phyll per hour were obtained for chloroplasts after 1 day in cell culture as well as for freshly isolated chloroplasts. Jagendorf and Evans (*10*) reported a similar value of about 300 μmole/mg per hour for spinach chloroplasts. Fluctuation of values was mostly due to a considerable dye reduction rate in the dark, which was subtracted from the light reaction. Contaminating L cell mitochondria were responsible for most of the dark reaction. The results suggest that the chloroplast lamellae retained some functional as well as structural integrity.

Tests of light-dependent, endogenous CO_2 fixation ($NaHC^{14}O_3$) of chloroplasts reisolated from L cells after 24 hours were carried out as described (*11*). The radioactivity was measured in acidified chloroplasts (*11*) and in a hot, 20 percent ethanol extract of the organelles, which would contain phosphoglyceric acid if present (*12*). The radioactivity in extracts of dark-kept chloroplasts was subtracted from that of illuminated preparations. Values corresponding to about 0.2 μmole of CO_2 fixed per milligram of chlorophyll per hour were obtained as total endogenous activity, and values of 0.05 to 0.1 μmole of CO_2 fixed per milligram of chlorophyll per hour were calculated from radioactivity in hot, 20 percent ethanol extracts. Similar activities were found for freshly isolated chloroplasts. Correspondingly, Spencer and Unt (*11*) reported a rate of total endogenous CO_2 fixation in the light of 0.23 μmole of CO_2 fixed per milligram of chlorophyll per hour for spinach chloroplasts without their outer membrane. Because of the lack of a limiting outer membrane of the chloroplasts, radioactive material seemed to diffuse from the chloroplasts into the medium. This was also apparent in autoradiographs of illuminated chloroplasts prepared for autoradiography as described (*13*).

The integrity of the DNA of chloroplasts after they resided intracellularly for 2 days was also studied. Chloroplasts were isolated as described above and were further purified by centrifugation in sucrose gradients (*8*). DNA was visualized after the chloroplasts were spread on a protein film by the osmotic shock technique (*8*). Clusters of DNA molecules, showing some free ends, were associated with ruptured membranes (Fig. 3). The DNA was resolved in some cases into linear molecules with molecular weights between 10^7 and 10^8 daltons. Similar values were obtained for the linear DNA isolated from purified fresh chloroplasts by osmotic shock techniques and centrifugation in cesium chloride to equilibrium.

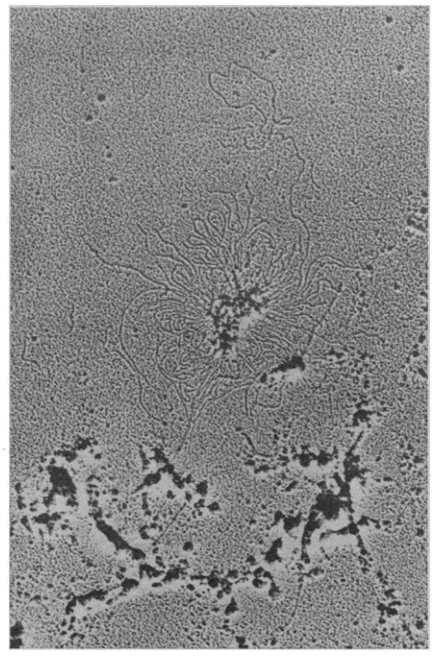

Fig. 3. Electron micrograph of the DNA of a spinach chloroplast reisolated after residing in an L cell for 2 days. Chloroplasts were lysed by osmotic shock on a protein film and rotary shadow-cast with vaporized platinum-iridium. The DNA is linear and several free ends are seen (× 20,000).

Isolated mitochondria of chicken liver, labeled with H^3-thymidine, were also taken up by L cells in 30 minutes, as observed by electron microscopy and by incorporation of the label into cells. The radioactivity of the cells declined in direct proportion to the increase of cell numbers during four cell generations.

Functional chloroplasts have been reported to occur naturally as symbiotic organelles in the digestive gland of the slug *Elysia* (*14*). We now see that animal cells can incorporate chloroplasts and mitochondria from other organisms. The degree to which these heterospecific organelles can function and multiply in the animal cells under various conditions of culture is being studied.

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Nerve Endings: Rapid Appearance of Labeled Protein Shown by Electron Microscope Radioautography

Abstract. *Electron microscope radioautographs of nerve ending fractions from mouse cerebrum show that radioactive protein is associated with nerve endings 15 and 90 minutes after intracerebral injection of tritiated leucine and lysine. The results are consistent with synthesis of protein at nerve endings, or with extremely rapid transport of particulate protein to nerve endings in the mouse cerebrum, or both.*

For days after intracerebral injection of a pulse of radioactive leucine, labeled soluble protein progressively appears in nerve endings (*1*, *2*). This delay is believed to be due to transport of soluble protein through axons from a perikaryal site of synthesis. Although there is very little labeled *soluble* protein in the nerve ending fraction 1 hour after injection, there is already substantial labeled *particulate* protein in this fraction at this time (*1*, *2*). Some of this appears to be mitochondrial protein made at nerve endings (*2*, *3*), but some is not associated with mitochondria (*2*). Since the nerve ending fraction is known to be

contaminated with membranes, the rapidly labeled protein associated with the particulate component of the nerve ending fraction could be in contaminants. To determine if there is truly rapid appearance of new protein at nerve endings, we examined radioautographs of nerve ending fractions with the electron microscope at several intervals after intracerebral injection of tritium-labeled amino acids.

Adult male Swiss albino mice, lightly anesthesized with ether, were mounted in a stereotaxic instrument and injected intracerebrally (*1*) with a mixture of 50 μc each of L-leucine-4,5,-H^3 (5 curie/mmole) and DL-lysine-4,5-H^3 (4 curie/mmole) delivered in a volume of 40 μl in 0.15*M* NaCl. At each time after injection three cerebrums were homogenized in 0.32*M* sucrose. An aliquot was removed for measurement of the specific radioactivity of protein in the whole brain by methods described previously (*1*, *2*). Maximum incorporation of radioactivity into protein of whole cerebrum was found within 90 minutes in these and preliminary studies. The nerve ending fraction was isolated by differential and sucrose gradient centrifugation (*1*, *2*), washed three times in 0.32*M* sucrose containing nonradioactive leucine and lysine, fixed in 4 percent formaldehyde, and washed three times with phosphate buffer containing nonradioactive leucine and lysine. One portion was precipitated with 6 percent trichloroacetic acid to determine the specific activity of proteins and the proportion of radioactivity which was not precipitated. The remainder of the pellet was postfixed in osmium tetroxide and embedded in epon, and the sections were radioautographed (*4*). In another experiment, mice were injected subcutaneously with saline with or without 5 mg of cycloheximide, an inhibitor of protein synthesis, 30 minutes before the intracerebral administration of 100 μc of leucine-H^3. Fifteen minutes after administration of the labeled amino acid the mice were killed, and nerve ending fractions were prepared as above.

Table 1. Radioactivity incorporated into brain and nerve ending proteins and number of grains overlying 10,000 nerve endings. The latter number was counted for each preparation.

Part	Postinjection time	
	90 min	3 days
Count/min per milligram of protein		
Nerve endings	89,000	92,000
Whole brain	230,000	151,000
Grains per 10,000 nerve endings		
Nerve endings	346	775

Radioactivity was associated with nerve endings within 90 minutes of injection of precursor (Table 1, Fig. 1). In the nerve ending fraction fixed in formaldehyde, 87 percent of the radioactivity in 90-minute samples and 94 percent of the radioactivity in 3-day samples was precipitated with 6 percent trichloroacetic acid. Therefore, the radioactivity artifactually bound to structures (*5*) might account for only 6 to 13 percent of the total radioactivity present in the nerve ending fraction. The radioautographs showed that 50 to 80 percent of the radioactivity counted in various preparations of nerve ending fractions from cerebrums homogenized 15 or 90 minutes after injection was specifically related to nerve endings rather than to contaminating membranes, mitochondria, ribosomes, or other structures. Fifteen minutes after injection of leucine-H^3, the number of silver grains seen over nerve endings was reduced by 70 percent in mice previously injected with cycloheximide. Therefore the grains reflect new protein rather than adsorbed amino acids.

The resolution achieved by radioautography does not permit accurate quantification of the distribution of silver grains over organelles present in a nerve ending. Nevertheless, by 15 and 90 minutes (Fig. 1, a and b), 20 percent of the silver grains overlay mitochondria in nerve endings. In numerous sections of labeled nerve endings, no mitochondrial profiles were observed (Fig. 1c). This indicates that some of the newly formed protein is related to the soluble protein, synaptic vesicles, or membranes. Only occasional labeled nerve endings had attached postsynaptic membrane (Fig. 1, a and d).

At 3 days, the total number of labeled nerve endings had doubled (Table 1). At this time several silver grains were frequently found over one nerve ending (Fig. 1d), whereas the radioactivity seen in the contaminants declined. This is consistent with the finding in the present (Table 1) and previous studies (*1*, *2*) that in the interval between 90 minutes and 3 days after injection, the specific activity of whole brain protein fell somewhat, whereas that of the protein in the nerve ending fraction rose slightly. The late accumulation of labeled protein in nerve endings presumably corresponds to the arrival of migratory protein, including particulate protein (*2*), synthesized in the perikaryon and transported through the axon.

The rapid appearance of labeled protein at nerve endings might be due to rapid transport from the perikaryal site of synthesis or to local protein synthesis. There is evidence that protein

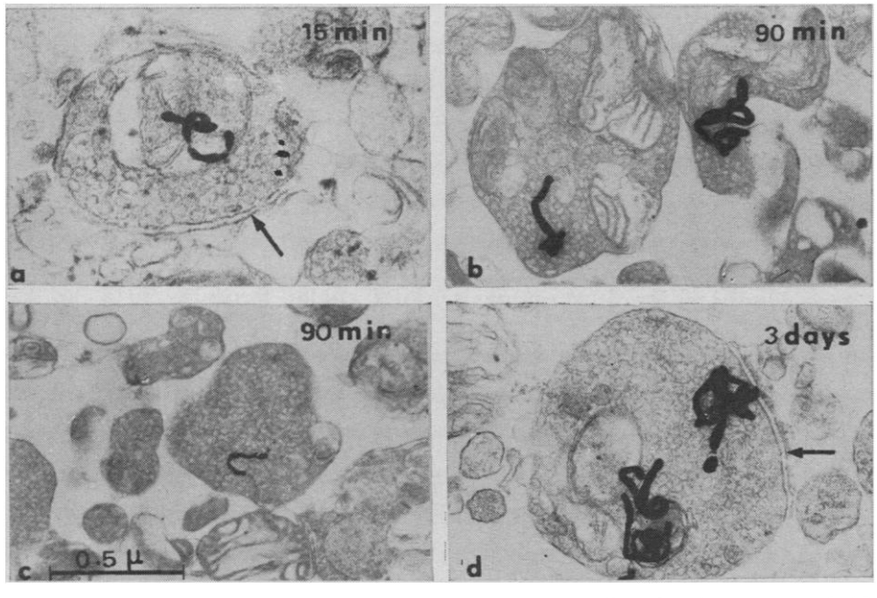

Fig. 1. Electron microscope radioautographs of nerve ending fractions prepared 15 minutes (a), 90 minutes (b and c), and 3 days (d) after intracerebral injection of tritiated amino acids. Contaminating membranes and mitochondria are intermingled with nerve endings. At 15 minutes (a) and 90 minutes (b and c) after injection, silver grains are located over and between mitochondrial profiles in nerve endings (a and b). Sections of labeled nerve endings, which are free of mitochondria, are also frequently observed (c). Three days later (d), nerve endings are often overlaid by two to four silver grains. The postsynaptic membrane, sometimes found attached to the presynaptic ending, is visible in a and d (arrow).

may be transported in axons at rates up to centimeters per hour (*6*). Since many neurons in the mouse cerebrum have short axons, rapid transport of protein from the perikaryon to nerve endings could be accomplished easily at such rates (*7*). McEwen and Grafstein (*8*) showed that particulate protein is transported more rapidly to nerve endings than soluble protein. The different behavior of soluble and particulate protein implied by these and previous studies (*1–3, 7*) might be due to such differential rates of transport.

An alternative explanation of these findings is that there is substantial local synthesis of protein at nerve endings. Although the nerve ending is devoid of detectable ribosomes, there are abundant mitochondria, and evidence consistent with local synthesis of mitochondrial protein at nerve endings has been presented (*2*, *3*). However, in the present study, grains overlying nerve endings were not usually associated with mitochondria. Therefore, the present findings cannot be explained in this way. Austin and Morgan (*9*) and Autillio *et al.* (*10*) have shown that nerve ending fractions can incorporate amino acids into protein in vitro. These studies are consistent with incorporation of amino acids into protein in nerve endings, but the possibility that the observed incorporation is attributable to contaminants has not been completely excluded. The potentiality for local protein synthesis at nerve endings is supported by radioautographic studies of in vitro incorporation of tritiated amino acids into protein of nerve endings of the ciliary ganglion of the chick (*11*), although extensive perikaryal synthesis of protein which migrated to these nerve endings was shown in vivo (*11*).

The present study does not unambiguously demonstrate local nonmitochondrial protein synthesis at nerve endings. However, it clearly shows that newly synthesized protein appears very rapidly at nerve endings of mouse brain. Therefore, new proteins may regulate nerve ending function within minutes of their synthesis.

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Adenosine-3′,5′-Phosphate: Identification as Acrasin in a Species of Cellular Slime Mold

Abstract. *Acrasin, the chemotactic agent responsible for aggregation in the cellular slime molds, has been identified for one species,* Dictyostelium discoideum, *as adenosine-3′,5′-phosphate.*

The cellular slime mold *Dictyostelium discoideum* has been used by a number of investigators to study the processes of cellular differentiation and morphogenesis. As long as adequate food is present the amoebas of this organism feed and reproduce by binary fission. When starved, the individual cells stream into central collecting points to form multicellular masses which become migrating pseudoplasmodia. These pseudoplasmodia later differentiate into fruiting structures consisting of both stalk and spore cells. The aggregation phase of the life cycle is mediated by chemotaxis (*1*).

The amoebas are also attracted to bacteria (*2*), and we showed that at least one of the attractants in *Escherichia coli* is adenosine-3′,5′-phosphate (3′,5′-AMP) (*3*). Subsequently, we found that the related species of slime mold *Polysphondylium pallidum* secretes 3′,5′-AMP, but is not attracted to it (*4*, *5*). Our first attempts to obtain sufficient acrasin from *D. discoideum* failed, presumably because of an acrasin-inactivating enzyme first discovered by Shaffer (*6*). Chang has isolated an extracellular enzyme capable of converting 3′,5′-AMP to 5′-AMP (*7*). This discovery suggested that if the activity of this enzyme could be curtailed, and if acrasin were a substrate for this enzyme, sufficient acrasin could be accumulated to effect a chemical identification. Two different approaches to this problem were initiated: the one reported here and another reported by Konijn *et al.* (*8*). Both approaches have led to the conclusion that 3′,5′-AMP is a naturally occurring acrasin of *D. discoideum*.

Amoebas of *D. discoideum* NC-4(H) were grown in liquid culture with the use of Hohl and Raper's (*9*) modification of Gerisch's (*10*) technique for growth on dead, autoclaved bacteria. When growth was completed, the cells were centrifuged at 2500*g* for 10 minutes and suspended in 1 percent Bonner's salt solution (*11*). These preparations could be treated in one of two ways.

In the first procedure, 40 to 50 ml (2×10^6 cell/ml) were poured onto a dialysis membrane placed over 1 percent salt solution and supported by a plastic frame (*12*). The cells settled and attached themselves to the membrane. After ½ hour, the excess liquid was siphoned off. Subsequently these cells aggregated normally, formed migrating

Table 1. Results of paper chromatography of acrasin. Chromatograms were developed for 16 hours at room temperature. Acrasin activity was determined on the Konijn bioassay after eluting 1-cm strips with water. Markers were detected with ultraviolet light. The solvents used were: A, 1*M* ammonium acetate and ethanol (3 : 7), ascending; B, isopropanol, ammonia, and water (7 : 1 : 2), ascending; and C, saturated ammonium sulfate, 1*N* sodium acetate, and isopropanol (40 : 9 : 1), descending.

Substance	R_F	Distance from origin (cm)
	Solvent A	
Acrasin	0.34 to 0.41	
3′,5′-AMP	.34 to .41	
5′-AMP	.07 to .10	
	Solvent B	
Acrasin	0.30 to 0.42	
3′,5′-AMP	.30 to .38	
5′-AMP	.08 to .11	
	Solvent C	
Acrasin		9 to 11
3′,5′-AMP		9 to 12
5′-AMP		22 to 27