centration of HNCO, identified by absorptions at 3495 cm^{-1} (strong, vibrational frequency ν_1), 2265 cm^{-1} (very strong, ν_2), and 790 cm^{-1} (strong, ν_4), all of which show small shifts to shorter wavelengths compared with the spectrum of gaseous HNCO (*11*).

The results for Pd are shown in Fig. 2. The maximum yield of HNCO was 60 percent at 350°C and the total yield of NCO was 73 percent. A prerequisite for a high yield is thorough reduction of the Pd. An effective procedure was to pass pure H_2 or H_2-He mixtures over the Pd at room temperature for several hours (*12*), followed by reduction in flowing pure H_2 at 450° to 500°C. A more moderate prereduction at 380°C was not effective. The effect of the concentrations of H_2 and H_2O in the inlet gas was studied. The yield of HNCO increased slightly when 3.4 percent H_2O was added to the standard NO-CO-H_2-He mixture with the Pd at 350°C. Replacing part of the H_2 with an equivalent concentration of H_2O decreased the yield of HNCO.

The reactions of NO, H_2, and CO over Ir or Pd described here provide a novel and practical synthesis of HNCO. Because of the large difference between the HNCO and NH_4OCN condensation temperatures, separation of the two products is simple. At a sufficiently low collection temperature (less than −20°C), HNCO is a stable liquid or solid, while at a higher temperature (20°C) it easily trimerizes to isocyanuric acid, cyanuric acid, and cyamelide (*13*). The mixture can simply be converted to pure isocyanuric acid (*14*) or reacted with NH_3 to melamine (*15*). Both are important raw materials in the chemical industry (*16*). In addition, conversion of HNCO to urea is long-known and straightforward (*17*). The mechanism of the formation of HNCO and NH_4OCN is under current investigation.

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Phagocytosis of Light- and Dark-Adapted Rod Outer Segments by Cultured Pigment Epithelium

Abstract. *Pigment epithelial cells in culture retain their ability to phagocytize rod outer segments. These cells phagocytize rod outer segments isolated from light-adapted rats, or from dark-adapted rats killed after the time at which the lights would normally be turned on. However, they phagocytize far fewer rod outer segments prepared in the dark from the retinas of rats killed before the onset of the normal light cycle. Phagocytosis of dark rod outer segments is variable, but that of light outer segments is reproducible. It is postulated that the effect of light is to synchronize the chemical events that occur at the surface of the rods to prepare them for phagocytosis. These processes also occur in the dark, but more slowly and irregularly than in the light.*

Vertebrate rod photoreceptor cells continually renew their outer segments by a balanced process of synthesizing new disks at the base of the outer segment and shedding packets of disks from the tip of the outer segment. These shed packets of disks are subsequently phagocytized by the adjacent pigment epithelial cells to form phagosomes, which are degraded by hydrolytic enzymes contained within the lysosomes of these cells (*1*).

It has recently been reported that the shedding of disks in rats and frogs is coupled to the light-dark cycle in which the animals are kept. In the rat retina a burst of rod outer segment (ROS) disk shedding occurs soon after the onset of light. However, the same process occurs at the same time without the onset of light, suggesting that disk shedding in this animal follows a circadian rhythm (*2*). In frogs it appears that most disk shedding is directly initiated by light, although cyclic shedding does occur at a reduced level when animals are kept in constant darkness for prolonged periods (*3*).

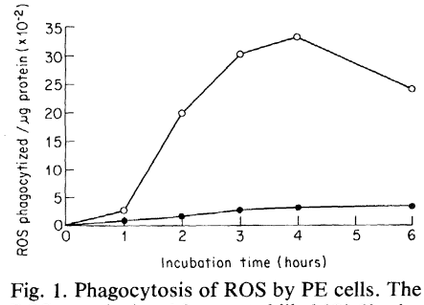

Fig. 1. Phagocytosis of ROS by PE cells. The ROS were isolated from rats killed (●) 60 minutes before the onset of light or (○) 30 minutes after the lights were turned on, and incubated with monolayer cultures of PE cells for the indicated periods. Each culture well received 2 × 10^6 ROS containing a measured amount of radioactivity. From the total radioactivity phagocytized by the monolayers, it was possible to calculate the number of ROS phagocytized at each time point.

Rod outer segment tips are not phagocytized by the pigment epithelium (PE) before being shed into the subretinal space. Since the phagocytic response of the PE cells appears to be triggered by this shedding, some chemical change must occur to the tip of the ROS that allows the PE to recognize the shed packet of disks as foreign and thus to initiate phagocytosis (*4*). This recognition must involve the plasma membranes of the shed outer segment fragments and the PE, and thus it is most probable that the plasma membrane of the ROS is chemically modified either before or just after shedding of a packet of disks.

To explore this problem, I examined the ability of PE cells grown in tissue culture to phagocytize ROS isolated at various times during the lighting cycle. I found that light acts as a trigger to prepare the ROS for phagocytosis by the PE.

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Long-Evans rats, 22 to 26 days old, were injected intraocularly with 40 μCi of [^{3}H]leucine per eye. The rats were kept in an approximately 12-hour-light:12-hour-dark cycle (lights on at 0645, off at 1800) and were killed 9 days after injection (*5*). Illumination in the cage was a maximum of 10 foot-candles (108 lm/m^2). Some animals were killed under dim red light 60 minutes before the onset of the normal light cycle; all subsequent operations involving retinas from these animals were done in the dark or under dim red light. Other animals were killed in the light, 30 minutes or more after the onset of the normal light cycle; all subsequent operations involving retinas from these animals were done in the light. Rod outer segments were liberated by stirring the isolated retinas in Hepes-buffered Hanks balanced salt solution (HBSS), *p*H 7.4, for 15 minutes at 4°C in a trypsinization flask. Retinal debris was allowed to settle for 10 minutes at 0°C and the ROS were collected from the supernatant by centrifugation. The ROS pellet was washed twice with HBSS, suspended in 1 ml of growth medium (*6*), and the number of ROS was counted. The suspension was then diluted to contain 2×10^6 ROS per milliliter of growth medium.

Pigment epithelial cells were grown as previously described (*7*). Confluent monolayers of cells were washed with growth medium before use. A 1.0-ml portion of ROS suspension was pipetted into each well, and the cells were incubated at 37°C in 5 percent CO_2 and 95 percent air for varying periods (*8*). Rod outer segments prepared in the dark were incubated with PE cells in a light-tight box provided with baffle ports to allow the entry of air. Rod outer segments prepared in the light were fed to PE cells in the light; however, incubation took place in a relatively dark incubator. At the end of each incubation period, the PE cell monolayer was rapidly washed four times with ice-cold HBSS, scraped from the bottom of the culture dish, and centrifuged. The pellet of PE cells was solubilized by boiling for 3 minutes in 3.3 percent sodium dodecyl sulfate and portions were taken for the determination of radioactivity and protein content (*9*). It was only at this point that the dark samples were exposed to light.

As can be seen from Fig. 1, PE cells avidly phagocytize ROS from rats that have been exposed to light, but only minimally phagocytize ROS prepared in the dark from the retinas of rats killed 1 hour before the onset of the normal light cycle. The PE cells always phagocytize two to ten times as many ROS isolated from rats exposed to light for 30 minutes as from rats killed 60 minutes before the lights are turned on (*8*). Thus exposure of the rats to light "activates" the ROS for phagocytosis by the PE. However, exposure to the light was not a prerequisite for this process. As the time approached at which the lights were normally turned on in the morning, more dark ROS where phagocytized by the PE cells. This is shown in Fig. 2. The number of ROS phagocytized increases in the dark up to and past the time at which the lights are normally turned on. Thus about twice as many ROS are phagocytized from rats killed at 0900 in the dark than from rats killed at 0530, and the number of ROS phagocytized from rats killed in the light at 0900 is about three times the dark value at 0530. This indicates that exposure to light is not a prerequisite for phagocytosis. Light may act to accelerate or synchronize the changes that occur to the surface of the rods to prepare them for phagocytosis. In the dark, this process occurs at approximately the same time each day, but in a less synchronous manner.

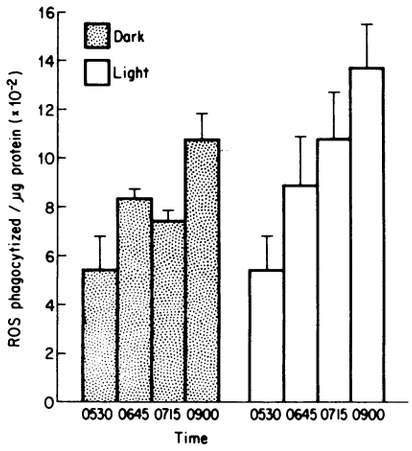

Fig. 2. The effect of time at which the animals were killed and of exposure to light on the phagocytosis of ROS by PE cells. Dark-adapted rats were killed in the dark at the times shown and ROS were prepared and fed to PE cells in the dark. As controls, ROS were prepared from rats killed in the light at the same time and fed to PE cells in the light. The lights were turned on at 0645. Incubation was for 4 hours.

To eliminate the possibility that these results were due merely to uptake of free [^{3}H]leucine and subsequent protein synthesis by the PE cells, incubation was carried out with light ROS in the presence or absence of puromycin (50 μg/ml), which was added to the monolayer 30 minutes before the addition of ROS and was present throughout the incubation period (*10*). With the inhibitor present, the phagocytosis of radioactive ROS continued at 80 percent of the level observed when puromycin was absent (*11*). Thus the observed uptake of radioactivity is not due to the synthesis of ^{3}H-labeled proteins by the PE cells. However, when iodoacetic acid ($6 \times 10^{-4}M$) and dinitrophenol ($2 \times 10^{-4}M$) were present throughout the incubation, phagocytosis was decreased by 86 percent. Thus metabolic energy is required for the phagocytosis of ROS. That the ROS are actually phagocytized, rather than merely adhering to the surface of the PE monolayer, is shown in Fig. 3,

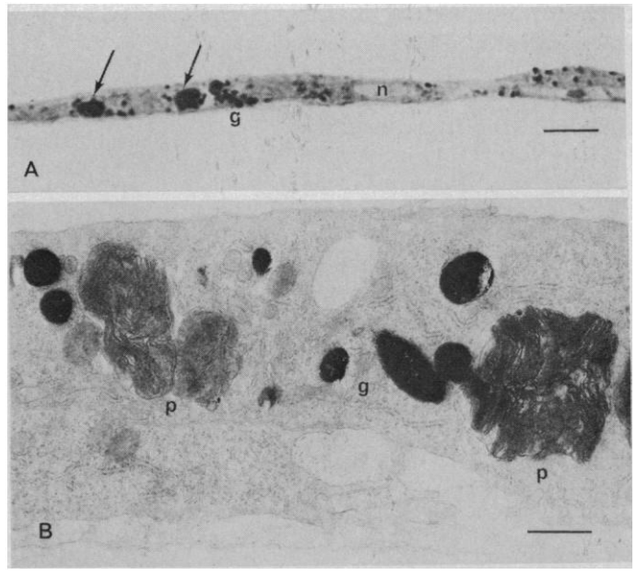

Fig. 3. (A) Light micrograph of monolayer PE cells incubated with ROS for 6 hours and washed four times with HBSS. The surface of the monolayer is free of adhering material. The arrows indicate particles that have been phagocytized by the cells. These particles are large in comparison with the melanin granules (*g*) and are darker than the flattened nuclei (*n*). The monolayer was fixed in 1 percent glutaraldehyde, post-fixed in 1 percent osmium tetroxide, dehydrated with graded ethanols, embedded in Epon, stained with toluidine blue, and sectioned at 1 μm. Scale bar, 5 μm. (B) Electron micrograph of monolayer PE cells incubated with ROS for 6 hours. The lamellar nature of the ingested particles (*p*) clearly identifies them as ROS fragments. Numerous pigment granules (*g*) are present within the cell. Scale bar, 1 μm.

where all of the ROS can be seen to be present as phagosomes within the PE cells.

A possibility that cannot be excluded by these experiments is that the PE cells are actually phagocytizing only shed packets of ROS disks, which is known to occur in vivo. I tried to minimize this possibility by preparing the ROS after exposing the rats to light for only 1/2 hour. Previous investigations with rats (*2*) and frogs (*3*) suggested that maximum shedding of ROS tips occurs after 1 to 2 hours of light exposure. The assumption made in choosing 30 minutes of light exposure was that activation of the ROS plasma membrane has occurred in this time, but only minimal shedding has taken place. Furthermore, the few tips that have been shed tend to remain with the eyecup, rather than with the excised retina. If shedding has not already occurred under the conditions of illumination used in these studies, it is possible that the activation affects the entire outer segment plasma membrane of light-exposed photoreceptors, any part of which may then be phagocytized. However, it is not possible to deduce from the results reported here which of these two alternatives is correct.

The nature of the activation required to make the ROS palatable to the PE is unknown. The chemical composition or the molecular architecture of the plasma membrane must be modified sufficiently for the PE to recognize it as foreign. Current theories suggest that it is the oligosaccharide side chains of surface glycoproteins that are responsible for the recognition of one cell by another (*12*). O'Brien suggested that the addition of galactose and fucose to rhodopsin in the ROS plasma membrane could be the signal that results in the shedding or phagocytosis of ROS fragments (*13*).

Whatever the chemical signal is for shedding and phagocytosis, these processes are synchronized by light, but can also occur in the absence of light, according to a circadian rhythm (*2*). The PE cells in tissue culture retain their ability to distinguish between ROS isolated from animals exposed to light and those from dark-adapted animals—that is, activated and nonactivated ROS. This system will thus prove useful for studying the involvement of surface components of the ROS and the PE in the phagocytic process.

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11. This 20 percent decrease in the extent of phagocytosis is probably not entirely due to the inhibition of synthesis of ^{3}H-labeled proteins by puromycin. It is known (*14*) that phagocytosis results in loss of plasma membrane from the cell surface. Continual protein synthesis is required to replace this internalized plasma membrane. Since protein synthesis is inhibited in the presence of puromycin, new plasma membrane cannot be assembled and inserted into the existing plasma membrane. Thus the rate of phagocytosis will gradually decrease under these conditions. It is probable that these constraints result in the leveling off and decrease in phagocytosis normally seen after 4 hours of incubation. The extensive internalization of plasma membrane during phagocytosis may result in a refractory period during which synthesis and assembly of new plasma membrane occurs.
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L-Canaline Detoxification: A Seed Predator's Biochemical Mechanism

Abstract. *The seeds of the Neotropical legume,* Dioclea megacarpa, *the sole food source for developing larvae of the bruchid beetle,* Caryedes brasiliensis, *contain about 13 percent* L-*canavanine (dry weight). Canavanine detoxification and utilization produces* L-*canaline, a potent neurotoxic and insecticidal amino acid. This seed predator has developed a unique biochemical mechanism for degrading canaline by reductive deamination to form homoserine and ammonia. In this way, canaline is detoxified; canavanine's stored nitrogen is more fully utilized and its carbon skeleton is conserved.*

About 13 percent of the seed dry matter of the Neotropical legume *Dioclea megacarpa* consists of L-canavanine (*1*), a structural analog and potent antimetabolite of L-arginine. L-Canavanine undoubtedly contributes to the effective chemical barrier against predation established by this leguminous plant, because the bruchid beetle *Caryedes brasiliensis* is the only known consumer of the seed (*2*). This seed predator possesses an arginyl-transfer RNA (tRNA) synthetase that does not charge canavanine (*3*). Consequently, *C. brasiliensis* avoids a principal cause of canavanine toxicity, that is, the production of canavanine-

$$H_2N\text{-}C(=NH)\text{-}NH\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H \rightarrow$$
L-canavanine

$$H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + H_2N\text{-}C(=O)\text{-}NH_2 \quad (1)$$
L-canaline urea

$$H_2N\text{-}C(=O)\text{-}NH_2 \rightarrow CO_2 + 2NH_3 \quad (2)$$

$$H_2N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + NADH + H^+ \rightarrow$$
L-canaline

$$HO\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)CO_2H + NH_3 + NAD^+ \quad (3)$$
L-homoserine

Fig. 1. Metabolic reactions of the bruchid beetle *Caryedes brasiliensis* whereby L-canavanine and L-canaline are detoxified and utilized.

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