52 mice that survived inoculation with SM-18 in the first several passages, 43 survived subsequent dengue challenge, which shows the acquisition of immunity, which, in turn, demonstrates the presence of dengue virus in the original inoculum. Virus strain SM-18 was reisolated successfully in mice from a stored sample of the original mosquito suspension.

The results of intracerebral neutralization tests in mice (Table 1) showed that SM-18 is a strain of dengue type 2 virus. Hyperimmune serums were prepared in adult mice by a series of five intraperitoneal inoculations with live virus, and in rabbits by a series of four intramuscular inoculations with live virus. Typing by the microprecipitin agar gel diffusion technique (*13*) confirmed the results of the neutralization tests. With SM-18 antigen (a 20-percent infant mouse brain suspension in borate-saline diluent at *p*H 9.0), precipitation occurred only with dengue type 2 and not with dengue types 1, 3, or 4, or with Japanese encephalitis hyperimmune mouse serum. No nonspecific precipitation occurred with normal mouse serum.

It has been established that *A. aegypti* is the primary vector of dengue-caused hemorrhagic fever in southeastern Asia, based on epidemiological evidence and numerous virus isolations. The significance of the single isolation of dengue virus from *A. albopictus* cannot be evaluated without further investigation, although epidemiological evidence suggests that this species is an important vector of endemic dengue in southeastern Asia (*8*).

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Enrichment of Serine-Acceptor Soluble RNA by Nucleic Acid Gels

Abstract. *Nucleic acid gels prepared by ultraviolet irradiation of polyuridylic acid on plastic beads can absorb a species of soluble RNA with increased serine-acceptor activity.*

Naturally occurring deoxyribonucleic acids can be immobilized on solid supports for the isolation of complementary strands of nucleic acid (*1*). Synthetic polynucleotides have been chemically coupled to solid supports and used (*2*) for the isolation of oligo- and polynucleotides on the basis of complementary hydrogen bonding. I now report further application of this technique for the isolation of a specific soluble RNA from mixtures with a nucleic acid gel.

Polyuridylic acid was made into insoluble gels by ultraviolet irradiations of polyuridylic acid (polyU) (*3*) on polyvinyl chloride beads (*4*) according to Britten's procedure (*5*), with the following modification. Ten grams of Geon 101 beads were coated with a solution containing 60 mg of polyU and dried with a heat lamp in a silicon-treated dish. The resulting powder was thinly spread in a large glass dish and irradiated with two 9-watt germicidal lamps (*6*). Six 30-second exposures were made at a distance of 7.5 cm, with stirring between exposures. About 100 mg of the polyU remained associated with 45 g of the beads after they were extensively washed with a warm citrate buffer (0.1*M* sodium chloride, 0.01*M* sodium citrate, *p*H 7.4) in a water-jacketed column (0.9 by 103 cm; void volume, 32 ml; bed volume, 65 ml). Soluble RNA from *Escherichia coli* (*7*) was purified with diethylaminoethyl cellulose chromatography (*8*), and a solution containing about 20 mg in the citrate buffer was recirculated through the column for 2 days at 0°C with a Sigmamotor T-8 pump. The unbound sRNA was flushed out from the column, at 0°C, with the citrate buffer until optical density (O.D.) readings of the collected fractions reached background. Then the column temperature was raised gradually to 45°C to displace material bound at the lower temperature (Fig. 1). An amount of solvent equal to one bed volume was allowed for elution at each temperature, and the elution was complete at 40°C with a midpoint around 20°C. No retention of sRNA was observed in a similar experiment with a column packed with only polyvinyl beads. To assure quick and complete displacement of the bound material, the elution temperature of 40°C was used in a typical experiment with the polyU gel column (Fig. 2).

The retained fractions accepted six times as much serine as either the original mixture or the fractions eluted at 0°C did. Although the

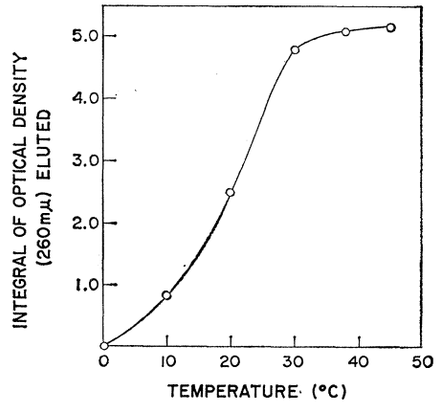

Fig. 1. Integral of the quantity of sRNA eluted from immobilized polyU gel as a function of temperature.

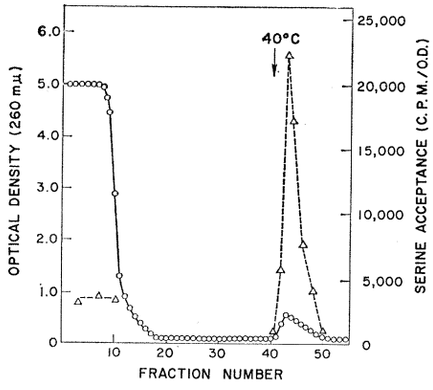

Fig. 2. Isolation of sRNA enriched in serine acceptance with a column containing polyU gel immobilized on plastic beads. A solution containing about 20 mg of sRNA from *Escherichia coli* was recirculated through the column at 0°C for 2 days and then was flushed out. At fraction 40, the column temperature was raised to 40°C. Portions from the collected fractions were tested for serine acceptance. Circles, optical density; triangles, specific activity for serine acceptance.

Table 1. Amino acid acceptor activity of sRNA isolated from polyU column. Reaction mixtures, in a total of 1.65 ml, were composed of 1 ml of sNRA solution containing about 0.5 O.D. unit (260 mμ, 10-mm light path) in the citrate buffer; and 0.65 ml of tris buffer (0.01*M*, *p*H 7.4) containing the following: adenosine triphosphate, 1.3 μmole; cytosine triphosphate, 0.25 μmole; phosphoenolpyruvate, 1.3 μmole; pyruvate kinase, 10 μg; amino acid activating enzyme, partially purified, from *E. coli* (*13*), 0.56 mg protein; $MgCl_2$, 5 μmole; KCl, 30 μmole; mercaptoethanol, 3 μmole; and one of C^{14}-labeled amino acids (specific activities from 13 to 360 mc/mmole), 1.0 μc. For the blanks, 1 ml of the citrate buffer was used to replace sRNA. The reaction was measured as radioactivity in the acid-insoluble fraction in comparison to that of blanks. After incubation at 37°C for 15 minutes, samples of 100 μl (in triplicate) were placed on Whatman No. 3 MM-paper discs to be washed with cold trichloroacetic acid (5 percent) five times and with ethanol twice (*14*). The washed discs were dried and counted with a scintillation spectrometer. OM, original mixture; RF, retained fraction.

C^{14}-Amino acid	μμmole of C^{14}-amino acid accepted by mμmole sRNA*		Total acceptance (%)	
	OM	RF	OM	RF
Serine	20.6	160.0	9.0	70.7
Leucine	80.0	47.7	35.2	21.1
Phenylalanine	12.0	0	5.2	0
Proline	20.5	4.5	9.0	1.9
Valine	20.0	0.7	8.8	0.3
Threonine	14.3	4.3	6.2	1.9
Glysine	8.5	1.2	3.7	0.5
Lysine	4.3	0.3	1.8	0.1
Isoleucine	3.0	0	1.3	0
Alanine	17.5	3.1	7.7	1.4
Tryptophan	0	0	0	0
Tyrosine	3.8	1.9	1.6	0.8
Arginine	7.9	0.9	3.4	0.4
Glutamic acid	4.3	1.4	1.8	0.6
Aspartic acid, plus methionine	10.5	0	4.6	0

* Molecular weight of 25,000 was assumed.

retained fractions showed increased ability to accept serine, they contained other species of sRNA because the acceptance of serine by these fractions varied when assayed individually. All the retained fractions were combined and examined for the ability to accept other amino acids (Table 1). There was a high specificity for serine (71 percent of total acceptance), but the material also accepted leucine (21 percent) and 14 other amino acids (less than 8 percent). Under the reaction conditions (Table 1), both the retained fraction and the original mixture showed the same total capacity to accept amino acids; about 23 percent of the RNA calculated from O.D. measurements was capable of accepting amino acids.

Physical forces influencing the retention of the serine-specific sRNA may be (i) van der Waals forces, (ii) hydrophobic bond formation, and (iii) complementary hydrogen bondings operating between the sRNA and the polyU gel. Among these forces, only the complementary hydrogen bondings would provide the selectivity needed for enriching a species of sRNA from mixtures. The complete sequence of alanyl sRNA (*9*) reveals that there are some oligonucleotide segments in the molecule available for intermolecular hydrogen bonding. The change in elution with temperature (Fig. 1) of the retained sRNA is consistent with the dissociation of a hybrid between some segment of the sRNA and the polyU gel. The observed melting temperature (T_m), 20°C, agrees with those T_m values (*10*) for dissociations of a hybrid between oligonucleotide and complementary polymer.

Subsequent to the completion of the work reported here, Erhan *et al.* (*11*) reported the retention of two species of charged sRNA's on polydeoxyoligothymidylic acid (poly-dT) and polydeoxyadenylic acid (poly-dA) columns. There was an apparent retention of C^{14}-phenylalanyl sRNA on a thymidylate column and of C^{14}-lysyl sRNA on an adenylate column at temperatures as high as 50°C. Only two "charged" species of sRNA were tested on each column. Therefore the heterogeneity of the retained material cannot be ascertained in their experiment because other C^{14}-aminoacyl sRNA's were not tested.

Grossman reported that two uracil photoproducts were formed when polyU was irradiated with ultraviolet light (*12*). A molecule of water may add at the uracil moiety, or a uracil-uracil dimer may be formed. When the irradiated polyU was compared with normal polyU in a cell-free system for protein synthesis, the hydrated form stimulated the incorporation of serine rather than phenylalanine. The formation of dimer resulted in the loss of incorporation of phenylalanine, which was not replaced by any other amino acid. Although it is attractive to associate the isolation of the serine-specific sRNA described above to Grossman's observation, such speculation must await further experimentation.

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Primary Lysosomes in Tetrahymena pyriformis

Abstract. *Primary lysosomes have been identified in the protozoon* Tetrahymena pyriformis, *and some evidence from their ultrastructure is offered for their origin.*

The protozoon *Tetrahymena pyriformis* contains numerous, slightly dense, oval or spherical vacuoles of variable size (0.5 to 1.0 μ). In the absence of cytochemical evidence, these vacuoles were tentatively identified as lysosomes (*1*). According to de Duve