Similar low-frequency values have been obtained by less sophisticated models of protein breathing motions. A globular protein possesses a number of vibrational motions that may be represented by a vibrating homogeneous elastic sphere. Suezaki and Go (*7*) calculated the fundamental vibration of such a sphere, assuming a radius of 20 Å, a density of 1 g/cm^3, and a Young's modulus of 10^{11} dyne/cm^2. For this simple elastic sphere the frequency of vibration for the radial pulsating mode was calculated to be 26 cm^{-1} (1.2 psec). deGennes and Papoular (*11*) used a spherical Bessel function of order 1 in analyzing the radial pulsations of a vibrating elastic material and reported similar values, varying with certain assumptions of the protein's elasticity (1.9, 1.3, and 0.44 psec; 18, 26, and 76 cm^{-1}). Longitudinal acoustic modes found in chain molecules with lengths comparable to that of BPTI also have frequencies on the picosecond time scale (*6*).

There is experimental evidence for the existence of such low-frequency breathing vibrations in other proteins. Strong Raman-active modes have been found at 29 cm^{-1} in films and crystals of native α-chymotrypsin (*12*). The 29 cm^{-1} mode in α-chymotrypsin was assigned to an elastic breathing mode of this globular protein, and the mode disappears when the protein is denatured. A 25 cm^{-1} mode in lysozyme is also probably due to an internal mode of the lysozyme molecule (*6*).

Since both the spectrogram and three-dimensional power spectrum present breathing motion data in a way that is easily understood by the biophysicist, characterization of the dynamic richness of proteins is greatly facilitated. The interactive nature of the research station allows rapid generation of these functions with a variety of input data scaling and windowing parameters. From this and other studies it is clear that a compact, rigid view of globular proteins is incomplete. Aside from the relatively fast processes, including collisions between neighboring atoms and localized group vibrations, proteins may undergo somewhat regular low-frequency breathing motions of varying complexity. These motions involve the collective movement of many different atoms. The functional importance of such breathing modes, and protein motion in general, has begun to attract the interest of an increasing number of physical chemists, as evidenced by proliferating spectroscopic, kinetic, and theoretical studies of protein dynamics (*1, 2*). It is hoped that these spectrographic methods will provide a useful tool for future investigation into the breathing motions of globular proteins and other internal motions of biomolecules.

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10. The user console consists of a vector graphics display (Tektronix 618) and a standard cathode-ray tube terminal (IBM 3277 GA). The user-written support software is implemented in PL/I. The Graphics Compatibility System (U.S. Army Engineer Waterways Experiment Station, 803F3-R0200), a package of general-purpose, device-independent computer graphics routines, is used to produce the plots. In both the three-dimensional power spectrum and the spectrogram, Hamming cosine tapers are first applied to each window of data before fast Fourier transformation. In the three-dimensional plot, a far-infrared filter is used to smooth the output display data. A system for converting the breathing motion data to sound has also been developed, enabling the biophysicist to "listen" to the R_g fluctuations.
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Microbial Transformation of Sulfate in Forest Soils

Abstract. *Incubation of forest soils containing sulfate labeled with sulfur-35 showed rapid conversion of the added sulfate to organic sulfur forms by microbial populations. Activity rates were highest in the forest floor, but significant activity was observed throughout the soil profile. The annual potential sulfur incorporation for forest floor and soil combined is estimated to be 30 kilograms per hectare. The metabolism of inorganic sulfate to organic forms can be a major process in the sulfur cycle, influencing sulfate accumulation and mobility in forest ecosystems.*

Sulfate has been identified as a major constituent of acid precipitation in several regions of the United States (*1, 2*). However, the fate of exogenous sulfate in forest ecosystems is not well known. Studies have shown that soil adsorption of sulfate can be an important component of the sulfur cycle for some forests (*3, 4*). In agreement with results obtained by Freney *et al.* (*5*) for agricultural soils, our research has suggested that the metabolism of sulfate to organic sulfur forms may also act as a major pathway in the sulfur cycle of forest ecosystems (*6*). In this report we present quantitative evidence substantiating the importance of biological transformations of sulfate in these ecosystems.

The study site is located at the Coweeta Hydrologic Laboratory in western North Carolina. The U.S. Department of Agriculture Forest Service facility is a site of long-term hydrologic and ecological research with collaboration between university and government scientists. During the past 14 years, the program has emphasized research on biogeochemical processes important in forest ecosystems, with the gaged watershed used as the unit of ecosystem investigation. On the basis of 80 gage-years (eight gages in operation for 10 years) of bulk precipitation chemistry measurements taken over the 2185-ha basin, sulfate makes up 68 percent of precipitation anions and the average annual *p*H of bulk precipitation is 4.45. During the period 1973 to 1982, the mean annual input of sulfate-sulfur in bulk precipitation was 10.0 kg ha^{-1} year^{-1} and export in streamflow for mixed hardwood-cover watersheds was 1.5 kg ha^{-1} year^{-1}; thus there was a large apparent accumulation of sulfate-sulfur of 8.5 kg ha^{-1} year^{-1}.

Our preliminary research with surface soil from four Coweeta watersheds indicated that exogenous $^{35}SO_4^{2-}$ was rapidly converted into nonsalt-extractable ester sulfate and carbon-bonded sulfur (*6*). More intensive sampling was initiated on watershed 18 in May 1981 to quantitatively assess the importance of transformations relative to sulfate accumulation within a watershed. Watershed 18 is a 12.5-ha north-facing catchment that has been a primary site of long-term research on nutrient cycling. Overstory vegetation is dominated by *Quercus*, *Carya*, and *Acer* species with *Rhododendron* and *Kalmia* in the understory. Several soil types occur over the watershed, but the dominant soil is a sandy loam Ashe, a

member of the mesic Typic Dystrochrept family with an A_1 horizon that averages about 10 cm in thickness. Permanent plots were established, and on each sampling date three samples of the A_1 horizon were taken at random on each plot (*7*).

Subsamples of soil (each 1 g, wet weight) were incubated with a range of 2.7 to 10.4 nmole of $Na_2{}^{35}SO_4$ (3.3×10^{10} becquerels per millimole) for 48 hours at 20°C (*8*). Soil water, salt extracts, acid hydrolyzates, and base fractions were obtained by established procedures (*9*). Fractions were analyzed by electrophoresis and radioactive components located with a scanner were determined with a scintillation counter. Details of the laboratory methodology have been reported (*6*).

Our earlier research has demonstrated that extraction procedures yield ≥ 90 percent of added ^{35}S. About 60 percent of the $^{35}SO_4{}^{2-}$ was found in the salt extract, which indicates a substantial sulfate adsorption capacity for these soils. This result is consistent with other research showing large adsorption capacity for this anion in Coweeta soils (*4*). However, between 8 and 27 percent of the radioactivity was recovered in the nonsalt-extractable fraction. As determined by electrophoresis, the major sulfur constituent was sulfate, an indication that ester sulfate was recovered by hydrolysis (*9*). Fitzgerald and Johnson have presented evidence demonstrating that this transformation is mediated by bacteria and fungi (*10*).

Table 1. Temporal variability in the formation of nonsalt-extractable sulfur in the A_1 soil horizon (0 to 10 cm) of Coweeta watershed 18; S.E., standard error. Values are based on three random samples taken at each of ten permanent plots ($N = 30$) distributed over the watershed. The incubation temperature was 20°C. We tested the means, using analysis of variance (Duncan's multiple range test, $P < 0.05$). Means followed by an asterisk or by a dagger or by a double dagger or by a section sign are not significantly different from each other.

Date	Mean (± S.E.) $^{35}SO_4{}^{2-}$ incorporated into nonsalt-extractable sulfur (nmole g^{-1} per 48 hours)
May 1982	1.25* (0.05)
June	1.28* (0.05)
July	1.49†‡ (0.06)
August	2.13§ (0.11)
September	1.70† (0.06)
October	1.56†‡ (0.07)
November	1.41*‡ (0.05)
December	1.57†‡ (0.05)
January 1983	1.60†‡ (0.06)
Average	1.55

Table 2. Formation of nonsalt-extractable sulfur within the forest floor and soil horizons on Coweeta watershed 18 in late August 1982.

Forest floor or soil horizon	Depth (cm)	Incorporation of $^{35}SO_4{}^{2-}$ (nmole g^{-1} per 48 hours)
O_1	4	11.68
O_2	2	8.73
A_1	0 to 10	1.85
A/B	11 to 25	0.61
B_W	26 to 65	0.44
C_r	66+	0.08

The activity for the surface soil on watershed 18 over a 9-month period in 1982 showed seasonal differences with highest activities occurring during August and September (Table 1). Substantial incorporation into nonsalt-extractable sulfur was also observed in samples collected in late spring and winter.

In late August 1982, activity was determined within the soil profile for one of the permanent plots and for the O_1 and O_2 forest floor layers at all ten plots (Table 2). The concentrations of $^{35}SO_4{}^{2-}$ added to soil samples varied by soil horizon because the incorporation rates were concentration-dependent. The determination of ambient litter and soil sulfate concentrations were based on annual concentrations of water collected by porous-cup lysimeters (*11*). The activity of sulfate incorporation was about sixfold greater in O_1 and O_2 layers than in the A_1 horizon, and activity declined rapidly with soil depth. The incorporation rate in the C_r horizon was only about 4 percent of that in the A_1 horizon (Table 2).

In an effort to place activity data in quantitative perspective from the standpoint of annual ecosystem flux, we made the following assumptions: (i) the activity data from incubation studies are representative of in situ data; (ii) the average activity of the A_1 horizon for the 9-month period (Table 1) can be extrapolated to the three remaining months; and (iii) activity data for the remaining soil horizons are representative of transformation rates over the entire year. Combining activity data with prior estimates of forest floor weights and soil bulk densities provides an estimate for the potential annual incorporation into nonsalt-extractable sulfur (Fig. 1). Although the forest floor exhibited high activity, the annual potential incorporation of sulfate is less than 0.5 kg ha^{-1} $year^{-1}$ because of the quantity of substrate involved (6×10^6 g ha^{-1} of O_1 and O_2). In contrast, each of the A_1 and B_W horizons provides a potential flux of about 11 kg ha^{-1} $year^{-1}$. When combined, the total annual flux of sulfate-sulfur in litter and soil is estimated as 30 kg ha^{-1} $year^{-1}$.

We emphasize that the fluxes in Fig. 1 represent potential rates since activity data are based on a standard incubation temperature of 20°C. Preliminary research has suggested lower incorporation rates at 5°C (*10*). Thus, it is reasonable to expect lower in situ fluxes than shown in Fig. 1, particularly below 10 cm in the soil profile, where ambient soil temperatures are 5° to 10°C lower than the assay temperature. Activity data are representative of other in situ variables important in regulating sulfate incorporation, such as moisture and soil sulfate concentrations. More precise estimates of annual in situ incorporation for an ecosystem must await detailed, long-term studies.

The annual incorporation of sulfate into organic sulfur forms substantially exceeds the sulfate-sulfur input in bulk precipitation (10 kg ha^{-1} $year^{-1}$) at Coweeta and the sulfur sequestered in vegetation (2 kg ha^{-1} $year^{-1}$) as documented for a similar forest ecosystem (*12*). The process of organic sulfate formation could account for part of the apparent sulfur accumulation indicated by ecosystem budgets. Moreover, the presence of this biological pathway in the sulfur cycle of a forest has implications for the interpretation of atmospheric sulfuric acid effects on forest ecosystems. That is, processes that reduce the mobility of sulfate also reduce cation leaching from forest soils (*13*). Incorporation of sulfate to organic forms could provide a buffer against the impacts of acid precipitation on forest soils. Conversely, the formation of organic sulfur

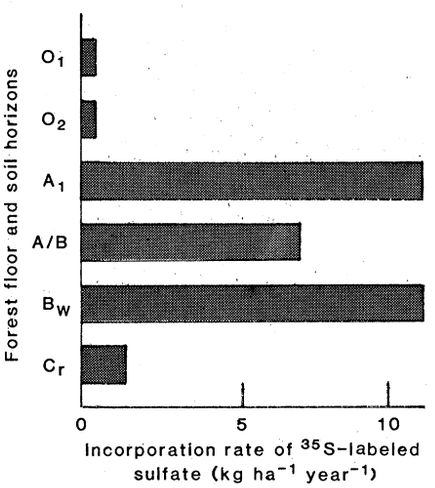

Fig. 1. Estimates of annual potential fluxes of inorganic sulfate converted to organic sulfur forms by microbial populations in forest floor and soil horizons of a forest ecosystem at Coweeta Hydrologic Laboratory.

provides a potential soil pool that could subsequently be remineralized. If mineralization rates were accelerated by chemical, physical, or biological factors, the released sulfate would increase cation leaching. Our analyses indicate that the formation of soil organic sulfur is of sufficient magnitude to warrant investigation in a variety of forest soils. Further study is also needed on the composition and turnover dynamics of this sulfur fraction.

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8. The incubation time was determined from a time series study of varying incubation periods which showed that $^{35}SO_4^{2-}$ incorporation into organic sulfur was complete after 48 hours.
9. After incubation, the soils were washed three times with water and the water was pooled and designated as soil water. Soils were then extracted three times each with 1*M* Na_2SO_4, NaH_2PO_4, and LiCl and washed three times with water to yield a salt extract. The sample was then hydrolyzed in 6*N* HCl at 121°C for 12 hours; the residue was washed in water once, and the sample was then held in contact with 2*N* NaOH for 12 hours at room temperature and was once again washed with water. In this report, the acid and base fractions were combined to yield a fraction designated as nonsalt-extractable sulfur.
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Light-Induced Phosphorylation of Retina-Specific Polypeptides of *Drosophila* in vivo

Abstract. *A moderate light stimulus induced isoelectric point (*pI*) changes in three classes of retina-specific polypeptides (80, 49, and 39 kilodaltons) of* Drosophila *in vivo. When inorganic phosphate labeled with phosphorus-32 was fed to flies, the radioactive label was incorporated into these polypeptides during the* pI *changes, indicating light-induced phosphorylation of the polypeptides. A 1-millisecond flash induced a detectable amount of phosphorylation in the 80- and 49-kilodalton polypeptides within 3 seconds. These results, and our previous results with* norpA *mutants, suggest that phosphorylation of these two polypeptides may be involved in some early stages of photoreceptor excitation or its modulation.*

Protein phosphorylation is thought to play an important role in a wide range of physiological functions, including metabolic, hormonal, and neural processes (*1*, *2*). For example, reversible phosphorylation regulates the activities of certain enzymes (*3*). Although protein phosphorylation also occurs in certain steps of synaptic transmission (*4*) and visual transduction (*5*), the molecular mechanisms underlying the regulation of these neuronal activities remain obscure.

We reported earlier (*6*) that in the compound eye of *Drosophila melanogaster* three classes of retina-specific polypeptides, having molecular sizes of 80, 49, and 39 kilodaltons, undergo light-dependent changes in their isoelectric points (*p*I's). The light-induced *p*I changes of three polypeptides are blocked (*6*) in the mutant, *norpA*, which is unable to generate the photoreceptor potential in response to light (*7*). This suggests a functional involvement of these polypeptides and their light-induced modifications in photoreceptor mechanisms. Because illumination shifts the *p*I's of the polypeptides toward the acidic direction and the shifts are reversible (*6*), we considered phosphorylation a likely cause of these light-induced polypeptide changes. In support of this hypothesis, the present studies demonstrate light-induced incorporation of ^{32}P into these polypeptides in vivo.

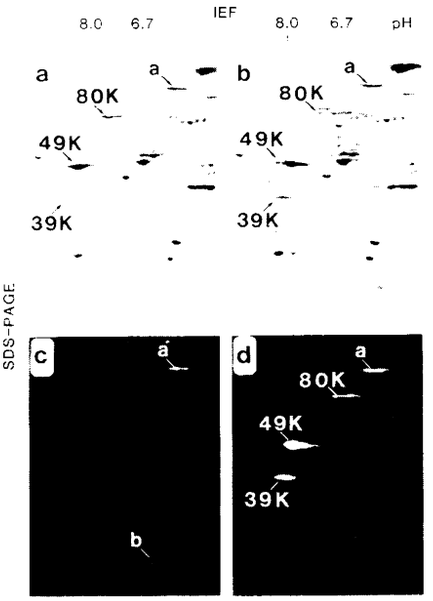

Fig. 1. In vivo incorporation of [^{32}P]phosphate into the light-adapted states of 80-, 49-, and 39-kD polypeptides. (Top) Coomassie blue–stained two-dimensional gels obtained from the compound eye preparation of (a) dark-adapted and (b) light-adapted flies. (Bottom) Corresponding autoradiograms for (c) dark-adapted and (d) light-adapted flies. *80K*, *49K*, and *39K* are polypeptides that undergo light-induced phosphorylation, designated according to their approximate molecular sizes, which are based on calibration by internal standard markers; *a* and *b* designate other major sites of [^{32}P]phosphate incorporation having molecular sizes of 130 kD and 2.3 kD, respectively.

Wild-type fruit flies (*D. melanogaster*) of the Oregon R strain were raised on cornmeal-yeast-agar medium at 25°C under a cycle of 12 hours of light and 12 hours of darkness. The flies were starved for 24 hours and then fed for 24 hours on 0.2 ml of 300 m*M* sucrose containing carrier-free ^{32}P-labeled inorganic phosphate (0.1 mCi) (New England Nuclear). The flies were separated into two groups of about 60 flies each. One group was dark-adapted for 12 hours, and the other group was exposed to room light (160 μW/cm^2) for 6 hours after being dark-adapted for 6 hours. These labeling protocols resulted in the incorporation of ^{32}P at about 1×10^5 count/min into each fly. One-sixth (16,000 count/min) of the radioactive label was taken up by the compound eyes. Two-dimensional gel electrophoresis of the compound eye preparation was carried out as described (*6*, *8*). The gels were stained with Coomassie blue and dried on a filter paper. Kodak XAR-5 film and a DuPont Lightning Plus screen were used for autoradiography; the film was exposed to the gels for 1 week at −80°C.

Figure 1, a and b, show the gels obtained from the compound eyes dissect-