transfers large-molecular label to the epithelial surface where ultrastructurally visible collagen occurs (*4*), and soluble tropocollagen does not replace salivary mesenchyme in its morphogenetic effect (*9*). These facts suggest that while the developmental influence of salivary mesenchyme on salivary epithelium may include the provision of soluble collagen, it involves additional components as well.

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Protein Synthesis in Rat Liver: Influence of Amino Acids in Diet on Microsomes and Polysomes

Abstract. *Rats were killed 1 hour after having been fed by stomach tube with amino acid mixtures that were either nutritionally complete or lacked tryptophan. Microsomes isolated from the livers of animals fed the incomplete mixture showed a reduced capacity to incorporate ^{14}C-leucine in vitro, and polysomes prepared from the same livers showed an increased proportion of monosomes and disomes. Prior treatment with actinomycin D did not prevent these differences in response to the two amino acid mixtures, an indication that synthesis of messenger RNA may not be involved in the cell mechanism which recognizes differences in amino acid pattern.*

The protein content of the liver changes rapidly when the intake of dietary protein is varied (*1*). The amounts of RNA and phospholipid in the liver cell vary with protein content of the cell (*2*), and structural changes have also been demonstrated in the nucleus (*3*) and in the endoplasmic reticulum (*4*). Consequently, these observations point to a complex regulation of protein synthesis in liver in relation to the supply of amino acids.

Clark, Naismith, and Munro (*5*) showed that there are considerable differences in uptake of precursors into liver RNA within a few hours of feeding protein to fasting rats. In later experiments the effect of feeding an amino acid mixture on labeling of liver RNA depended on the nutritional properties of the amino acids in the mixture; deletion of tryptophan from the mixture led to reduced incorporation of ^{14}C-orotic acid into the pyrimidine bases and of ^{14}C-glycine into the purine bases (*6*). The most likely explanation for these observations is that the absorption of a complete mixture of amino acids stimulates synthesis of messenger RNA (mRNA), whereas the incomplete mixture fails to do so. Consequently, the formation of polysomes in the cytoplasm may be favored by the additional messenger, and this should be reflected in greater protein synthesizing activity of the microsome fraction in the livers of such animals. We have accordingly fed rats with an amino acid mixture that was either nutritionally complete or was deficient in tryptophan. The microsomes prepared from the livers of each group of animals were tested for their capacity to incorporate labeled amino acids, and the pattern of polysomes in the microsomes was also examined.

Male albino rats (150 g) were fasted overnight. Groups of rats were fed by stomach tube with mixtures of amino acids (*6*) in which the complete mixture provided all ten essential amino acids, and also cystine, tyrosine, aspartic acid, glutamic acid, proline, and alanine. One group of rats received the nutritionally complete mixture, whereas a second group were given the mixture with tryptophan omitted. The complete mixture of amino acids, administered in 3 ml of water to each rat, consisted of the L-isomers of valine, 100 mg; leucine, 120 mg; isoleucine, 80 mg; cystine, 20 mg; methionine, 80 mg; threonine, 70 mg; phenylalanine, 120 mg; tyrosine, 60 mg; lysine (HCl), 25 mg; histidine (HCl), 95 mg; arginine (HCl), 50 mg; alanine, 20 mg; proline, 20 mg; aspartic acid, 20 mg; glutamic acid, 200 mg; and tryptophan, 50 mg; together with $NaHCO_3$, 127 mg. One hour after feeding, the animals were stunned, and the livers were removed and homogenized in two volumes of medium A (*7*). Cell debris, nuclei, and mitochondria were precipitated and removed by centrifugation at 19,000*g* for 10 minutes; the remaining supernatant was divided into two portions. The first was used for studies of amino acid incorporation into microsomes, and the second for preparation of polysomes.

Microsomes were prepared from the first portion of this supernatant by centrifugation at 135,000*g* for 70 minutes. The final supernatant, after removal of the microsomes, was recovered from the homogenate from both groups and mixed to provide the cell-sap fraction used for incorporation studies. The microsome pellets were suspended in 0.25*M* sucrose buffer and recovered by centrifugation. The new pellet was suspended in a small volume of sucrose buffer, and heavy aggregated particles were removed by centrifugation at 850*g* for 10 minutes. The microsomes remaining in suspension were used for the study of amino acid incorporation in vitro. Samples of the microsomes containing 2 mg protein were incubated at 37°C with cell sap (7.5 mg protein) which had been passed through Sephadex G25 to remove inhibitors (*8*). The incubation medium consisted of 0.25*M* sucrose-tris buffer at *p*H 7.6 and contained 2 μmole adenosine triphosphate, 0.5 μmole guanidine triphosphate, 14.8 μmole creatine phosphate, 20 μg creatine phosphokinase, and 1 μc ^{14}C-DL-leucine. At the end of the incubation, the tubes were chilled and treated with an equal volume of cold 0.6*N* $HClO_4$ containing 1 mg of ^{12}C-DL-leucine. After two washings with 0.3*N* $HClO_4$, the RNA was hydrolyzed by incubation in 0.3*N* KOH for 1 hour at 37°C. The protein was then recovered quantitatively by acidification of the digest, separated by centrifugation, and redissolved in alkali for measurement of radioactivity in a gas-flow counter. The results were calculated as counts per minute per milligram of microsome protein in the sample.

In order to obtain polysomes, the second portion of the supernatant fraction (from which mitochondria had been removed) was treated with deoxycholate to a final concentration of 1 percent, and C-ribosomes (polysomes) were prepared by the procedure of Wettstein *et al.* (*9*). The deoxycholate-treated fraction (2.3 ml) was layered on to an equal volume of 0.5*M* sucrose in medium-A buffer, which in turn had been layered over the same volume of 2.0*M* sucrose in the same buffer. This

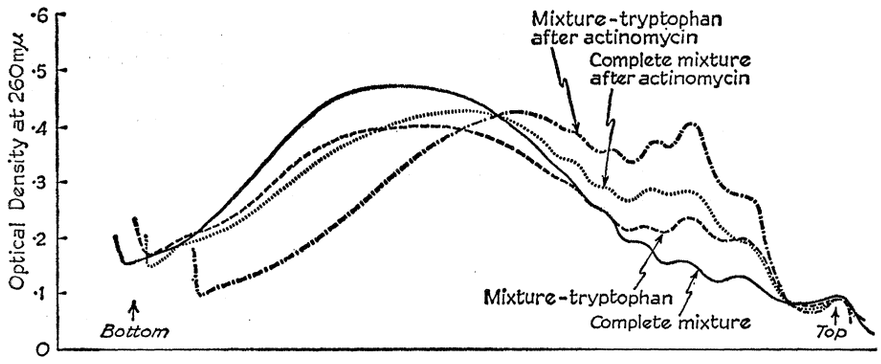

Fig. 1. Sucrose-gradient analysis of the C-ribosome (polysome) fraction prepared from the livers of rats which had been fed either a complete amino acid mixture or a mixture deficient in tryptophan. Some animals also received actinomycin D at 1½ hours before having been fed the amino acid mixtures. Each gradient represents the polysome fraction prepared from the pooled livers of three animals. The heaviest polysomes are to the left and the lighter aggregates to the right of the diagram.

was centrifuged at 158,000*g* for 4 hours at 1°C. The pellet was gently suspended in 0.15 ml medium-A buffer that contained no sucrose; 50 μl were layered on to 4.6 ml of a sucrose-density gradient (15 to 34 percent) and centrifuged for 1 hour at 118,000*g* at 1°C. The ultraviolet absorption of the gradient at 260 mμ was recorded automatically by a flow-cell device in conjunction with the Spectronic 505 spectrophotometer.

Uptake of ^{14}C-leucine into protein by microsomes prepared from animals fed the complete mixture was considerably greater than uptake by the group given the deficient mixture (Table 1). The microsomes had been recentrifuged before incubation in order to remove traces of cell sap and were incubated with a common cell-sap preparation. Consequently, the difference in ^{14}C-leucine uptake by the systems prepared from the two nutritional groups must be due to a difference in the incorporating capacity of the microsome fractions.

Table 1. Uptake of ^{14}C-leucine by microsomes prepared from the livers of rats fed a complete amino acid mixture, or a similar mixture lacking in tryptophan, 1 hour before death. Some animals were given actinomycin D (75 μg/100 gm body weight) 1½ hours before the feeding of the amino acid mixtures. Incorporation of leucine was significantly greater in the case of rats fed the complete amino acid mixture ($P<0.01$), and this response to the nutritional quality of the amino acid mixture was not affected by prior treatment with actinomycin.

Expts. (No.)	Incubation time (min)	Radioactivity (count min^{-1} mg^{-1} of microsome protein)		Difference (±S.E.)
		Amino acid mixture		
		Incomplete	Complete	
Not treated with actinomycin				
6	10	3690	4630	940±232
	40	4600	5580	980±291
Treated with actinomycin				
4	10	2020	2540	520±330
	40	2520	3550	1030±535

The general aggregation pattern of C-ribosomes for fasting animals was similar to that reported by Wettstein *et al.* (*9*). This pattern was also obtained in the animals fed the incomplete amino acid mixture (Fig. 1). Rats given the complete mixture had fewer monosomes and disomes, and had a larger proportion of material heavier than pentasomes. In eight such experiments, the monosomes and disome peaks were about 40 percent less in the case of the rats fed the complete amino acid mixture. Since the method of preparing C-ribosomes results in the loss of a large proportion of monosomes and low-molecular-weight aggregates, the magnitude of the effect of tryptophan deletion may not be fully indicated by these patterns. In recent experiments, we have in fact demonstrated that the monosomes and disomes, left in suspension after sedimentation of C-ribosomes, undergo extensive increases in amount when tryptophan is omitted from the mixture (*10*). From studies of polysome gradients, Noll *et al.* (*11*) have shown that uptake in vitro of ^{14}C-leucine increases with size of aggregate up to 10 to 12 ribosome units. Consequently, our observation of an enhanced capacity of liver microsomes, prepared from animals fed the complete amino acid mixture, to incorporate ^{14}C-leucine into their protein (Table 1) is compatible with the difference in polysome patterns (Fig. 1).

If these responses to the complete amino acid mixture are due to the formation of new mRNA, it should be prevented by effective doses of actinomycin D. Accordingly, fasting rats were given 75 μg of actinomycin D per 100 g of body weight 1.5 hours before feeding either amino acid mixture. This interval and dose of actinomycin are effective in preventing synthesis of RNA (*12*). Actinomycin depressed the capacity of the microsomes to incorporate ^{14}C-leucine. Nevertheless, on feeding the amino acid mixtures to actinomycin-treated animals, the microsomes of those rats fed the complete amino acid mixture were still increased (Table 1). In agreement with Staehelin *et al.* (*13*), polysomes prepared from the livers of the actinomycin-treated animals showed a larger quantity of monosomes and disomes (Fig. 1). However, there was still some reduction in the size of these two peaks after feeding the complete amino acid mixture to actinomycin-treated animals (Fig. 1). Changes in polysome pattern have been attributed to differences in quantity of mRNA (*9, 13*). In the case of our experiments, either there must be synthesis of more mRNA that participates in the response to the complete amino acid mixture and this synthesis must be resistant to actinomycin D, or else the response to amino acids does not require synthesis of new mRNA. We favor the latter view.

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