tissues, discouraged us from attempts to demonstrate such widening. On the other hand, the notion of labile "pore" size is consistent with the increase in the concentrations of proteins which occurs in the interstitial fluids of the limb as venous pressure is increased (*18*) as well as with the sudden increase in the concentrations of albumin and dextran in the right thoracic lymphatic duct, which occurs in the dog after rapid expansion of the plasma volume by intravenous infusions (*19*).

In addition to its theoretical importance, change in the endothelial pore size due to hemodynamic factors is of practical interest because of its role in predisposing to hemodynamic edema by enhancing the passage of proteins into the interstitial space, thereby influencing the balance of forces that is involved in Starling's law of capillary exchange. Moreover, the presence of "tight junctions" between squamous alveolar cells (*2*) which favors the accumulation of fluid in the interstitial space, plus the existence of "collagen" sumps in the interstitial space where fluid may accumulate, is consistent with the clinical observation that, early in the course of pulmonary edema, interstitial edema (stiff lung) may exist without evidence of alveolar edema (rales).

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Rhapidosomes: Absence of a Highly 2′-O-Methylated RNA Component

Abstract. *Rhapidosomes of* Saprospira grandis *do not contain RNA that is highly methylated at the 2′-oxygen of the sugar residues. Neither is such an RNA associated with the particles as a contaminant. A cursory examination of the rhapidosomes of two other marine organisms gave similar results.*

Rhapidosomes, rod-shaped particles produced by various marine flexibacteria (*1*), have been reported by Correll and Lewin (*2*, *3*) to be composed of 70 percent protein and 30 percent RNA of which 40 to 90 percent of the sugar residues are methylated at the 2′–oxygen position. However, we found only a small amount of nucleic acid [less than 2 percent relative to protein by weight (*4*)] in purified preparations of the particles. Furthermore, the nucleic acid is more than 95 percent labile to standard alkaline hydrolysis, thus ruling out a high degree of methylation on the 2′–oxygen (*5*). In addition, crude rhapidosome preparations isolated according to Correll and Lewin (*2*) contained no RNA that was unusual with respect to the amount of *O*–methylation (*6*). The results of three groups of experiments, in which rhapidosomes of an increasing degree of purity were examined, support these conclusions.

In two separate experiments, RNA was extracted with phenol from rhapidosomes of *Saprospira grandis* DAW-2 by the procedure of Correll and Lewin (*2*) and chromatographed on diethylaminoethyl(DEAE)-cellulose (Cl^-) (*3*). In each case, that fraction, which, like the presumed rhapidosomal RNA (*2*, *3*) and transfer RNA (tRNA), was eluted between 0.3 and 0.7*M* NaCl, was hydrolyzed in alkali (0.3*N* NaOH, 18 hours, 37°C). The neutralized hydrolyzates were absorbed on DEAE-cellulose (CO_3^{-2}). With both preparations, essentially all of the material was eluted between 0.03 and 0.15*M* $(NH_4)_2CO_3$ (Fig. 1) in three partially resolved peaks, the components of which were identified by their spectral and electrophoretic properties as mononucleotides. Insignificant quantities of di- or oligonucleotides stable to alkali were detected, in marked contrast to the report (*3*) which stated that 93 percent of the nucleic acid isolated from the rhapidosomes remained as oligonucleotide fragments after alkaline hydrolysis.

The rhapidosome fractions were also isolated (*2*) from *S. grandis* WH, *Microscilla tractuosa* EG-13, and *Cytophaga diffluens* GOL-12 (*3*). A small amount of nucleic acid was obtained from the *S. grandis* WH preparation via phenol extraction (*2*), but it was almost completely (greater than 95 percent) labile to alkali. No nucleic acid could be isolated from the rhapidosomes of *M. tractuosa* or *C. diffluens*, although material absorbing in the ultraviolet, but exhibiting only end absorption (that is, increasing absorption from 320 to 220 nm with no minimum between), was obtained in all cases. We are thus unable to support the claim (*3*) that the rhapidosomes of these organisms contain RNA which is 75 to 97 percent resistant to alkali.

Rhapidosomes from *S. grandis* DAW-2 of a higher degree of purity were isolated as follows. Cells (116 g, wet weight) were harvested in late log phase from 40 liters of medium, suspended in distilled water, and allowed to lyse (*7*). After the bulk of the cell debris was removed by centrifugation at 5000*g*, the supernatant liquid was centrifuged and the fraction sedimenting

between 10,000*g* (20 minutes) and 65,000*g* (30 minutes) was taken. The material was resuspended in water and the differential centrifugation step repeated. The final suspension was brought to a density of 1.29 g/ml by the addition of solid CsCl, and a gradient was developed (*7*). Rhapidosomes, containing 280 mg of protein, recovered from bands below the bulk of the residual cell debris, were extracted with phenol (*8*). About 4 mg of nucleic acid, representing an 80 percent recovery of total phosphate, were obtained. After alkaline hydrolysis and chromatography on DEAE-cellulose (Cl^-), 80 percent of the optical density (O.D.) units (measured at 260 nm) were eluted before the linear gradient (NaCl) reached a concentration of 0.05*M* and was subsequently identified as mononucleotide. By rechromatography on DEAE-cellulose (CO_3^{-2}), the residual 20 percent was shown to consist primarily of nonnucleotide components showing only end absorption, with a small amount of alkali-stable nucleic acid (representing 4 percent of the original O.D. units). After the latter material was incubated with deoxyribonuclease, it was eluted from DEAE-cellulose (CO_3^{-2}) at a lower $(NH_4)_2CO_3$ concentration than that required before treatment. It was thus presumed to be contaminating DNA which is frequently obtained via phenol extraction at high *p*H (*8*).

Finally, a highly purified preparation of rhapidosomes from the WH strain of *S. grandis* (800 g, wet weight), containing 55 mg of protein and 0.009 mmole of phosphate, was incubated with deoxyribonuclease and ribonuclease. The particles were sedimented at 65,000*g*, resuspended in water, and sedimented again. Although neither the morphology (*7*, *9*) of the particles as seen in the electron microscope (phosphotungstate stain) nor their density in CsCl (*9*) was altered by the enzymic treatment, analysis of the treated particles for phosphate and examination of the ultraviolet absorption spectrum of the 65,000*g* supernatant indicated that about 1 mg of nucleic acid (34 percent of the phosphate) was removed from the particles and remained in the relatively protein-free supernatant as nucleotides. The only nucleic acid (about 0.15 mg) that could be recovered via phenol extraction (*2*, *8*) of the rhapidosomes treated with nuclease was of very low molecular weight (less than 2000) emerging with Cl^- ions from Sephadex G-200 and G-100 (Fig. 2). These data suggest that the enzymic

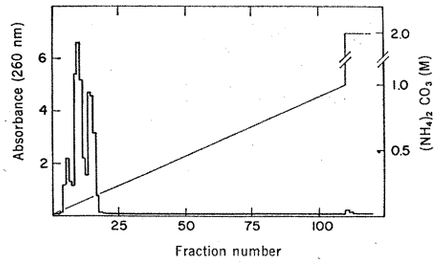

Fig. 1. Chromatography of alkaline hydrolyzate of nucleic acid isolated from impure rhapidosomes on DEAE-cellulose (CO_3^{-2}). The column was 1.1 by 19 cm with a 300 ml [0.02 → 1.0*M* $(NH_4)_2CO_3$, *p*H 9] linear gradient; flow rate was 1.5 ml/min. The three partially resolved peaks contained cytidine monophosphate, uridine monophosphate and adenosine monophosphate (AMP), and guanosine monophosphate with a small amount of AMP, respectively.

treatment and subsequent sedimentation of the rhapidosomes were only partially effective in removing contaminating nucleic acid. Again our results are inconsistent with those (*2*, *3*) stating that rhapidosomes contain an RNA largely resistant to ribonuclease. Furthermore, degradation of a highly *O*-methylated RNA by deoxyribonuclease is unlikely, because this enzyme is inactive toward tRNA and ribosomal RNA (rRNA).

The nucleic acid associated with rhapidosomes of *S. grandis* and of other marine flexibacteria, whether as an integral component or impurity, is a minor constituent relative to protein (less than 2 percent by weight based on total phosphate). Our difficulty in establishing the exact amount of nucleic acid present resides in the variable recovery of phosphate as RNA by phenol extraction and the unusual stability of the particles which have thus far defeated

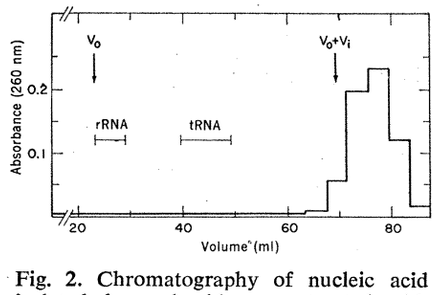

Fig. 2. Chromatography of nucleic acid isolated from rhapidosomes treated with nuclease on Sephadex G-100. The column was 1.5 × 48 cm [0.05*M* $NH_4C_2H_3O_2$, *p*H 5]; flow rate was 0.4 ml/min. The V_o (void volume) and $V_o + V_i$ (internal volume) were determined with Blue Dextran 2000 (Pharmacia) and Cl^- ions, respectively. The elution positions of rRNA and tRNA are indicated by the horizontal bars.

attempts to dissociate them by other conventional means. Spectral data suggest that the nucleic acid content based on total phosphate is an overestimation (*9*). However, there is no evidence that the RNA isolatable from rhapidosome preparations by the phenol method (*2*, *3*) has an unusual 2′-*O*-methyl content. Although Norton and Roth (*10*) presented evidence supporting the presence of a highly 2′-*O*-methylated RNA in rhapidosomes of *S. grandis*, Roth's attempts to repeat some aspects of this work have been unsuccessful, and, consequently, it is entirely possible that a highly methylated RNA was never isolated from rhapidosomes by Norton and Roth (*11*). Furthermore, Roth suggested that the nucleases that were isolated from *Anacystis nidulans* may not have the specificities reported (*10*). Thus, their data can in no way support the existence of 2′-*O*-methyl RNA in the particles. While our results are at variance with reports of Correll and Lewin (*2*) and Correll (*3*), they are in complete agreement with the data of Price and Rottman (*12*) obtained in independent studies. Price and Rottman were unable to demonstrate the presence of a highly 2′-*O*-methylated RNA in rhapidosome fractions prepared in their laboratory or in nucleic acid samples supplied to them by Correll.

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