per 100 mg protein. This is apparently a low molecular weight RNA (S-RNA) with different metabolic properties from the high molecular weight RNA of the ribonucleoprotein of the microsomes. When the amino acid activating enzyme preparation is incubated with ATP and $^{14}$C-carboxyl labeled leucine, at pH 7.5, the S-RNA subsequently isolated from this fraction is found to be labeled (0.02 to 0.05 $\mu$moles leucine per mg RNA). The time curve of labeling is linear for 2 min, maximal at 10 min, and thereafter the $^{14}$C-leucine label is rapidly lost. Microsomal RNA is labeled at approximately a tenth this rate. 0.005 $M$ leucine and 0.01 $M$ ATP saturate the system, and ribonuclease is completely inhibitory. Labeling is additive when 0.005 $M$ $^{14}$C-valine and 0.005 $M$ $^{14}$C-glycine are also incubated in the same preparation. Yeast RNA, microsomal RNA, and degraded microsomal RNA (prepared by mild alkaline hydrolysis of microsomes) do not give increased labeling when added to the system.

Leucine-labeled S-RNA so obtained from the activating enzymes preparation is non-dialysable and is charcoal and Dowex-1 adsorbable. The $^{14}$C-leucine-RNA bond is acid stable and alkali labile, and does not exchange with free $^{12}$C-leucine. The ninhydrin-$CO_2$ method indicates no free $^{14}$C-leucine. When labeled S-RNA is incubated with anhydrous hydroxylamine and the products are chromatographed on paper a spot corresponding to leucine hydroxamic acid contains all the radioactivity.

The activating enzyme preparation labeled with $^{14}$C-leucine (and reprecipitated twice at pH 5.2 from dilute solution to remove free $^{14}$C-leucine and ATP) will transfer the bound $^{14}$C-leucine to microsome protein upon subsequent incubation with microsomes and GTP (Table I). The GTP effect is apparently specific and is potentiated by addition of a nucleotide triphosphate generating system. With this complete system a high percentage of the S-RNA bound $^{14}$C-leucine is transferred to protein, the rest remaining in S-RNA.

$^{14}$C-Leucine-S-RNA prepared by a phenol method[3] has been found to transfer $^{14}$C-leucine to microsome protein in the absence of added activating enzymes, provided GTP is present.

Preliminary results, using an ascites tumor *in vivo* incorporation system[4], reveal that S-RNA becomes labeled with $^{14}$C-leucine more rapidly than does the protein of the ribonucleoprotein particles of the microsomes, the most rapidly labeled protein fraction in this system.

These experiments suggest that incorporation of labeled amino acids into protein is indeed dependent upon the amino acid activation system. The initial formation of an enzyme-bound amino acyl-AMP compound, as originally suggested, accounts for hydroxamic acid formation and PP–ATP exchange[1]. It is now further postulated that this initial activation of amino acids is followed by a transfer of activated amino acid to S-RNA. This latter reaction is ribonuclease sensitive, while the former is not. GTP mediates the transfer of this activated amino acid to peptide linkage via the microsome by a mechanism as yet unknown.

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1 M. B. HOAGLAND, E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 218 (1956) 345.
2 J. A. DEMOSS AND G. D. NOVELLI, *Biochim. Biophys. Acta*, 22 (1956) 49.
3 K. S. KIRBY, *Biochem. J.*, 64 (1956) 405.
4 J. W. LITTLEFIELD AND E. B. KELLER, *J. Biol. Chem.*, 224 (1957) 345.
5 E. B. KELLER AND P. C. ZAMECNIK, *J. Biol. Chem.*, 221 (1956) 45.
6 L. I. HECHT AND V. R. POTTER, *Cancer Research*, 16 (1956) 988.

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## N-Succinyl-L-diaminopimelic acid, an intermediate in the biosynthesis of diaminopimelic acid*

$\alpha,\varepsilon$-Diaminopimelic acid (DAP) has been shown to occur quite generally in bacteria and blue green algae[1]. In these organisms it appears to serve not only as a cell constituent but also as a precursor of lysine[2,3]. However, little is known concerning the intermediates which participate in DAP biosynthesis. In an effort to gain such information, several mutants of *E. coli* were obtained

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with an absolute requirement for DAP. One of the mutants (D-1) was found to accumulate a compound which was nutritionally active for the same mutant, but only after acid hydrolysis. The accumulated compound was isolated as a barium salt in a state of about 60% purity. After acid hydrolysis of the barium salt, an acidic substance could be extracted with ether. This was obtained in crystalline form and was identified as succinic acid (m.p. 187–188°, no depression on mixture with authentic succinic acid). The other moiety was identified as L-DAP, by the nutritional response of a DAP auxotroph (M-173-25)[3] and correspondence with known L-DAP by paper chromatography in the solvent of RHULAND *et al.*[4]. This solvent distinguishes L- from *meso*-DAP. Analytical data on the composition of the compound are given in Table I.

TABLE I

COMPOSITION OF BARIUM SALT OF COMPOUND ACCUMULATED BY D-1
(Hydrolysis was carried out in 4 *N* HCl at 100° C for 2 h)

| | *Succinate** μmoles/mg | *DAP*** μmoles/mg |
|---|---|---|
| Before hydrolysis | 0 | 0*** |
| After hydrolysis | 1.4 | 1.7 |

* Determined spectrophotometrically with succinoxidase and cytochrome *c*.
** Determined colorimetrically. Details of this method will be published elsewhere.
*** No activity towards DAP auxotroph 173–25.

The unhydrolyzed material gave a positive acyl amine test[5] and the chromogen was identified as succinhydroxamic acid by paper chromatography using butanol-water as solvent system.

These observations suggested that the unknown compound was N-succinyl-L-DAP. This was confirmed by preparing synthetic N-succinyl-L-DAP through the action of succinic anhydride on L-DAP. The synthetic material possessed the same $R_F$ as the natural compound in several solvent systems.

The compounds which auxotrophic mutants accumulate are frequently biosynthetic intermediates. Such a conclusion can be strengthened if enzymic conversion to the proposed end product can be demonstrated *in vitro*. Both synthetic and natural N-succinyl-L-DAP are rapidly split by extracts prepared from either wild type *E. coli* or a lysine-requiring mutant (M-26-26)[3]. The enzyme could not be detected in extracts prepared from the mutant D-1 (Table II).

TABLE II

ENZYMIC HYDROLYSIS OF N-SUCCINYL-L-DAP

100 μmoles imidazole buffer pH 6.8, 1 mg barium salt; final volume 0.5 ml; 37° C; 1 h. In the experiment with the extract from M-26-26, the products of the reaction were shown to be succinic acid and DAP by spectrophotometric assay and paper chromatography respectively.

| *Extract source* | *Protein (mg)* | *% hydrolyzed* |
|---|---|---|
| Wild type *E. coli* ATCC 9637 | 6.1 | 85 |
| M-26-26 | 6.1 | 100 |
| D-1 | 5.0 | 0 |

Cleavage of N-succinyl-L-DAP must yield L-DAP. However, the substrate of DAP decarboxylase is known to be *meso*-DAP[1]. These observations are easily reconciled since an enzyme that interconverts L- and *meso*-DAP has been detected in bacterial extracts[6].

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[1] E. WORK, in W. D. MCELROY AND B. GLASS, *Amino Acid Metabolism*, The Johns Hopkins Press, Baltimore, 1955, pp. 462–492.
[2] D. L. DEWEY AND E. WORK, *Nature*, 169 (1952) 533.
[3] B. D. DAVIS, *Nature*, 169 (1952) 534.
[4] L. E. RHULAND, E. WORK, R. F. DENMAN AND D. S. HOARE, *J. Am. Chem. Soc.*, 77 (1955) 4844.
[5] J. KATZ, I. LIEBERMAN AND H. A. BARKER, *J. Biol. Chem.*, 200 (1953) 417.
[6] D. S. HOARE, *Biochem. J.*, 59 (1955) xxii.

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* Scholar in Cancer Research, the American Cancer Society.