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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References and Notes

1. J. N. Galloway, G. E. Likens, E. S. Edgerton, *Science* **194**, 722 (1976).
2. P. L. Brezonik, E. S. Edgerton, C. D. Hendry, *ibid.* **208**, 1027 (1980).
3. D. W. Johnson and G. S. Henderson, *Soil Sci.* **128**, 38 (1979).
4. D. W. Johnson, J. W. Hornbeck, J. M. Kelly, W. T. Swank, D. E. Todd, in *Atmospheric Sulfur Deposition: Environmental Impact and Health Effects*, D. S. Shriner, C. R. Richmond, S. E. Lingberg, Eds. (Ann Arbor Press, Ann Arbor, Mich., 1980), pp. 507–520.
5. J. R. Freney, G. E. Melville, C. H. Williams, *Soil Biol. Biochem.* **3**, 133 (1971).
6. J. W. Fitzgerald, T. C. Strickland, W. T. Swank, *ibid.* **14**, 529 (1982).
7. A 280-m transect was established across the watershed at mid-elevation and was transversed from ridge to stream to ridge. The transect was segmented into ten equally spaced 0.01-ha circular plots. Samples of the A_1 horizon were routinely collected on a monthly basis; forest floor and other soil horizons were sampled in late summer.
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.
10. J. W. Fitzgerald and D. W. Johnson, in *Sulphur 82*, A. I. More, Ed. (British Sulphur Corporation, London, 1982), vol. 1, pp. 411–426.
11. The average annual sulfate concentrations of soil water collections in a mixed hardwood forest at Coweeta decreased from 105 μeq liter^{-1} at a soil depth of 5 cm to 69 and 17 μeq liter^{-1} at 30 and 100 cm, respectively.
12. D. W. Johnson *et al.*, *Oecologia (Berlin)* **54**, 141 (1982).
13. D. W. Johnson, D. W. Breuer, D. W. Cole, *J. Environ. Qual.* **8**, 246 (1979).
14. Support for this research was provided in part by NSF grant BSR-8215259 and in part by the Southeastern Forest Experiment Station, U.S. Department of Agriculture, Forest Service. We thank D. W. Johnson, J. E. Douglass, and T. C. Strickland for helpful comments.

25 July 1983; accepted 5 October 1983

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.

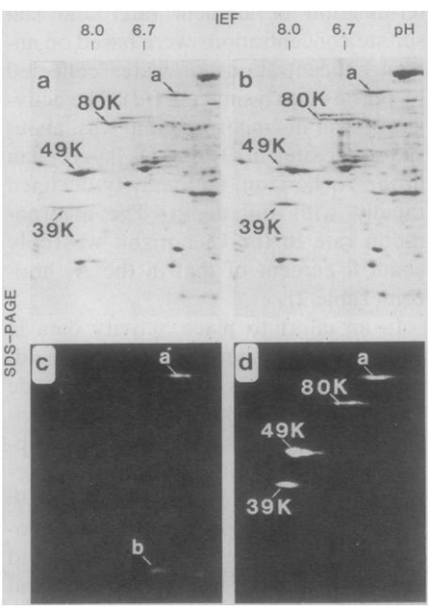

Fig. 1. In vivo incorporation of [^{32}P]phosphate into the light-adapted states of 80-, 49-, and 39-kD polypeptides. (Top) Coomassie blue–stained two-dimensional gels obtained from the compound eye preparation of (a) dark-adapted and (b) light-adapted flies. (Bottom) Corresponding autoradiograms for (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-

ed from the dark- and light-adapted wild-type flies, respectively. The arrows indicate the three classes of polypeptides, 80, 49, and 39 kD, that exhibit light-dependent *p*I changes (*6*). Only that portion of the gels in the region of the three polypeptides is shown in these figures. The autoradiograms corresponding to Fig. 1, a and b, are shown in Fig. 1, c and d, respectively. The autoradiograms show clearly that the 80-, 49-, and 39-kD polypeptides are labeled by [^{32}P]phosphate only in the light-adapted states. In the case of the polypeptide designated "a" in Fig. 1, c and d, both light-dependent and light-independent incorporation of ^{32}P appears to take place, even though no *p*I shift of this polypeptide is apparent in Coomassie blue–stained gels. Thus, the polypeptide is labeled in both the dark- and light-adapted states (Fig. 1, c and d), and the labeling appears to be slightly heavier in the light-adapted state (Fig. 1d). The ^{32}P activity designated "b" in Fig. 1c, by contrast, undergoes a loss of the activity after light adaptation. The corresponding polypeptide was difficult to identify in Coomassie blue–stained gels (Fig. 1, a and b).

Because the two-dimensional gel electrophoresis was carried out under denaturing conditions for proteins (the first dimension in a mixture of Triton X-100 and urea and the second dimension in sodium dodecyl sulfate), the polypeptides that appear labeled in Fig. 1, c and d, are most likely linked to [^{32}P]phosphate by covalent bonds, that is, phosphoester bonds, that are stable under these conditions. The results, thus, indicate that the 80-, 49-, and 39-kD polypeptides undergo light-induced phosphorylation, which appears as a shift in the *p*I to acidic direction in two-dimensional electrophoretic patterns (Fig. 1, a and b).

To obtain some idea of the speed of the reactions, we subjected flies to a brief strobe flash, froze them as quickly as possible, and examined them by two-dimensional gel analysis. For this purpose, wild-type flies that had been dark-adapted for 24 hours were placed in a test tube, exposed to a white strobe flash less than 1 msec in duration, and frozen by plunging the test tube into liquid nitrogen as soon after the flash as possible. Temperature measurements made with a small copper-constantan thermocouple indicated that the flies could be frozen within 3 seconds after the flash by this method. Figure 2b shows the results along with the control electrophoretic patterns obtained from dark-adapted (Fig. 2a) and light-adapted (Fig. 2c) flies (*9*). Figure 3, a to c, shows computer-generated, densitometric contour map profiles of the three two-dimensional electrophoretic patterns (*10*). The results show that the 1-msec flash was sufficient to phosphorylate about 25 percent (*11*) of the 49-kD polypeptide within 3 seconds (Figs. 2b and 3b). The flash also phosphorylated a fraction of the 80-kD polypeptide (Figs. 2b and 3b). The phosphorylation of this polypeptide, however, is to an intermediate state. In the dark-adapted flies the 80-kD polypeptide is primarily in the dephosphorylated state, 80A (Fig. 3a), and shifts to states 80C, 80D, 80E, and 80F upon complete light adaptation (Fig. 3c). In the flash-illuminated flies, on the other hand, the polypeptide shifted to states 80B, 80C, and, perhaps, very slightly to 80D, but not to 80E or 80F (Fig. 3b). In contrast to 80- and 49-kD polypeptides, phosphorylation of the 39-kD polypeptide was not detectable in the flash-illuminated flies (Figs. 2b and 3b). It became detectable only after the flies were illuminated with steady room light (160 μW/cm^2) for several tens of seconds.

An important point is that the phos-

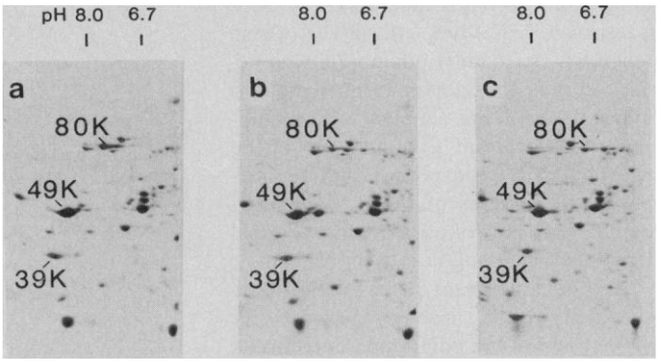

Fig. 2. Phosphorylation of 49- and 80-kD polypeptides by a strobe flash. Coomassie blue–stained two-dimensional gel patterns of (a) dark-adapted, (b) flash-illuminated, and (c) light-adapted flies.

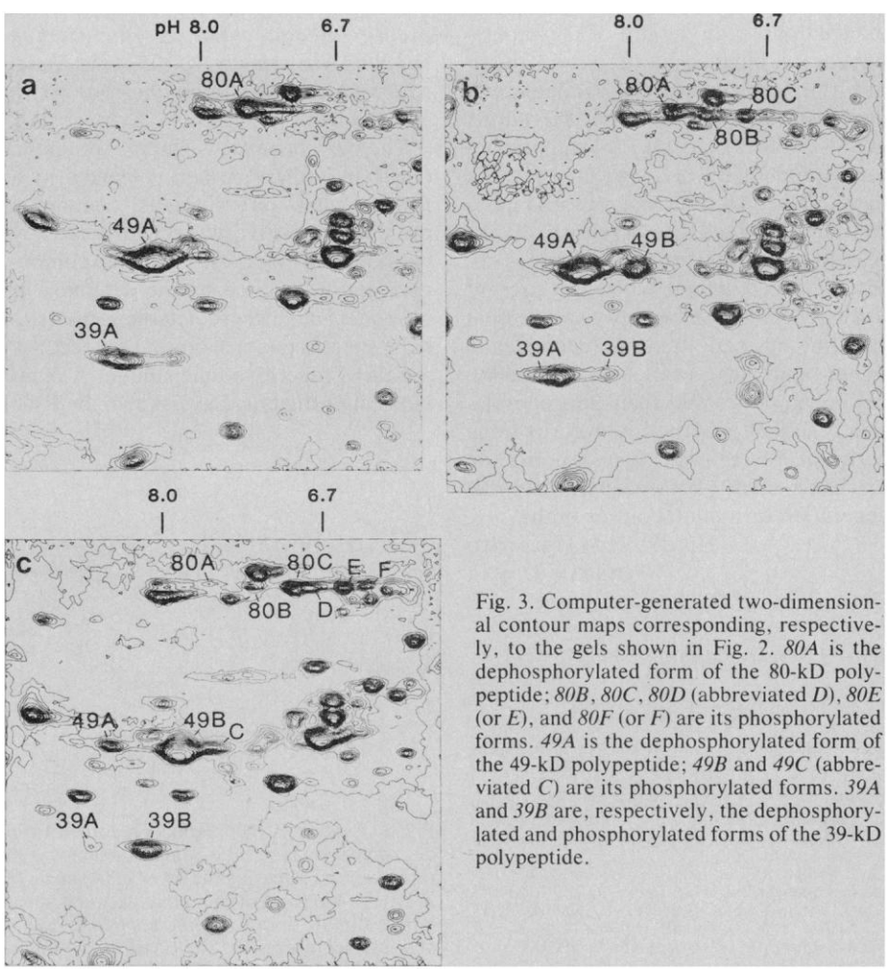

Fig. 3. Computer-generated two-dimensional contour maps corresponding, respectively, to the gels shown in Fig. 2. *80A* is the dephosphorylated form of the 80-kD polypeptide; *80B*, *80C*, *80D* (abbreviated *D*), *80E* (or *E*), and *80F* (or *F*) are its phosphorylated forms. *49A* is the dephosphorylated form of the 49-kD polypeptide; *49B* and *49C* (abbreviated *C*) are its phosphorylated forms. *39A* and *39B* are, respectively, the dephosphorylated and phosphorylated forms of the 39-kD polypeptide.

phorylation and dephosphorylation reactions of the 80-, 49-, and 39-kD polypeptides occur in vivo. Moreover, these reactions are readily inducible by exposing the flies to ambient room light. No unusual stimuli are necessary. These observations lead one to conclude that the light-induced phosphorylation-dephosphorylation cycles of these three polypeptides are part of normal physiological reactions occurring in a living system.

Another important point is the tissue specificity of the three polypeptides. By dissecting out the compound eye into its individual components, it was shown that all three polypeptides arise specifically from the photoreceptor layer (*6*). The lamina, which contains the synaptic endings of photoreceptors, in particular, does not make any contribution to these polypeptides in two-dimensional gels stained with Coomassie blue. The tissue specificity makes it highly unlikely that the three polypeptides are involved in general metabolism or housekeeping functions. Nor is it likely that any of these polypeptides are involved in synaptic mechanisms. Rather, the polypeptides are probably part of the molecular machinery underlying photoreceptor mechanisms.

We reported earlier that the light-induced shift in the *p*I (that is, the light-induced phosphorylation) of these polypeptides is blocked by the *norpA* mutation (*6*). Inasmuch as the available evidence strongly suggests that the *norpA* mutation blocks an essential step in phototransduction (*7*), we suggested that the light-induced modification of these polypeptides requires the integrity of the phototransduction process (*6*). These observations, together with our present finding that the phosphorylation of at least the 80- and 49-kD polypeptides is rather rapid, suggest that the 80- and 49-kD polypeptides and their phosphorylation reactions may be involved in some early molecular process underlying the mechanisms of photoreceptor potential generation or modulation (or both).

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References and Notes

1. P. Greengard, *Science* **199**, 146 (1978); P. Cohen, *Nature* (*London*) **296**, 613 (1982).
2. O. M. Rosen and E. G. Krebs, Eds., *Protein Phosphorylation* (Cold Spring Harbor Conferences on Cell Proliferation, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), vol. 8, A and B.
3. P. Cohen, *Mol. Aspects Cell. Regul.* **1**, 1 (1980).
4. P. Greengard, *Harvey Lect.* **75**, 277 (1981); C. G. Paris, V. F. Castellucci, E. Kandel, J. H. Schwartz, in (*2*), vol. 8B, p. 1361; M. B. Kennedy, *Annu. Rev. Neurosci.* **6**, 493 (1983).
5. J. Hermolin, in (*2*), vol. 8B, p. 1347; D. B. Farber, *Prog. Brain Res.* **56**, 435 (1982).
6. H. Matsumoto, J. E. O'Tousa, W. L. Pak, *Science* **217**, 839 (1982).
7. W. L. Pak, S. E. Ostroy, M. C. Deland, C.-F. Wu, *ibid.* **194**, 956 (1976); W. L. Pak, in *Neurogenetics: Genetic Approaches to The Nervous System*, X. O. Breakefield, Ed. (Elsevier/North-Holland, New York, 1979), p. 67.
8. The dark- or light-adapted ^{32}P-labeled flies were frozen in liquid nitrogen and dehydrated in acetone at −20°C. After dehydration, 100 compound eyes were dissected out from each group of flies and homogenized in 60 μl of a mixture containing 8*M* urea, 2 percent Triton X-100, and 2 percent Bio-Lyte 3/10 (Bio-Rad). The supernatant (about 55 μl), recovered after a brief centrifugation (3000 rev/min for 10 minutes), was subjected to two-dimensional gel electrophoresis by the method of P. H. O'Farrell [*J. Biol. Chem.* **250**, 4007 (1975)] with a slight modification by K. Miyazaki et al. [K. Miyazaki, H. Hagiwara, M. Yokota, T. Kakuno, T. Horio, in *Isoelectric Focusing and Isotachophoresis*, N. Ui and T. Horio, Eds. (Kyoritsu Shuppan, Tokyo, 1978), p. 183 (in Japanese)]. The isoelectric focusing (IEF) gels contained: 1.6 ml of 30 percent acrylamide, 1.5 percent *N,N'*-methylenebis (acrylamide), 1.5 ml of 0.004 percent riboflavin, 0.45 percent *N,N,N',N'*-tetramethylethylenediamine, 0.08 ml of 1.5 percent ammonium persulfate, 0.6 ml of 40 percent Bio-Lyte 3/10, 6.13 g of urea (Ultrapure grade, Schwarz/Mann), 1.2 ml of 20 percent Triton X-100, and 2.5 ml of distilled water. The gels were polymerized by illumination. The sample was applied from the acidic end (0.02*M* H_3PO_4, anode; 1*M* NaOH, cathode) and was focused with constant current mode at 0.5 mA per gel until the voltage reached 300 V and, thereafter, with constant voltage mode at 300 V for 16 hours and finally at 700 V for 2 hours. The second dimension, sodium dodecyl sulfate–10 percent polyacrylamide gel electrophoresis (SDS-PAGE), was carried out on a slab gel (14 by 13 cm) according to L. K. Laemmli [*Nature* (*London*) **227**, 680 (1970)].
9. Wild-type flies that had been dark-adapted for 24 hours were separated into three test tubes of 60 flies each. The first group was frozen in the dark (dark-adapted; Fig. 2a). The second group was subjected to a white strobe flash of < 1 msec duration (National Panashot PE-200, Matsushita Electric Co., Japan) and frozen as described in the text (flash-illuminated; Fig. 2b). The third group was exposed to room light for 5 minutes and frozen in room light (light-adapted; Fig. 2c). The gel analyses were carried out as described (*8*).
10. To obtain contour profiles, we dried the Coomassie blue–stained gels on filter paper and photographed them. The grain density of the photographic negatives was scanned to 100 μm resolution with Optronix Colorscan System C-4100. The data were stored on a magnetic tape and processed on the Purdue MACE operating system connected to a PDP 11/70 computer with the program developed by M. Laris.
11. The amount of phosphorylation was estimated by integrating each of the two contour peaks in Fig. 3b, representing dephosphorylated and phosphorylated forms of the 49-kD polypeptide.
12. We thank M. Laris for his help with computer analyses of two-dimensional gel electrophoretic profiles. Supported by grant BNS 80-15599 from the National Science Foundation and grant EY 00033 from the National Eye Institute of NIH.

12 September 1983; accepted 10 November 1983

Task-Relevant Late Positive Component of the Auditory Event–Related Potential in Monkeys Resembles P300 in Humans

Abstract. *A long-latency (300-millisecond), vertex-positive component of the event-related potential recorded from monkeys was present only when the eliciting stimulus was relevant to the task. The amplitude of this component varied inversely with stimulus probability and was dissociable from motor responses.*

The event-related brain potential (ERP) in humans, which is evoked by a task-relevant, rare stimulus, contains a prominent positive wave with a latency of about 300 msec (*1*). This P300 component can be elicited by the detection of infrequent and unpredictable target signals, such as pitch changes, that occur in a train of repetitive tone stimuli. A number of investigators have shown the P300 to be relatively independent of physical stimulus parameters and significantly related to cognitive processing of task-relevant stimulus information (*2*). These findings have led to attempted clinical application of the P300 component of the auditory ERP in the evaluation of cognitive functioning associated with aging, dementia, and alcoholism (*3*). Although the neural processes responsible for the

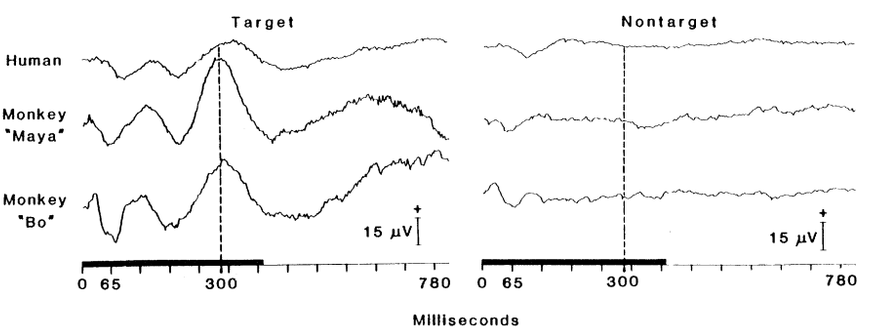

Fig. 1. Event-related potentials recorded from a human and two monkeys engaged in a similar discrimination task. The human subject was instructed to operate a lever and to respond to the rare event by releasing the lever in the same fashion as the monkeys. Note the strong similarity in ERP wave form morphology between species and the presence of a P300 component to the target stimuli that does not appear to the nontarget stimuli. The thickened line along the abscissa represents the period of tonal stimulation.