side of the membrane. Some evidence suggests that adenosine triphosphate (ATP) may be associated with the carrier in its high Na^+-affinity state and that conversion of the carrier to a low Na^+-affinity state is the result of the hydrolysis of the ATP by adenosine triphosphatase (*7, 8*).

The precise localization of the lead phosphate end-product of the adenosine triphosphatase reaction (*9*) and $NaSb(OH)_6$ (Figs. 1 and 2) on opposite sides of the lateral plasma membrane of corneal endothelial cells supports this theory. The increase of $NaSb(OH)_6$ precipitate after exposure of the cornea to ouabain may be the indirect result of adenosine triphosphatase inhibition. If this inhibition stops the removal of Na^+ from the carrier, all of the carrier may, therefore, remain in its high Na^+-affinity state and continue to concentrate Na^+ on the cellular side of the membrane until the carrier is saturated. The precipitate which occurs at the basal and apical membranes after ouabain treatment may indicate sites which are capable of transport but which are not active under normal conditions.

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Sulfur: Incorporation into the Transfer Fraction of Soluble Ribonucleic Acid

Abstract. *When S^{35}-labeled soluble RNA from* Escherichia coli *K 38 is subjected to gel filtration, four fractions of RNA are obtained by elution. Only one RNA fraction, the transfer RNA, contains sulfur, presumably as thionucleotides. Treatment with ribonuclease suggests that the incorporated sulfur is an integral part of the polynucleotide chain; digestion with alkali yields a mixture of products containing sulfur, the major one being eluted in a position similar to uridine diphosphate upon Dowex-1 chromatography. Analysis by countercurrent distribution of S^{35}-labeled transfer RNA from* E. coli *B reveals that the incorporated sulfur is found in many RNA's that accept amino acids, but the possibility remains that not all acceptor RNA's contain sulfur.*

The presence of sulfur-containing nucleotides in the sRNA (*1*) of *Escherichia coli* has recently been established (*2, 3*). However, the sRNA of several strains of *E, coli,* prepared by phenol extraction and elution from DEAE-cellulose with molar NaCl, is a composite of RNA's of various sizes, only one of which has the biologically important ability to accept amino acids (*4, 5*). In view of this, experiments were undertaken to determine the distribution of radiosulfur in the fractions of differing sizes in sRNA from *E. coli* K 38 (*6*).

Figure 1 shows the elution patterns after gel filtration on Sephadex G-100 equilibrated with 1.0*M* NaCl (column, 0.9 by 150 cm, *7*) of H^3-uracil-labeled sRNA and S^{35}-labeled sRNA (*8*). The sRNA's were obtained by phenol extraction (*9*) of *E. coli* K 38 grown to the late logarithmic phase in Difco leucine-assay medium (10 g/liter) containing 0.1*M* phosphate buffer (*p*H 7.0) supplemented with either H^3-uracil (5 μc/ml, 8 μg/ml) or S^{35}-sulfate (25 μc/ml, total sulfate 0.4m*M*). The H^3-uracil labels elution region 1, which is a blend of messenger and ribosomal RNA (*4*); region 2, which contains an RNA of unknown identity (*4*); region 3, which is an RNA of major nucleotide composition more similar to tRNA than to either messenger or ribosomal RNA, but lacking minor nucleotides (*4*); and region 4 which is tRNA (*4*). Such a distribution of H^3-uracil indicates biosynthesis of each of these fractions. The S^{35} label, on the other hand, appears at elution region 4 (the tRNA) and is also present to the extent of 30 percent of the total S^{35} radioactivity in elution region 5, the low-molecular-weight area of the elution profile.

That sulfur is an integral part of the RNA emerging in elution region 4 is illustrated in Fig. 2, which shows the gel filtration pattern of S^{35}-labeled sRNA digested with ribonuclease. Digestions were carried out by addition of 50 μg of ribonuclease A per milliliter (Worthington, protease free) to the S^{35}-labeled sRNA solution (2 mg/ml) in 1.0*M* NaCl and subsequent incubation of the mixture at 35°C for 27 hours. All of the label (*8*) shifts with the

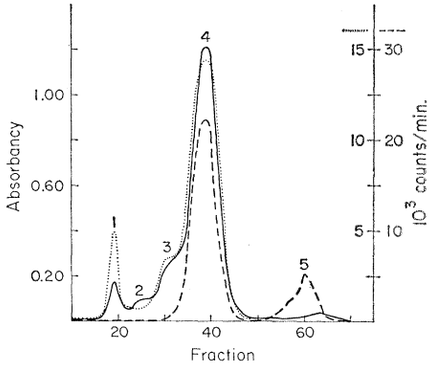

Fig. 1. Elution profiles for S^{35}-labeled sRNA and H^3-uracil–labeled sRNA after gel filtration on Sephadex G-100. Solid line, absorbancy at 260 mμ; dotted line, radioactivity of H^3, counts per minute; broken lines, radioactivity of S^{35} counts per minute. The presenece of H^3-uracil in region 5 was not determined.

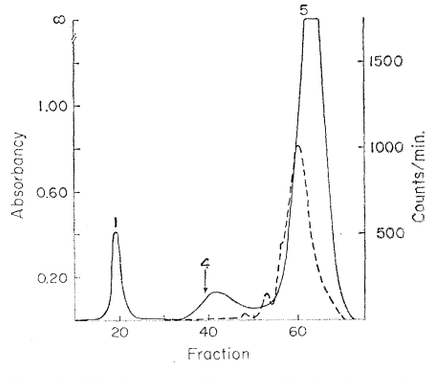

Fig. 2. Elution profile (on Sephadex) for ribonuclease-digested S^{35}-labeled sRNA. Peak 1 (void volume), in this instance, is the absorbancy of blue-dextran dye which is added to the digestion mixture prior to gel filtration. Solid line, absorbancy at 260 mμ; broken line, radioactivity, counts per minute.

absorbancy to the low-molecular-weight area of the elution profile (region 5). This observation eliminates the possibility that the S^{35} label which appears in elution region 4 is the result of a fortuitous elution of protein with tRNA.

Column chromatography on Dowex-1 (*5*) of an alkaline digest (0.3*N* KOH, 18 hours at 37°C) of S^{35}-labeled sRNA (Fig. 3) indicates a major sulfur-containing component (50 percent of the total S^{35} radioactivity) eluting in a position adjacent to pUp, a finding in nice agreement with Lipsett's work (*2*) which suggests that the major nucleotide containing sulfur in the sRNA of *E. coli* is 4-TUMP. In addition to a number of other unidentified sulfur-containing compounds small amounts of methionine and cystine to the extent of 6 percent of the total S^{35} radioactivity are also present. The latter arise from the alkaline cleavage of amino acid 3′(2′)ester linkages (*12*) and amino acid N^6-acyl linkages (*13*) and make their appearances early in the Dowex-1 column effluent stream.

We next examined the partition of these sulfur-containing compounds among the various tRNA's that accept amino acids. To do this, sRNA doubly labeled from S^{35} and H^3-uracil was prepared from *E. coli* B grown to late logarithmic phase in Difco leucine-assay medium (2.5 g/liter). The medium also contained 0.1*M* phosphate buffer (*p*H 7.1) supplemented with NH_4Cl (2 g/liter), NaCl (5 g/liter), $FeCl_3$ (trace), $MgCl_2 \cdot 6H_2O$ (100 mg/liter), glucose (2 g/liter), H^3-uracil (5 μc/ml, 10 μg/ml), and S^{35}-sulfate (50 μc/ml, total sulfate 0.1 m*M*.

Peak 4, the tRNA fraction containing this double label, was isolated by gel filtration and subjected to countercurrent distribution for 476 transfers in a solvent system composed of 1.7*M* phosphate buffer (*p*H 6.8), formamide, and isopropanol (*10*). Both isotopes were counted in every fifth tube (*8*) of the distribution train (Fig. 5). That both isotopes are distributed throughout the train indicates that sulfur-containing compounds are not confined to one amino acid acceptor species of tRNA. The partition of these acceptors has already been studied by the same method (*10*). However, differences do exist between the distribution of H^3-uracil and the S^{35} label (the sum of Up and ψp decreases 4 percent from low- to high-partition region tRNA (*11*)), suggesting that different tRNA species have varying amounts of thio-containing compounds.

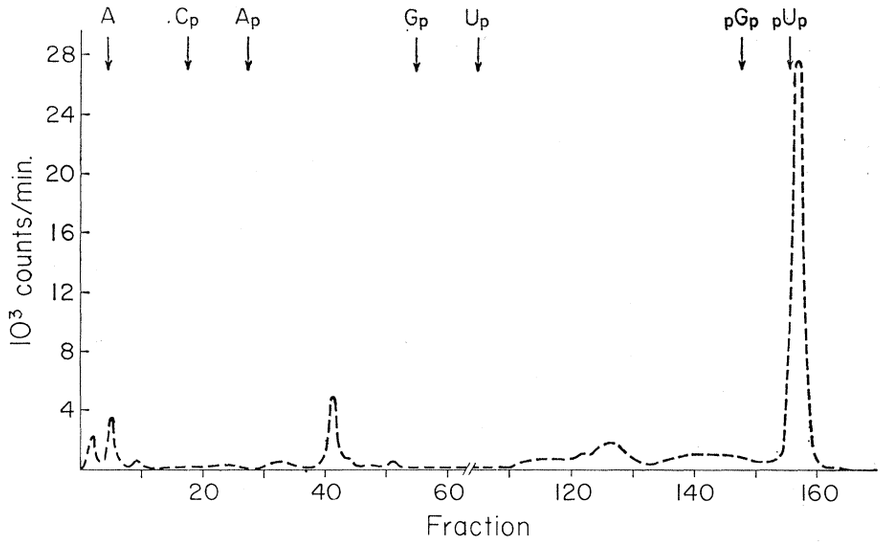

Fig. 3. Column chromatography (Dowex-1) of an alkaline digest of S^{35}-labeled sRNA. Elution positions of the nucleotides are indicated by arrows. The first two peaks represent methionine and cystine (6 percent of total radioactivity) whereas the major peak accounts for 50 percent of the total radioactivity. The remaining components have not been identified.

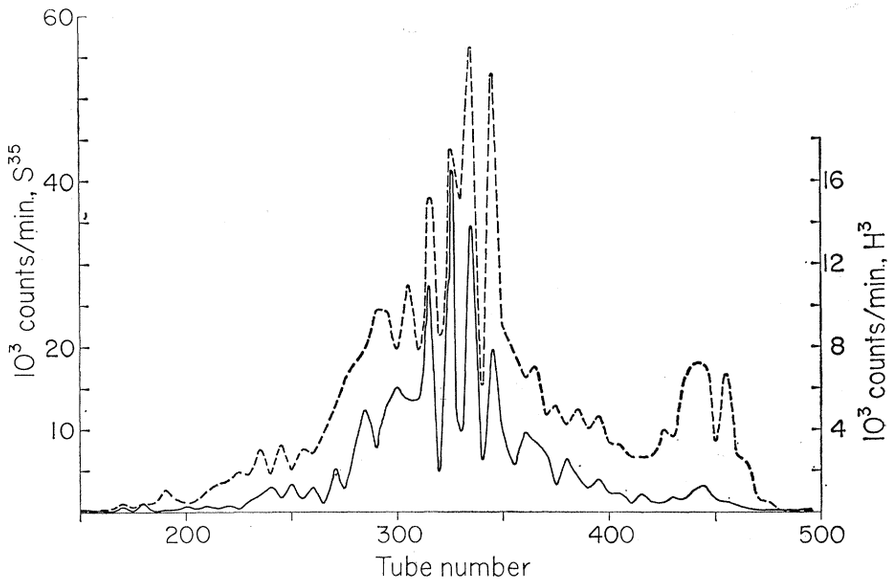

Fig. 4. Countercurrent distribution profile after 476 transfers for tRNA, from *E. coli* B doubly labeled with S^{35} and H^3-uracil. Broken line, radioactivity of S^{35}, counts per minute; solid line, radioactivity of H^3, counts per minute.

Thus the sulfur labeling is confined to the tRNA fraction of the sRNA and does not appear to arise primarily from the binding of sulfur containing amino acids, but rather by the synthesis of sulfur-containing compounds, presumably thionucleotides, with the major sulfur-containing product having similar electrical-charge properties to pUp. The absence of sulfur in elution region 3, an RNA of somewhat similar major nucleotide composition to tRNA but having 122 nucleotides per chain (*4*), precludes the possibility that this molecule is composed of subunits held together by thio linkages. Furthermore, thionucleotides appear to be a part of many, but possibly not all, tRNA species.

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References and Notes

1. Abbreviations: sRNA, soluble ribonucleic acid; tRNA, transfer ribonucleic acid, that is the fraction of sRNA which has the ability to accept and consequently transfer amino acids; DEAE, diethylaminoethyl; A, adenosine; Cp, 3′(2′)-cytidylic acid; Ap, 3′(2′)-adenylic acid; Gp, 3′(2′)-guanylic acid; Up, 3′(2′)-uridylic acid; ψp, 3′(2′)-pseudouridylic acid; 4-TUMP, 3′(2′),4-thiouridylic acid; pGp, 3′(2′),5′-guanosine diphosphate; pUp, 3′(2′),5′-uridine diphosphate; PPO, 2,5-diphenyloxazole; POPOP, *p*-bis [2-(5-phenyloxazolyl)]-benzene; BSA, bovine serum albumin; TCA, trichloroacetic acid.
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Flavonoids from the Moss Mnium affine Bland

Abstract. *Eight different flavone* C*-glycosides from* Mnium affine *were tentatively identified by chromatography and spectroscopy. Quercetin-3-diglycoside and other unidentified flavonoids were found in* M. arizonicum. *The flavonoid chemistry of* Mnium *is complex.*

Anthocyanin pigments in mosses have been reported (*1*), but the evidence has not been convincing until recently, when four anthocyanins from *Bryum* species were described (*2*); the pigments were reportedly 5-monoglucosides and 5-diglucosides of the rather uncommon anthocyanidins, apigeninidin and luteolinidin, which lack the 3-hydroxy substituent. Other types of flavonoids have not been reported from mosses, and the consensus has been that mosses are not a rich source of flavonoids.

We now report eight different flavone *C*-glycosides from the moss *Mnium affine*, additional flavone *C*-glycosides from *M. cuspidatum*, and the flavonol, quercetin-3-diglycoside, and other unidentified flavonoids in *M. arizonicum*. The flavonoid chemistry of *Mnium* species examined by us is comparable in complexity to that of angiosperms, and significant interspecific differences occur.

Gametophytic tissue of *M. affine* (*3*) was used to obtain two-dimensional chromatograms by methods described previously (*4*). Individual flavonoid compounds were eluted from replicate series of chromatograms and purified by repeated chromatography as necessary. Hydrolysis was effected by heating in 0.5*N* HCl in methanol for about 2 hours. Identification was confirmed by ultraviolet absorption, chromatographic data, and selected spray reagents.

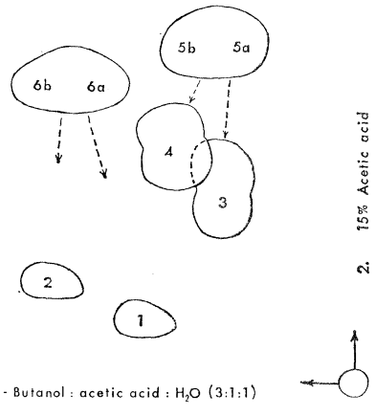

Fig. 1. Major *C*-glycosides in unhydrolyzed extracts of *Mnium affine*; arrows indicate the course of hydrolysis of specific flavonoids. [Drawn from chromatogram]

Compound 1 (Fig. 1, drawing of a two-dimensional chromatogram) runs at a position close to that of orientin (luteolin 8-*C*-glucoside) but yields ultraviolet spectra, obtained in methanol, sodium ethoxide, sodium acetate, and aluminum chloride (*5*), that although similar, are not identical with those of orientin.

The compound gives a negative result when the chromatogram is sprayed with Benedict's solution and observed in ultraviolet light, and thus it cannot have *o*-dihydroxy substituents —that is, it cannot be orientin. Scoparin, which is the 3′-methyl ether of orientin, has the chromatographic and spectral characteristics of compound 1. Compound 1 was therefore tentatively identified as scoparin, a *C*-glycoside (*6*).

Compound 2 (Fig. 1) is, by chromatography and absorption spectrum, identical with vitexin (apigenin-8-*C*-glycoside), a compound studied in the Lemnaceae (*7*) by these methods.

Compounds 3 and 4 may be chrysoeriol-6,8-di-*C*-glycoside and vicenin (apigenin-6,8-di-*C*-glycoside), respectively, on the basis of spectral and chromatographic data. Compound 3 is tentatively identified by analogy to the position and spectral features of lucenin; it gives a negative result by Benedict's test.

Compounds 1 to 4 resist acid hydrolysis. Compounds 5a and 5b partially overlap, are negative by Benedict's test, yield compounds 3 and 4 on acid hydrolysis, and are therefore considered to be *O*-glycosides of vicenin and the corresponding *C*-glycosidic derivative of chrysoeriol, respectively. Compounds 6a and 6b also partially overlap, are negative by Benedict's test, and yield compounds 1 and 2 on acid hydrolysis. Compounds 5a and 5b and 6a and 6b have not yet been separated; their spectra were otherwise clean, but had mixed characteristics.

We have little further information on the positions of attachment of the *O*-glycosides or on their identities. The 7-glucosides and 4′-glucosides of 6-*C*-glycosyl flavones are common, but the positions of the spots representing corresponding *O*-glycosides of 8-*C*-glycosyl flavones cannot be deduced from our present knowledge (*8*).

Related *C*-glycosides plus *O*-glyco-