ber of liver cells was inadequate to produce a response. However, after removal of 70 percent of the liver in the single animal, which resulted in a disturbance of the relationship between the PBF and parenchymal cells, the amount of PBF that reached the liver directly via portal blood and by recycling was adequate to stimulate DNA activity in the remnant. When cross-circulation was maintained between an animal with 70 percent hepatectomy (as well as one with 86 percent hepatectomy) and a normal member, the amount of PBF that would ordinarily return to the partially hepatectomized liver from the systemic circulation was reduced by the quantity reaching the whole liver and being removed by it (Fig. 2, B and D). Such a reduction resulted in a diminished response in the partially hepatectomized liver as compared with the activity of similar livers in single animals, yet the addition of the PBF to that going to the liver via its own portal blood was sufficient to produce some stimulation of the liver cells of the whole liver. The addition of a portacaval shunt to partial hepatectomy in one partner would be expected to divert more of the PBF of that animal to the intact liver of the other member, which would result in a greater parenchymal cell response in the other member, as was the case (Fig. 2C). Since the partially hepatectomized liver had a markedly reduced blood flow, that is, only the flow via its hepatic artery, the amount of PBF available to it was inadequate to effect a response similar to the one that would occur in such a liver in a single animal. Indeed, the DNA activity was less than that observed in intact livers of single or cross-circulated animals. Following total hepatectomy all of the PBF entered the systemic circulation to reach the intact liver of its partner (Fig. 2E). When this PBF was added to the PBF being produced by the partner, a maximum response resulted. Further investigations into the nature, site of origin, mechanism of action, and regulation of concentration of PBF are needed.

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Specialization of Rabbit Reticulocyte Transfer RNA Content for Hemoglobin Synthesis

Abstract. *The amount of acceptance of each amino acid per absorbancy unit of rabbit reticulocyte transfer RNA was determined. The results were compared with the amino acid composition of rabbit hemoglobin and with a similar determination of the transfer RNA content of rabbit liver. The histidine and isoleucine transfer RNA content of reticulocytes is specialized for the synthesis of hemoglobin, in which histidine is unusually common and isoleucine unusually scarce compared to most proteins.*

Reticulocytes, the anucleate precursors of circulating erythrocytes, are among the most specialized cells from the point of view of protein synthesis since 90 percent of the protein made in them is of one kind, hemoglobin (*1*). The amino acid composition of the mammalian hemoglobins has some unusual features (*2, 3*), the most notable being the abundance of histidine, a generally uncommon amino acid, and the scarcity of isoleucine, a generally common amino acid. Isoleucine is entirely absent in many hemoglobins including adult human hemoglobin, and it accounts for very few residues in any mammalian hemoglobin.

Many studies have been done seeking to relate the transfer RNA (tRNA) content of various kinds of cells to biological processes such as differentiation, viral infection, and neoplasia. If tRNA content is specialized to serve the translation of proteins characteristic of particular kinds of cells, then reticulocytes with their unusual protein product should provide one of the most unambiguous animal cell systems for examination. Moreover, globin synthesis is subject to a degree of posttranscriptional regulation in that it is coupled to hemin synthesis and to the availability of iron (*4*). It has long been considered likely that tRNA may have a role in the control of protein synthesis at this level. The possibility that the amount of hemoglobin synthesized is closely dependent on the tRNA content of reticulocytes was first raised by Itano (*5*) to explain the observation that, in heterozygotes for most of the hemoglobinopathies, the protein product of the mutant allele compared to that of the corresponding normal allele is present in circulating red cells in smaller quantities.

We have determined the relative content of tRNA accepting each amino acid for rabbit reticulocytes, and the results are considered in view of the amino acid composition of rabbit hemoglobin as well as in comparison with the tRNA content of rabbit liver. The liver was chosen for this comparison because the variety of proteins it synthesizes for both internal use and for extracellular transport would suggest that it is relatively nonspecialized in its utilization of amino acids and therefore in its tRNA content. Evidence is presented that the histidine and isoleucine tRNA content of reticulocytes is specialized for hemoglobin synthesis.

The relative content of tRNA's accepting each of the amino acids in liver and reticulocytes was determined by measuring the enzymatic aminoacylation of limiting amounts of tRNA with radioactive amino acids (*6*). Assays were performed as described in Table 1. For each amino acid, an optimum Mg^{2+} concentration and an optimum ratio of Mg^{2+} to adenosine triphosphate (ATP) were determined. To avoid spurious results due to contaminated radioactive amino acids, acceptance of each amino acid was first determined in the presence of an approximately tenfold excess of the same amino acid in nonradioactive form. When the acceptance was not depressed to an appropriate level, a mixture of all nonradioactive amino acids in tenfold molar excess except the one being assayed was added. In general, the enzyme preparation used for the assays was from the same source as

the tRNA. With a few amino acids, however, lability of the synthetases in the homologous preparations resulted in levels of enzyme activity which were inadequate to provide satisfactory determinations of amino acid acceptance. In these cases, enzyme from the other kind of cells was used, as indicated in Table 1. In no case examined, including that of isoleucine and histidine, did the source of the enzyme affect the degree of tRNA amino acid acceptance.

Our determinations of acceptance are based on proportional acceptance by several different amounts of tRNA. With one exception (tryptophan), this proportionality extended to radioactivity counts several times those of the blank determinations in which there was no tRNA. Values for amino acid acceptance result from determinations with three liver and two reticulocyte tRNA preparations. Determination of amino acid acceptance agreed within 10 percent for reticulocyte preparations and within 15 percent for liver preparations. The values are expressed as the number of picomoles of amino acid accepted per absorbancy unit at 260 nm of tRNA, 1 absorbancy unit being defined as 1 ml of tRNA solution having an absorbancy of 1.0 in a 1-cm light path.

The results of determinations of amino acid acceptance for the tRNA preparations are shown in Table 2. The results are considered below from two points of view.

1) In comparison with the amino acid composition of rabbit hemoglobin: In Table 2 the number of residues for each amino acid in a whole hemoglobin molecule (two alpha and two beta chains) is shown (*2*) along with the ratio of acceptance activity to residues per hemoglobin molecule for each amino acid. In general, the more abundant amino acids are represented by larger relative amounts of tRNA. Among the amino acids asparagine, lysine, phenylalanine, serine, and tyrosine, for example, the relative amounts of their acceptance by tRNA are very closely paralleled by their abundance, as indicated by the constancy of the ratios. Similarly among the amino acids isoleucine, proline, threonine, and valine, relative tRNA abundance and amino acid frequency in hemoglobin are closely parallel; in fact, the two groups are reasonably consistent with each other. There are, however, some conspicuous exceptions to a relation between amino acid abundance in hemoglobin and relative tRNA abundance. For leucine, which is the most abundant amino acid in hemoglobin, there is no correspondingly large amount of tRNA. In the case of methionine, tRNA appears to be disproportionately abundant for the four residues per molecule found in hemoglobin, even if allowance is made for an additional initial methionine removed later in the synthesis of each subunit chain (*7*).

2) In comparison with the tRNA content of liver: For many amino acids the tRNA content of reticulocytes and of liver is similar, with the ratio of acceptance activity per absorbancy unit for tRNA from the two sources being close to unity. In the cases of isoleucine and histidine tRNA's, the ratios show a difference in content by factors of more than 2, with isoleucine tRNA being relatively less abundant and histidine tRNA being more abundant in reticulocytes as compared to liver. As stated above, these differences can be related to the unusual abundance of

Table 1. Conditions for determining amino acid acceptance by tRNA. Assay mixtures contained the following in a total volume of 0.40 ml: ^{14}C-labeled amino acid (^{3}H in the case of tryptophan) (New England Nuclear and Amersham/Searle), 2 μM; ATP, 0.75 mM; tris, *p*H 7.5, 12.5 mM; bovine serum albumin, 0.0125 mg (per assay); tRNA, 0.006 to 0.30 absorbancy unit; and enzyme preparation, 0.005 ml (protein concentration, 25 mg/ml). Nonradioactive amino acids and $MgCl_2$ were added as indicated below. The reaction mixtures were incubated for 20 minutes at 37°C, and the reactions were terminated by the addition of 5 ml of cold 5 percent trichloroacetic acid. The precipitate formed was collected as described previously (*14*) on membrane filters (Millipore type HA), and the acid insoluble radioactivity was determined in a liquid scintillation spectrometer.

Amino acid	Optimum $MgCl_2$ (mM)	Mg^{2+}/ATP ratio	Nonradioactive amino acids	Enzyme source
Alanine	1.25	1.67	Yes	Same as tRNA
Arginine	10.00	13.33	No	Same as tRNA
Asparagine	5.00	6.67	Yes	Same as tRNA
Aspartic acid	2.50	3.33	Yes	Same as tRNA
Glutamine	10.00	13.33	Yes	Liver
Glutamic acid	1.25	1.67	Yes	Same as tRNA
Glycine	7.50	10.00	Yes	Same as tRNA
Histidine	1.25	1.67	Yes	Same as tRNA
Isoleucine	7.50	10.00	No	Same as tRNA
Leucine	0.50	0.67	No	Reticulocytes
Lysine	1.25	1.67	No	Same as tRNA
Methionine	1.25	1.67	Yes	Same as tRNA
Phenylalanine	10.00	13.33	No	Liver
Proline	1.25	1.67	Yes	Same as tRNA
Serine	5.00	6.67	Yes	Same as tRNA
Threonine	1.25	1.67	No	Same as tRNA
Tryptophan	1.25	1.67	No	Same as tRNA
Tyrosine	7.50	10.00	No	Reticulocytes
Valine	1.25	1.67	No	Same as tRNA

Table 2. Acceptance of amino acids by preparations of tRNA from rabbit reticulocytes and livers.

Amino acid	Residues per hemoglobin molecule	Acceptance activity (picomoles per absorbancy unit)		Ratio of acceptance activity of reticulocyte tRNA to residues per hemoglobin molecule	Ratio of acceptance activities of reticulocyte tRNA to liver tRNA
		Reticulocyte tRNA	Liver tRNA		
Alanine	4	49	54	12.25	0.91
Arginine	32	37	19	1.16	1.94
Asparagine	22	41	39	1.87	1.05
Aspartic acid	42	50	62	1.19	0.81
Cysteine	32	58	49	1.81	1.18
Glutamine	6	19	21	3.17	0.91
Glutamic acid	12	16	15	1.33	1.07
Glycine	58	95	38	1.64	2.50
Histidine	32	33	35	1.03	0.94
Isoleucine	40	99	48	2.48	2.06
Leucine	38	35	11	0.92	3.18
Lysine	8	14	31	1.75	0.45
Methionine	70	34	53	0.49	0.64
Phenylalanine	48	60	61	1.25	0.98
Proline	56	125	57	2.23	2.19
Serine	12	41	52	3.41	0.79
Threonine	24	31	36	1.29	0.86
Tryptophan	22	52	48	2.36	1.08
Tyrosine	4				
Valine	12	19	18	1.58	1.06

histidine and the scarcity of isoleucine in hemoglobin.

The ratio of relative abundance of tRNA in reticulocytes compared to that in liver is approximately 2 for valine, alanine, glycine, and phenylalanine. It is more difficult, however, to evaluate the translational needs of reticulocytes compared to the liver with respect to these amino acids than it is in the cases of isoleucine and histidine.

There are hazards in a study of tRNA content. The reliability of an assessment of tRNA content is dependent on the quantitative or at least representative extraction of all tRNA species from the cells being studied. It is also dependent on the intactness of all of the tRNA with respect to amino acid acceptance, at least to the extent that the molecules are intact within the cell. That these criteria may not have been fully met in this study is suggested by the sum of acceptance of all amino acids per absorbancy unit of tRNA. The value for tRNA from both sources is substantially less than stoichiometric (1400 to 1800 pmole). This could indicate alteration of some of the tRNA during extraction so that it cannot accept amino acids. Acceptance of amino acids even by some purified individual tRNA species falls far short of 100 percent for reasons that are not fully understood (*8*). It is likely that the tRNA preparations in our study contain fragments of larger nucleic acid molecules, causing a decrease in apparent total acceptance activity but not altering the validity of the results for comparative purposes.

Gilbert and Anderson (*9*) have published determinations of amino acid acceptance by reticulocyte and liver tRNA, though not from the point of view of specialization of tRNA content for hemoglobin synthesis. Their data show some differences from ours, possibly because their preparations were made from supernatants of homogenates centrifuged at 105,000*g* which would not include the tRNA associated with the ribosomes and that shown to be attached to reticulocyte membranes (*10*). In the cases of isoleucine and histidine their results and ours are in close agreement.

Our results are offered as evidence for the specialization of the content of certain tRNA species in reticulocytes for hemoglobin synthesis. Studies in other laboratories have shown a tRNA content in other specialized cells which is consistent with the synthesis of proteins of unusual amino acid composition. These studies include the silk glands of silkworms, which synthesize fibroin that is composed almost entirely of four amino acids (*11*); fibroblasts of healing wounds that make collagen, which is rich in proline (*12*); and the livers of roosters treated with estrogenic hormones causing them to synthesize large amounts of a serine-rich phosphoprotein (*13*). We suggest that in general the relative tRNA content of different kinds of cells in the same animal is similar, but that modification of the content can occur in a manner consistent with unusual amino acid requirements in protein translation. The modifications in some cases are the result of temporary functional adaptations and in other cases (such as reticulocytes) are an aspect of differentiation.

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Specific Enzymic Methylation of an Arginine in the Experimental Allergic Encephalomyelitis Protein from Human Myelin

Abstract. *A cytoplasmic enzyme from guinea pig brain was shown to transfer methyl groups from* S*-adenosylmethionine to only one of 19 arginine residues in the basic protein from human brain. The products were* ω-N*-monomethylarginine and* ω-N,N′*-dimethylarginine. These methylated arginines are adjacent to the main encephalitogenic determinant in the protein. Methylation may aid in the transfer of this region of the protein into the nonpolar environment within myelin and in maintaining the integrity of myelin.*

Myelin from the central nervous system contains an unusual basic protein that will induce experimental autoimmune encephalomyelitis. From their studies on the plaques of multiple sclerosis Adams and Hallpike (*1*) consider it to be the most vulnerable part of myelin. During sequence studies (*2*) on the encephalitogenic basic protein from human brain microheterogeneity was observed at position 107. An arginine at this position occurs either in the dimethylated, monomethylated, or unmethylated form (*3*). Eylar (*4*)