ^{14}C in bound valine and leucine shows that leucine biosynthesis by a pathway of the type proposed represents an important source of leucine in this tissue.

Attempts to demonstrate the participation of α-isopropylmalate as an intermediate were inconclusive. [^{14}C]Isopropylmalate could not be detected in aq. ethanol extracts of seedlings administered L-[^{14}C]valine. A competition experiment was carried out with tops of green flax seedlings in which the amount of label incorporated into free and bound leucine from L-[^{14}C]valine was compared in the presence and absence of synthetic DL-α-isopropylmalate. It will be seen from Table II that a competitive or inhibitory effect of isopropylmalate was evident from the specific activity of bound leucine, but not in the case of free leucine. It is of interest in this connexion that STRASSMAN AND CECI[5] found synthetic DL-α-isopropylmalate was not converted to α-ketoisocaproic acid by yeast extracts whereas the natural α-isopropylmalate was converted.

TABLE I

PERCENTAGE INCORPORATION OF ^{14}C IN VALINE AND LEUCINE OF ETHANOL EXTRACTS AND HYDROLYSATES OF SORGHUM SEEDLINGS AFTER L-[^{14}C]VALINE ADMINISTRATION

L-[^{14}C]Valine administered by method (a). Radioactivity determined by planimetric measurement of the areas of peaks from chromatogram scanning and comparison with standard amounts of L-^{14}C valine.

Amino acid	Ethanol extract		Hydrolysate	
	^{14}C (%)	[^{14}C]Valine / [^{14}C]Leucine	^{14}C (%)	[^{14}C]Valine / [^{14}C]Leucine
Leucine	1.3	33	3.5	2.7
Valine	42.3		9.8	

TABLE II

SPECIFIC ACTIVITIES OF FREE AND BOUND ^{14}C LEUCINE AFTER L-[^{14}C]VALINE ADMINISTRATION TO FLAX TOPS WITH AND WITHOUT DL-α-ISOPROPYLMALATE

L-[^{14}C]Valine administered by method (b). Leucine separated by paper chromatography[8] and determined by a ninhydrin method[9]. Specific activity of administered L-[^{14}C]valine was 4.8 μC/μmole.

Treatment	Specific activity (mμC/μmole)	
	Free leucine	Bound leucine
L-[^{14}C]Valine, (0.21 μmole)	33	26
L-[^{14}C]Valine, (0.21 μmole) and DL-α-isopropylmalate, (10 μmoles)	33	10

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Department of Biochemistry and Biophysics, University of California, Davis, Calif. (U.S.A.) G. W. BUTLER* LAURA SHEN**

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* U.S. Public Health Service International Post-Doctoral Research Fellow (FF-246). Present address: Plant Chemistry Division, D.S.I.R., Palmerston North (New Zealand).
** U.S. Public Health Service Pre-Doctoral Trainee (2G119).