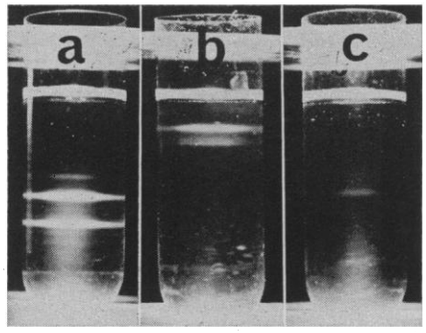

Fig. 2. Rate sedimentation in 10 to 40 percent sucrose density gradients for 1.75 hours at 24,000 rev/min in a Spinco SW-25.1 rotor. (a) Purified TRV in 0.1*M* phosphate, *p*H 7.0; (b) extracted protein in 0.2*M* glycine, *p*H 8.0; (c) extracted protein and RNA in 0.1*M* phosphate, *p*H 7.0.

cal protein ultraviolet spectrum with a maximum at 284 nm and a minimum at 254 nm, the ratio of the absorption at 260 and 280 nm being 0.50 to 0.60. Electron micrographs of preparations stained with uranyl acetate indicated clumps of protein aggregates and a few ring structures (Fig. 1c). With dialysis at 30°C in 0.05 to 0.5*M* glycine adjusted to *p*H 7.5 to 9.5, the solution became opalescent, and long tubular structures as well as large numbers of rings were observed in electron micrographs (Fig. 1d). The tubular and ring structures banded near the meniscus after sedimentation in 10 to 40 percent sucrose density gradients in 0.25*M* glycine at *p*H 8.0 (Fig. 2b). Purified TRV was partially degraded by similar conditions of sedimentation. When dialyzed against 0.1*M* phosphate, *p*H 7.0, and sedimented in gradients of the same medium, the reassembled tubular forms dissociated.

The phenol-extracted TRV-RNA was added to the protein preparation; the mixture was dialyzed against 0.25*M* glycine, *p*H 8.0, at 9°C and then dialyzed against 0.1*M* phosphate, *p*H 7.0. Particles with a sedimentation rate similar to native 85-nm rods (Fig. 2c) were obtained. Staining and shadow-casting confirmed the presence of typical 80- to 90-nm particles (Fig. 1, e and f). Reduced temperatures enhanced production of the nucleoprotein particles but not the protein tubes. Also, a ratio of protein to RNA in the foregoing mixture less than 10 : 1 (by weight) results in the most homogeneous product, as judged by banding in gradient columns.

Although the phenol extract of the purified virus contains the RNA moieties from the three particle lengths, no evidence of distinct banding could be observed among the 50- to 190-nm particles. No infection resulted when reconstitution mixtures were inoculated to the systemic host, *Nicotiana clevelandii* Grey. Both observations may reflect the differential structural instability of the RNA extracted from the 190-nm particles, as compared to that of the 85-nm particles (*7*), thereby permitting the 85-nm particles to be assembled more readily.

The biological activity of the reconstituted particles was demonstrated by inoculating plants previously inoculated with the unstable form of TRV with the density-gradient banded rods (Fig. 2c). All plants inoculated with the unstable form produced symptoms; however, typical viral particles could be recovered only from plants also inoculated with either reconstituted or native short particles. Extracted protein alone was not effective in changing the unstable to the stable form of infection. In that these reconstituted particles code for the distinct function of coat protein synthesis (*8*), perhaps the nucleic acid might display an affinity for the protein subunit.

On the basis of sedimentation properties, particle morphology, and biological activity, our results indicate that native protein subunits can be extracted from tobacco rattle virus and then polymerized into protein tubes and nucleoprotein particles resembling naturally occurring short rods.

Note added in proof: Reconstitution of long rods (190 nm) has been accomplished under the same condition presented here except with much larger quantities of extracted protein (about 500 μg) and RNA (about 100 μg). The reassembled particles were judged identical to native long rods on the basis of sedimentation rates, particle morphology, and biological activity.

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Aminoacyl Transfer Ribonucleic Acid Synthetases from Cell-Free Extract of Plasmodium berghei

Abstract. *Aminoacyl transfer ribonucleic acid synthetases for leucine, tyrosine, histidine, valine, proline, threonine, and lysine were obtained from cell-free extract of* Plasmodium berghei. *The leucyl-tRNA synthetase can charge tRNA from liver or* Escherichia coli *with leucine-C^{14}, liver tRNA being a better substrate. The amount of aminoacylation increases linearly with respect to the quantity of tRNA added from either source and is dependent on the amount of enzyme added. The rate of aminoacylation is constant for 10 minutes and then decreases. It is enhanced by polyvinylsulfate. One-tenth millimolar pyrimethamine, hydroxystilbamidine, quinacrine, and acriflavine inhibited the formation of C^{14}-valyl-tRNA. Species specificity between tRNA and its charging enzyme with respect to the recognition site is discussed.*

This report is concerned with the study of protein synthesis in extracts from invertebrates (*1–4*). Despite the fact that in all species studied the first reaction leading to protein synthesis is the charging of transfer ribonucleic acid (tRNA) with amino acids and the formation of aminoacyl-tRNA, there are species differences with heterologous interactions. For example, tyrosyl-tRNA from *Escherichia coli* cannot interact with tyrosyl-tRNA synthetase from yeast (*5*). Species differences in charging were observed by using aminoacyl-tRNA synthetase from liver or *E. coli* with heterologous tRNA (*6*). However, once tRNA is charged with an amino acid, it can serve as a precursor for polypeptide

Table 1. Aminoacyl tRNA synthetase activity for different amino acids in cell-free extract of *P. berghei*. Experimental conditions were as described in Fig. 1 except that only liver tRNA (1 mg/ml) was used. Supernatant enzymes were added at a concentration of 3 mg/ml. Each tube contained 0.25 μc of labeled amino acid. Incubation time was 12 minutes.

C^{14}-Amino acid	C^{14}-Aminoacyl-tRNA (count/min)	
	Endogenous	Liver tRNA
Tyrosine	876	1335
Histidine	432	1546
Valine	95	4072
Proline	238	404
Threonine	374	1484
Lysine	582	3578

synthesis regardless of its origin. Thus, aminoacyl-tRNA from *E. coli* when added to reticulocyte ribosomes, can support hemoglobin synthesis in mammals (*6*) or when added to insect ribosomal cell-free protein-synthesizing system can support protein synthesis in insects (*1*, *4*).

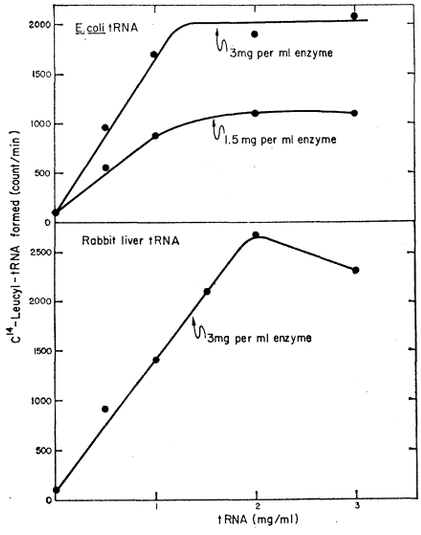

Fig. 1. Effect of tRNA concentration on the formation of leucyl-C^{14}-tRNA by cell-free extract from *P. berghei*. Blood was removed from mice infected with *P. berghei* (*10*) by cardiac puncture on the 4th day of infection and centrifuged for 5 minutes. The red cells were suspended in 0.9 percent NaCl and centrifuged again for 5 minutes; they were suspended again in 0.4 percent NaCl and centrifuged for 5 minutes at 10,000*g*. The last step was repeated three times, and the leukocytes and ghosts, which sedimented above the packed free parasites, were removed by means of a Pasteur pipet after each sedimentation. The parasites were suspended in 2 ml of buffer A (*4*) containing 0.4 g of glass beads (0.2 μ in diameter) and 0.2 ml of 2 percent macaloid, and homogenized in a Sorvall micro Omni-Mixer for 1 minute at top speed. After the addition of deoxyribonuclease (5 μg/ml) the homogenate was centrifuged for 10 minutes at 15,000*g*. The supernatant was centrifuged at 150,000*g* for 2 hours. The supernatant, which served as enzyme, was then dialyzed overnight against 2 liters of buffer containing 10 m*M* mercaptoethanol, 10 m*M* imidazol (*p*H 7), and 5 percent glycerol. The buffer was changed three times in the course of dialysis. All above operations were carried out at 0° to 4°C. Incubations were carried out at 30°C for 30 minutes. The incubation mixture (0.1 ml) contained per milliliter: 50 μmole of imidazole (*p*H 7), 10 μmole of $MgCl_2$, 4 μmole of ATP, 2 μc of leucine-C^{14} (248 mc/mmole), rabbit liver or *E. coli* tRNA, and supernatant enzyme protein as indicated above. The reaction was terminated by the addition of 5 ml of cold 5 percent trichloroacetic acid (TCA); the precipitate was washed on a glass filter with 50 ml of cold TCA and dried; the radioactivity was determined by counting (*4*). Results are expressed as counts per minute per incubation tube.

Species specificity of tRNA for its charging enzymes must lie in the recognition site. There are at least three specific sites on tRNA. One of these is the anticodon that is engaged with messenger RNA to place the amino acid in its proper sequence in a growing polypeptide chain. A second site is required for attachment to ribosomes and is perhaps common to all tRNA's. A third site is that of the recognition of the correct aminoacyl-tRNA synthetase. A mistake in recognition will result in the synthesis of mutant protein. Since the genetic code is universal, species specificity for tRNA and its charging enzymes must be due to structural changes in the recognition spot of tRNA or in specific charging enzyme, or to both, which have taken place during the course of evolution.

The availability of sufficient tRNA from malarial parasites presents a problem. However, we were encouraged by the fact that extract from a trypanosomatid can aminoacylate tRNA from *E. coli* or from liver (*3*). When the supernatant fraction (*P. berghei*) from which the ribosomes have been removed is incubated with tRNA from either liver or *E. coli* and C^{14}-leucine, C^{14}-leucyl-tRNA is formed (Fig. 1). The amount of aminoacylation increases proportionally with respect to the quantity of tRNA added from either source and is dependent on the amount of enzyme added. Aminoacylation is restricted to a relatively narrow range of magnesium concentration with an optimum between 4 to 12 m*M* $MgCl_2$. The rate of formation of leucyl-C^{14}-tRNA is constant for 10 minutes and then decreases (Fig. 2). Maximum activity is observed at 12 to 20 minutes; there is then a sharp decline, which may be due to deacylation or to the presence of nucleases. Addition of polyvinyl sulfate results in a higher rate as well as an elevated maximum (Fig. 2a). Addition of an adenosine triphosphate (ATP) regenerating system (phosphoenolpyruvate and pyruvate kinase) does not enhance the reaction significantly, which means that adenosine triphosphatase is not an important factor under these experimental conditions.

Liver tRNA is a better substrate for plasmodial leucyl-tRNA synthetase. The rate of the reaction, the degree of leucine esterification, and the extent of dependency on added tRNA are higher with liver tRNA. Liver leucyl-tRNA

Table 2. Effect of drugs on valyl-tRNA synthetase activity. Experimental conditions were as given in Table 1 except that only C^{14}-valine was used and drugs were added as indicated. Incubation time was 12 minutes.

Additions	Concentration (mmole/liter)	C^{14}-Valyl-tRNA (count/min)
None		3458
Pyrimethamine	0.5	422
	0.1	2243
Hydroxystilbamidine	0.5	363
	0.1	1230
Quinacrine	0.5	418
	0.1	2620
Acriflavine	0.5	391
	0.1	2792
Chloroquine	0.5	2874
	0.1	3074
None (0° control)		99

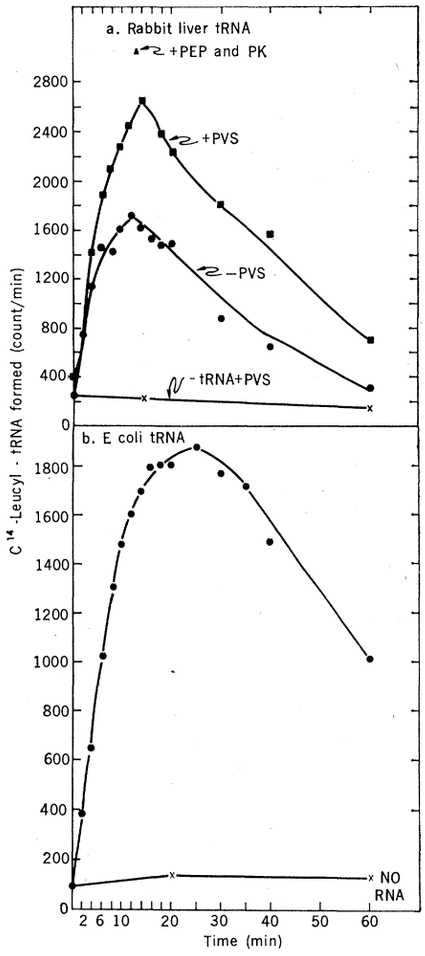

Fig. 2. Formation of leucyl-C^{14}-tRNA by *P. berghei* enzyme. Experimental conditions as in Fig. 1 except for the time of incubation and the source of tRNA. Closed circles, *E. coli* or rabbit liver tRNA (1 mg/ml); closed squares, liver tRNA (1 mg/ml) and 4 μg of polyvinylsulfate (PVS) per milliliter; crosses, without RNA; closed triangle, with liver tRNA, PVS, 5 μmole of phosphoenolpyruvate (PEP) per milliliter, and 20 μg of pyruvate kinase (PK) per milliliter.

synthetase cannot charge *E. coli* tRNA with leucine and *E. coli* leucyl-tRNA synthetase cannot charge liver tRNA. The fact that the plasmodial enzyme can charge tRNA from both sources suggests that the major structural change with respect to the enzyme and its recognition site on the tRNA is inborn in the enzyme. It was shown that malarial parasites can take up host cytoplasm by pinocytosis (*7*). Therefore, the lack of charging specificity may permit the parasite to use host tRNA for its protein synthesizing system. However, tRNA is synthesized very rapidly in *P. berghei* in vivo (*8*).

Aminoacyl tRNA synthetase activity was found in the *P. berghei* extract for the following amino acids: tyrosine, histidine, valine, proline, threonine, and lysine (Table 1). High synthetase activity was observed for valine and lysine. Relatively high endogenous activity (without added tRNA) was recorded for almost all amino acids tested. However, when the extract was treated with 0.1 volume of 2 percent streptomycin sulfate, centrifuged for 15 minutes at 30,000*g* to remove RNA, and then passed through a Sephadex G-25 column, no endogenous activity could be observed and there was an absolute dependency on added tRNA.

It should be emphasized that the determinations of aminoacyl-tRNA synthetase activities for the above amino acids were carried out under conditions optimum for leucyl-tRNA synthetase. For example, in *E. coli* the optimum ratio of magnesium ion to ATP for leucyl-tRNA synthetase is 10 while the optimum ratio for the prolyl-tRNA synthetase is 30 (*9*).

Pyrimethamine, hydroxystilbamidine, quinacrine, and acriflavine inhibit the esterification of valine with tRNA significantly at a concentration as low as 0.1 m*M* (Table 2). Chloroquine was without effect. Elucidation of the reactions leading to protein synthesis in malarial parasites may explain the specificity of certain drugs and provide the rationale for the synthesis of new ones.

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Circadian Rhythm of Optic Nerve Impulses Recorded in Darkness from Isolated Eye of Aplysia

Abstract. *The isolated eye of the sea hare* Aplysia californica *shows a circadian rhythm of optic nerve impulses when kept in total darkness. Peak activity on the first day of isolation occurs during the projected "dawn" of the light-dark cycle to which the whole animal had previously been entrained. Eyes from animals previously exposed to constant light show a free-running rhythm. This simple photoreceptor provides a quantized output with an ideal control (the other eye) for studies on rhythms.*

A circadian rhythm in the rate of spontaneous impulses from an isolated eye has not been described for any animal, although a rhythmic migration of pigment occurs in the compound eyes of intact arthropods (*1*). The eyes of certain beetles show a "diurnal rhythm" (circadian) in electrical response to illumination (*2*). The circadian rhythm was thought to be a consequence of pigment migration. Recently, the response of the "sustaining" fibers of the optic nerve of the intact crayfish to light flashes has been demonstrated to show a circadian rhythm as well as the electroretinogram (ERG) (*3*). In the crayfish the analysis of the origin of the rhythm is complicated by the probable influence of several systems within the animal.

The isolated eye and optic nerve of *Aplysia,* described here, has a circadian rhythm of optic nerve activity when kept in constant darkness. Eighteen eyes removed from animals that had been subjected to either constant white fluorescent light (LL) of 195 lux or light-dark cycles (LD, 12 hours light to 12 hours dark) of 165 lux to 0 lux, were used in the experiments. The duration of the exposure to either of these conditions varied from 2 to 10 days. The animals were kept in groups of 4 to 50 at 14° to 15°C in tanks of seawater (380 liters) that were part of a 5680-liter circulating system. They were killed at various times during the day in order to test for the complication of dissection time as a factor in the rhythm. The optic nerves were severed at the cerebral ganglion, and the eyes were removed from the surrounding body wall tissues, leaving 1 cm of optic nerve and the attached eye as the isolated preparation (*4*). Dissection, performed under white light or red light from a 6-volt lamp, was completed in 15 to 20 minutes. The eye was placed in a 100-ml chamber thermostatically regulated at 15°C and containing either seawater filtered through millipore filters (0.22 μ) or sterile culture medium (*5*). This culture medium contained Eagle's minimum essential medium made up in seawater consisting of 20 percent filtered *Aplysia* blood. The medium was buffered at *p*H 7.8; it maintained normal electrical activity of the parieto-visceral ganglion of *Aplysia* for up to 6 weeks (*5*).

The eye was stapled through peripheral connective tissue to a silastic platform, and the severed end of the optic nerve was picked up in a suction electrode consisting of Intermedic polyethylene tubing (PE 20) for recording. The electrical activity was led off by a stainless steel needle and amplified by a Tektronix 122 preamplifier, monitored on an oscilloscope, and recorded with a Grass polygraph. Usually two eyes, either from the same animal or different animals, were tested in the same chamber, but as many as four were sometimes tested together. The surface of the chamber was left open to permit free gas exchange during the 1 to 3 days of continuous recording. Special precautions were taken to insure conditions of total darkness during the recording sessions. After the recording apparatus was properly functioning, the chamber was sealed inside a double light-tight box. This was usually completed within 1 hour after dissection had started.

The optic-nerve activity consists of impulses which are compound action