plasts was demonstrated recently by Ongun and Mudd (*3*).

Chloroplasts from whole green spinach leaves were prepared according to the aqueous method of Walker (*4*). Purification of intact chloroplasts (class 1) was carried out according to a modification of the method of Leech (*5*). The envelope, thylakoid, and stroma of the chloroplasts were prepared and characterized as described previously (*6*). The method makes use of the fact that gentle swelling of intact chloroplasts caused rupture and total detachment of the envelope with the liberation of the stroma material.

The following enzyme assays were carried out on the various fractions: trypsin-activated Ca^{2+}-dependent adenosine triphosphatase (ATPase) activity of coupling factor 1 (*7*), Mg^{2+}-dependent ATPase insensitive to *N,N'*-dicyclohexylcarbodiimide (*6*), fructose-1,6-diphosphatase (*6*), and reduced nicotinamide adenine dinucleotide (NADH) : cytochrome c oxidoreductase (*6*). Galactolipid synthesis was measured at 37°C in a 0.4-ml incubation mixture containing tricine-NaOH buffer (*p*H 7.5), 4 μmole; $MgCl_2$, 0.8 μmole; diglyceride, 0.4 μmole; and appropriate amounts of enzyme. Reactions were initiated upon addition of uridine diphosphate—[U-^{14}C]galactose (18 nmole, 500,000 count/min), and incubated for 20 minutes. The reaction mixtures were extracted according to the method of Bligh and Dyer (*8*). Labeled galactolipids were isolated from the chloroform-methanol phase and identified by thin-layer chromatography (*6*). The ^{14}C in the isolated lipids was measured in a liquid scintillation counter, and the amount was converted to picomoles of galactose for each lipid. Protein, chlorophyll, and polar lipids were determined according to the methods of Douce *et al.* (*6*).

Trypsin-activated Ca^{2+}-dependent ATPase of coupling factor 1 and chlorophyll content were markers for the thylakoid membrane fraction; fructose-1,6-diphosphatase was the marker for the stroma; Mg^{2+}-dependent ATPase insensitive to *N,N'*-dicyclohexylcarbodiimide was the marker for the chloroplast envelope (*6*). The marker activities were distributed among the fractions as anticipated, thus validating the fractionation procedure used (Table 1). The galactolipid synthesis occurred primarily in the envelope. The galactolipid synthesis activity found in the envelope is not attributable to microsomal contamination; the envelope is strikingly different from the microsomal subfractions in that it exhibits no NADH : cytochrome c oxidoreductase activity, no b-type cytochrome (*6*), and no phosphatidylethanolamine (Table 2). During the chloroplast preparation some envelope material released from the broken chloroplasts contaminated the other cell fractions. This may explain why other investigators observed galactolipid synthesis activity in the microsomal fraction obtained from spinach leaves (*3*). It should be pointed out that the demonstration of the preferential association of galactolipid synthetic activity with the envelope does not preclude association of a small fraction of this activity with the thylakoid system.

Table 2. Polar lipid composition of chloroplast envelopes and microsomes of spinach. The identification of the polygalactolipids was determined by the method of Webster and Chang (*12*). Microsomes from whole green leaves were prepared by the method of Marshall and Kates (*9*); there was some contamination by chloroplast material.

Polar lipid	Lipid (percent by weight)	
	Envelopes	Microsomes
Monogalactosyldiglyceride	24	12
Digalactosyldiglyceride	32	8
Trigalactosyldiglyceride	5	0
Tetragalactosyldiglyceride	1	0
Sulfolipid	7	2
Phosphatidylcholine	21	35
Phosphatidylethanolamine	0	30
Phosphatidylglycerol	8	3
Phosphatidylinositol	2	7
Phosphatidylserine	0	3

It is well established that the main location of the polar lipid synthesizing enzymes in plant cells (*9*) and animal cells (*10*) is the endoplasmic reticulum. However, the data presented here show that the outer membrane of the chloroplast, like that of the mitochondrion (*11*), can be a site of synthesis of an organelle's major structural lipids.

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13 August 1973; revised 31 October 1973

Amorphous Semiconductor Switching in Melanins

Abstract. *Melanins produced synthetically and isolated from biological systems act as an amorphous semiconductor threshold switch. Switching occurs reversibly at potential gradients two to three orders of magnitude lower than reported for inorganic thin films, and comparable to gradients existing in some biological systems. Of a number of other biological materials tested, only cytochrome c acted similarly, but at the high potential gradients reported for thin film amorphous semiconductors.*

There has been a suggestion that the biological pigment melanin may qualify as an amorphous semiconductor (*1*). Recent experimental evidence has demonstrated that this material exhibits properties consistent with a more exotic form of an amorphous semiconductor, a threshold switch. Threshold switching has become a central issue in the development of amorphous electronic devices, but until now only inorganic materials have been demonstrated to possess these properties.

Synthetic melanins were produced

both by the enzymatic action of mushroom tyrosinase (Worthington Biochemical) on tyrosine incubated in a buffer at *p*H 7.0 for 4 days at 37°C and by the autoxidation of L-dopa (3,4-dihydroxyphenyl-L-alanine) in 1*M* NaOH for 1 week. The product was adjusted to a neutral *p*H, dialyzed against twice distilled water, and lyophilized.

Melanosomes were isolated from 8 g of human melanoma tumor material obtained at autopsy. The tumor material was strained through a 200-mesh grid to form a single cell suspension in a phosphate buffer. This suspension was then homogenized in a Dounce homogenizer, breaking the cell membrane but leaving the nuclear membranes intact. The homogenate was centrifuged at 1000*g* for 10 minutes to remove the remaining intact cell nuclei and other cell debris. The supernatant was centrifuged at 5000*g* for 10 minutes, and the new supernatant was again centrifuged for 30 minutes at 15,000*g*. The pellets from these centrifugations were then resuspended and layered on 30 to 50 percent sucrose density gradients in a Beckman SW-27 rotor and spun at 10,000 rev/min for 30 minutes; the gradients were fractionated and examined microscopically for their contents. The center one-third of each gradient was found to contain particles in the size range 0.1 to 1.0 μm. The central fractions were pooled and centrifuged at 50,000*g* for 4 hours to form black pellets weighing approximately 20 mg, which were used for further study on melanosomes.

Melanin was also isolated by suspending 50 g of homogenized tumor in 6*M* HCl and centrifuging at 10,000*g* for 30 minutes. The pellet was subsequently washed with three rinses each of dimethyl sulfoxide and acetone. In this case no effort was made to separate free melanin and melanosomes. The yield was approximately 50 mg.

The melanin samples were compressed into cylinders 3 mm in diameter and 0.1 to 10 mm in length. The cylinder of melanin was compressed in a quartz tube (inner diameter, 3 mm) between carbon, copper, or aluminum electrodes.

The electrical properties of all the melanin preparations were essentially the same. Figure 1 shows the typical electrical characteristics of melanin samples in series with a load resistor and an applied potential. As the applied potential is increased, the current through the melanin sample increases monotonically up to a voltage V_T (the threshold voltage), at which point the material switches.

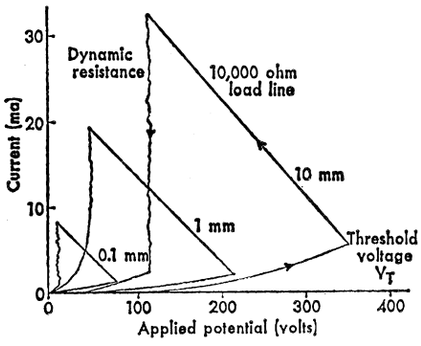

Fig. 1. Current-voltage properties of melanin prepared by autoxidation of L-dopa for various sample thicknesses; copper electrodes were used. Data were obtained by using an X-Y plotter and circuitry as described in the text. The curves are typical of results obtained with other electrodes and melanins. Materials that do not switch (in a sense, have no V_T) either never leave the lower curve, or break down irreversibly. The current through a melanin sample, however, is dependent on its history, and will be given by the lower curve unless V_T has been exceeded, in which case the dynamic resistance line applies. This process is reversible and is not a breakdown in the usual sense. The load line (negative slope) merely reflects the X-Y trace as the current and voltage adjust from the "off" to the "on" state and is inversely proportional to the value of the resistor in series with the melanin sample; the potential therefore represents the voltage across the sample.

Most materials do not switch. In this case the current at a particular voltage is always the same, resulting in a single curve. Materials that switch exhibit two separate voltage-current characteristics, the "on" and "off" states. At the point of switching (V_T), the X-Y recorder leaves the "off" curve and follows a line whose slope is inversely proportional to the load resistor (the load line) until it reaches the curve characteristic of the "on" state, producing the triangular pattern in Fig. 1.

This sequence may be repeated indefinitely; for example, a 10,000-ohm load resistor was sufficient to prevent a shift in the dynamic load line, even though V_T was found to decrease about 20 percent on the first firing and then stabilize. [A decrease in V_T by a factor of 5 to 15 has been reported in inorganic amorphous semiconductors on first firing; however, 50 percent was considered typical (*2*).] As Fig. 1 also shows, V_T increases as a function of sample thickness, which indicates a dependence on the applied electrical field strength. Switching may also depend on the presence of absorbed water. When samples were dried for 30 minutes at 200°C, they would not switch until rehydrated and dried at room temperature. Rosenberg and co-workers (*3*) have shown that the conductivity of the melanins (presumably in the "off" state) has a protonic component. Water, however, is also capable of lowering the activation energy for conduction, apparently as a result of local modification of the dielectric constant of the material. Since the current density in the "on" state is several orders of magnitude higher than that in the "off" state for the same applied potential, one can obtain an estimate of the protonic contribution as follows: approximately 10 mg of water must be dissociated to produce a current of 25 ma for 1 hour. A 30-mg sample (25 mg of melanin and 5 mg of H_2O) was switched "on" and maintained at 25 ma for 3.5 hours, at which time the current was still being maintained at the initial voltage. This would require hydrolysis of over 30 mg of water, if 100 percent protonic conduction is assumed. The sample was found to exhibit switching properties at the end of this time. It appears that the protonic contribution to the conductivity in the "on" state is negligible, and the water probably acts by altering the local dielectric constant of the material.

We attempted to measure the switching time by adjusting the applied voltage to a value just below V_T. At this potential, the material oscillated between the "on" and "off" states. The oscillations were viewed with an oscilloscope and found to be square waves with roughly a 1-msec lifetime and a rise time of less than 10^{-6} second.

The conductivity of dopa-melanin and isolated melanosomes was relatively high [10^{-5} (ohm-cm)$^{-1}$], resulting in a resistance of 10^4 ohms for a sample 1 mm thick. The conductivity in the "on" state is increased by a factor of 100 to 1000. Hence in the "on" state melanin is a relatively good conductor. Using the same techniques, we investigated the electrical characteristics of a number of other biological molecules. Bovine serum albumin, myoglobin, lecithin, polytryptophan, bilirubin, and oxidized cholesterol were not observed to switch (at least at potential gradients below 5×10^5 volt/cm). Equine cytochrome c switched at 4×10^5 volt/cm, three orders of magnitude higher than the potential gradients of melanins and comparable to those of inorganic amorphous semiconductors (*4*).

Switching phenomena in thin amorphous films and chalcogenide glasses have been extensively studied (*4*, *5*). Since more information is becoming available on these materials, we wished to determine to what extent the results for melanins parallel those reported for some inorganic amorphous semiconductors. Switching behavior in these materials is usually reported for samples less than 10 μm thick and with potential gradients greater than 10^5 volt/cm (*4*). Melanins, however, switch at 3.5×10^2 volt/cm and through at least 1 cm of material. It should be noted that this potential gradient exists in some biological systems.

Switching at low gradients and through bulk samples poses interesting theoretical questions. However, the consistent appearance of melanin in living organisms at locations where energy conversion or charge transfer occurs (the skin, retina, midbrain, and inner ear) is of particular interest in view of the evidence for a role for melanin in such human disorders as parkinsonism (*6–8*), schizophrenia (*7*), and deafness (*8*). The role of melanin in these disorders may be in some way related to its ability to function as an electronic device. This possibility is supported by the observation that the electronic properties of the melanin persist in intact melanosomes.

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9. We thank M. Romsdahl and S. Moss for their assistance in this work. Supported in part by PHS training grant CA-05099 and by AEC contract AT-(40-1)-2832.

14 December 1973

Hormonal Control of Neutrophil Lysosomal Enzyme Release: Effect of Epinephrine on Adenosine 3′,5′-Monophosphate

Abstract. *Human neutrophilic leukocytes release neutral protease and β-glucuronidase during cell contact with, and phagocytosis of, zymosan particles treated with rheumatoid arthritic serum. Release of lysosomal enzymes is inhibited by epinephrine and adenosine 3′,5′-monophosphate (cyclic AMP), but not by phenylephrine or adenosine 5′-monophosphate. Inhibition of enzyme release by epinephrine may be mediated by cyclic AMP because the cyclic AMP in the neutrophils is increased by epinephrine treatment at the time when enzyme release is reduced.*

Extracellular release of lysosome granule enzymes from polymorphonuclear leukocytes is provoked by cell contact with various immunologic reactants (*1*, *2*). Local tissue injury is probably a direct consequence of the release of granule constituents (*3*), as lysomal proteins are capable of mediating inflammation (*4*) and cartilage degradation (*5*). Therefore, inhibition of lysosomal enzyme release from leukocytes in contact with immune reactants might be of value in attenuating the severity of inflammation and tissue destruction.

Catecholamines, and adrenergic mechanisms in general, are thought to be involved in the regulation of the inflammatory process. In fact, certain catecholamines can elicit anti-inflammatory effects in various models of acute and chronic inflammation, including polyarthritis (*6*). Moreover, specific actions of catecholamines at the cellular level include inhibition of allergic release of histamine from sensitized human leukocytes (*7*) and lung tissue (*8*). At the subcellular level, catecholamines inhibit the release of enymes from isolated lysosome granules (*9*), probably via a mechanism involving adenosine 3′,5′-monophosphate (cyclic AMP).

The findings that catecholamines and cyclic AMP inhibit the phagocytic release of lysosomal enzymes from human mixed leukocytes (*10*) prompted an analysis of the effects of these agents on purified human neutrophils. We report here that the adrenergic neurohormone epinephrine inhibits the extracellular release of β-glucuronidase and neutral protease from human neutrophils, in contact with zymosan particles treated with rheumatoid arthritic (RA) serum, and that inhibition of enzyme release is accompanied by a concomitant elevation of intracellular cyclic AMP. Thus, the influence of epinephrine on neutrophil function may be mediated by changes in the endogenous concentration of cyclic AMP.

Human neutrophils were isolated from fresh heparinized venous blood of healthy volunteers (*11*). Final leukocyte suspensions were prepared in Hanks balanced salt solution containing 1 percent glucose (weight to volume), and final cell concentrations were adjusted to 5×10^6 neutrophils per milliliter. Neutrophils constituted 95 to 98 percent of all cells present. Mononuclear cells were present to the extent of 0 to 3 percent, and erythrocytes and platelets were absent. Viability of the neutrophils was greater than 99 percent, as determined by eosin Y or trypan blue exclusion.

Zymosan particles (0.5 to 3.0 μm in diameter) were boiled in Hanks balanced salt solution (10 mg/ml), resuspended in RA serum (1 : 20,480 titer rheumatoid factors as determined by latex agglutination) at a concentration of 25 mg/ml, and incubated at 37°C for 30 minutes (with slight agitation). The treated zymosan particles were washed with cold saline and suspended at 10 mg/ml in the Hanks-glucose solution (4×10^8 particles per milliliter).

Neutrophils (5×10^6 in 1.0 ml of Hanks-glucose solution) and test agent (or agents) were incubated at 37°C for 5 minutes prior to the addition of 0.1 ml of zymosan suspension, and were then incubated at 37°C in a Dubnoff metabolic shaker (120 excursions per minute). After incubation, samples were centrifuged at 200*g* for 10 minutes at 4°C, and the supernatants were assayed for neutral protease, β-glucuronidase, and lactate dehydrogenase (LDH) activities (*12*). Determinations of total neutrophil enzyme activities were made after cell lysis, by eight freeze-thaw cycles, from a Dry Ice–acetone mixture to cool tap water, and centrifugation as described above. Epinephrine solutions contained 0.01 percent (weight to volume) sodium metabisulfite to prevent spontaneous oxidation of the catecholamine, and were used immediately.

Cyclic AMP in neutrophils was determined by a protein-binding method (*13*), after rapid freezing of individual incubation mixtures containing cells,