I, II, or III. Electrophoretically the anti *P. vulgaris* components corresponded to components V and VIII of the whole granule (Fig. 2) and were different from those that inhibited *S. aureus* and *S. faecalis*. The specific activity of the anti *P. vulgaris* components was low (200 IEP_{50} units/mg of protein).

The antibacterial activity of polymorphonuclear lysosomes is not vested in a single entity but is associated with a heterogeneous mixture of cationic components to which the microorganisms are differentially susceptible. By use of sucrose density gradient electrophoresis a measure of homogeneity was achieved for the various lysosomal protein fractions, whereby it was possible to attribute anti *S. faecalis* activity to components I and II, anti *S. aureus* activity to component III, and activity against *P. vulgaris* to components V and VIII of the lysosomal cationic proteins. It is too early to define the nature and the extent of this specificity. The components are not exclusively inhibitory to the strains of microorganisms used in the assays herein described. Other strains of the same species show similar sensitivity patterns. However, in the case of *Escherichia coli* strains differences in the susceptibility to the lysosomal components do exist. In the past, the poorly defined antibacterial activity of polymorphonuclear leukocytes has frequently been specified in terms of Gram reactivity of microorganisms (*10*). In our study there was no indication that the specificity is related to the Gram reaction of the bacteria.

In view of the highly cationic nature of the lysosomal components, a general electrostatic interaction has been previously suggested as the mechanism of antibacterial activity (*1*). However, the demonstration of an element of specificity in the antibacterial action of these lysosomal components shows the need to reinvestigate the mechanisms of antibacterial action of these substances and to assess their place in relation to the known humoral and cellular substances that play a part in the defense mechanisms of the host.

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References and Notes

1. H. I. Zeya and J. K. Spitznagel, *Science* **142**, 1085 (1963); *J. Bacteriol.* **91**, 750, 755 (1966).
2. A. Janoff and B. W. Zweifach, *Science* **144**, 145 (1964); *J. Exptl. Med.* **120**, 747 (1964); E. S. Golub and J. K. Spitznagel, *J. Immunol.* **95**, 1061 (1965).
3. J. C. Herion, J. K. Spitznagel, R. I. Walker, H. I. Zeya, *Am. J. Physiol.* **211**, 693 (1966).
4. J. K. Spitznagel and H. I. Zeya, *Trans. Assoc. Am. Physicians* **77**, 126 (1964).
5. Z. A. Cohn and J. G. Hirsch, *J. Exptl. Med.* **112**, 983 (1960).
6. H. I. Zeya and J. K. Spitznagel, *Proc. Soc. Exptl. Biol. Med.* **121**, 250 (1966).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1952).
8. D. Shugar, *Biochim. Biophys. Acta* **8**, 302 (1952).
9. L. H. Muschel and H. P. Treffers, *J. Immunol.* **70**, 1 (1956).
10. R. C. Skarnes and D. W. Watson, *Bacteriol. Rev.* **21**, 273 (1957).
11. Supported by USPHS grant AE-02430 and a grant from the Leukemia Society. We thank Dr. J. L. Irvin (University of North Carolina) for invaluable suggestions and help. We gratefully acknowledge the expert technical help of Marjorie Huck Cooney.

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Light-Stimulated Electrical Responses from Skin

Abstract. *When skin is exposed to an intense flash of light, an early electrical response can be detected from its surface. The signals that occur during the first milliseconds after the flash are similar to electrical signals recently observed in the eye from the cell layers containing melanin. Possibly the melanin in skin augments, but does not directly generate, this early electrical response. In addition, a late response, which arises hundreds of milliseconds after the flash, also occurs in skin. Unlike the early response, the late response is sensitive only to violet and shorter wavelengths of light and hence is probably mediated by a pigment other than melanin.*

When the retina in a vertebrate eye is stimulated by an intense light flash, fast electrical signals (the early receptor potentials) are generated from the visual pigment in the photoreceptors (*1, 2*). Intense light flashes also evoke similar signals from the cell layers immediately behind the retina, the pigment epithelium-choroid complex (*3*), but in this case the signals appear to be mediated primarily by melanin (*4*). This led us to investigate other melanin-containing tissues, and we report here the discovery of two light-stimulated electrical responses from skin. Quite unexpectedly, we have found such signals not only in pigmented skin, but also in albino skin. The early skin response is larger in melanin-containing skin than in albino skin, but is otherwise similar. Hence, the early electrical response in skin appears to be augmented by melanin, but not solely dependent upon it. We have also observed a late response which begins to appear hundreds of milliseconds after the flash, reaching its maximal amplitude several seconds later. This late response appears to be mediated by pigments that absorb light only at the violet end of the spectrum (*5*).

Intense light flashes from a xenon gas–discharge tube (200 joules input energy, 120 μsec flash duration) were focused onto excised patches of skin bathed in Ringer solution. Silver–silver chloride electrodes on opposite sides of the skin picked up the electrical signals, which were amplified and recorded photographically from an oscilloscope. Electrical artifacts from the flash lamp were eliminated by isolating the preparation in a copper box, while photovoltaic artifacts resulting from the flash were eliminated by completely shielding the electrodes from light. A microscope condensing lens (numerical aperture 1.3) mounted in one side of the box was used to focus the stimulus light on the skin, and the stimulus energy and wavelength were controlled by filters.

Figure 1 shows oscilloscope tracings of the early and the late electrical responses in skin from the frog *Rana pipiens*. The early response has been observed in every animal investigated (frog, guinea pig, rat, axolotl, and black mollie) (*6*). In every case, the first peak of the early response lasts for about 1 msec, and the electrode on the outside surface of the skin becomes positive. This peak is often followed by more slowly developing peaks of differing polarities. The late response, such as shown to the right in Fig. 1, has been observed only in frog skin. The late response lasts for several seconds (see time bases in Fig. 1) and sometimes has both positive and negative peaks.

The amplitudes of both the early and the late responses are strictly proportional to the energy of the stimulating flash. In some cases we have observed this linear relationship over a thousand-fold increase in the amplitude. No falloff or saturation of the response amplitudes occurs even for the maximum available flash energy (approximately 0.1 joule/cm^2 incident

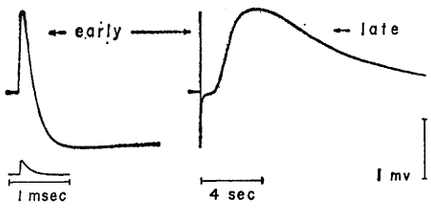

Fig. 1. Light-stimulated electrical responses of frog skin. The early and late responses are shown on different time bases. The light flash was incident on the outside surface of the skin sample, and signals of positive polarity with respect to this surface are shown as upward deflections. The skin sample is from a black spot on the dorsal surface of *Rana pipiens*. Time course of the flash is shown by a photodiode trace. Approximate flash energy incident on skin: 0.05 joule/cm². The flash was white with an effective bandwidth of 350 to 680 nm. Amplifier bandwidth: d-c to 30 khertz. Temperature: 18°C.

on the skin). The response amplitudes are not significantly affected by a long series of maximum early flashes nor by the simultaneous presence of steady intense light. Thus these responses appear to be photostable and are not damaged by the unusually intense light stimuli used here. When the skin is inverted between the fixed electrodes, the polarities of both the early and the late responses are correspondingly inverted. This implies that the orientations of the signal-generating structures are fixed in the skin, producing the same polarity of response regardless of the direction of the incident light.

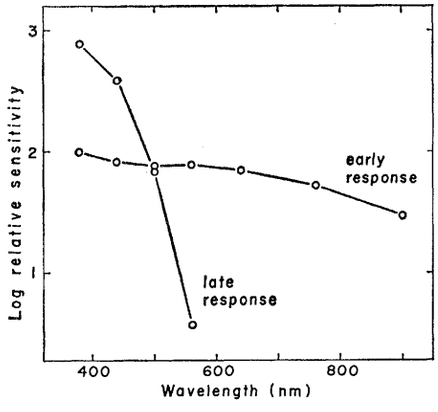

Fig. 2. Action spectra of early and late responses from frog skin. The sensitivity is the reciprocal of the incident flash energy (relative joules) needed to produce a response of a given amplitude. Well-blocked interference filters were used to limit spectral bandwidths to about ± 10 nm for most points, but the points at the ends of each action spectrum were obtained with wide-band glass filters (approximately ± 50 nm). Skin sample: black spot from the dorsal skin of *Rana pipiens*. Flash duration: 120 μsec. Temperature: 18°C.

Figure 2 shows the action spectra of both the early and the late responses from frog skin. In every animal investigated, the action spectrum for the early response is nearly flat over the entire observable wavelength range. This type of action spectrum would be expected from a black pigment such as the melanin present in the melanosomes. On the other hand, as shown in Fig. 2, the late response in frog skin is far more sensitive to violet light than to other visible wavelengths. Though we have observed some small differences between action spectra of the signals of the late response in frog skin, in every case their action spectra slope rapidly upwards to the violet end of the spectrum. This almost certainly rules out melanin as the photopigment of the late response, because the concentration of melanin in melanosomes is so great that all visible wavelengths are absorbed about equally. There are several other pigments in skin that might be responsible, however, for example, pteridines, flavins, and carotenoids (*7*), but further work will be needed to identify the photopigment.

Since the flat action spectrum of the early response strongly suggested that the photopigment was melanin, we also examined skin from albino animals of the same species (guinea pig, rat, and black mollie) (*8*). Surprisingly, we found that the early response could be observed in albino skin as well as in pigmented skin. Figure 3 shows the early response in pigmented (A) and albino (B) guinea pig skin. In trace C the skin sample was replaced by a piece of white saline-soaked cotton to help indicate that the skin's responses were not distorted by electrical or photovoltaic artifacts. In every pair of animals the signals from albino skin were invariably smaller than those in the corresponding pigmented skin (note differences in amplification in Fig. 3) but were otherwise fairly similar. Unfortunately, the signals from the albino skin were too small for us to determine their action spectra, but significantly, their polarity is the same as in pigmented skin and also is independent of the direction of the light.

That the early response is larger and has a flat action spectrum in skin containing melanin strongly suggests that melanin in some way mediates this response. However, since the early response is similar in both albino and pigmented skin, it may be that melanin simply augments a process that occurs in both types of skin. The evidence so far available is insufficient to determine the actual mechanisms that produce this early response, but there is one very likely mechanism worth mentioning, namely, heat. A simple calculation indicates that for an incident flash energy of 0.1 joule/cm², the temperature of a melanosome (melanin granule) in the skin would rise on the order of 10°C during the flash. If the early response is generated by the heating of a cellular structure, then melanin could well augment this effect, because it would absorb more of the flash energy.

The early response in skin is quite similar to the fast electrical signals generated in the pigment epithelium-choroid complex of the eye under comparable stimulus conditions (*3, 4*). Amplitudes, wave forms, action spectra, and photostability are much the same. In both cases the polarity is independent of the direction of the light. Moreover, in albino animals similar photostable signals occur in both the eye (*4*) and the skin. On the other hand, both these signals differ markedly from the early receptor potentials

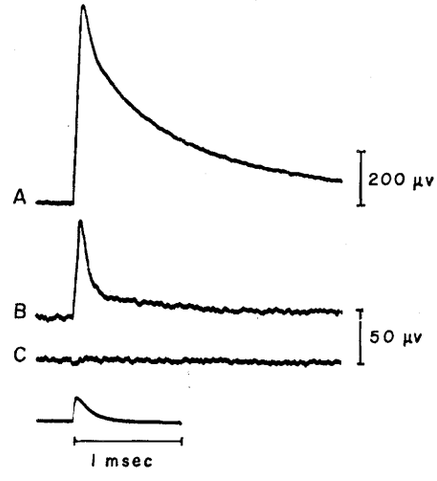

Fig. 3. Early responses from black pigmented guinea pig (A), albino guinea pig (B), and control trace with white cotton in place of skin (C). Skin samples were taken from the dorsal surface of the tip of the ear. Time course of the flash is shown by a photodiode trace. Approximate flash energy: 0.1 joule/cm². The white flash had an effective bandwidth of 350 to 1000 nm. Amplifier bandwidth: 10 to 10,000 hertz. Temperature: 37°C.

generated by the visual receptors. The amplitudes of the early receptor potentials can be hundreds to thousands of times larger for a given flash energy (and hence their threshold flash energies are correspondingly lower), their action spectra correspond to the appropriate visual pigments (*2*, *9*), they saturate at high flash energies (*2*), and they are completely photolabile (*2*, *4*). However, these differences do not rule out the possibility that a similar mechanism may generate all of these fast electrical signals.

We expect these new electrical responses in skin will help reveal the mechanisms that generate the fast electrical signals in the eye. Moreover, the late response in skin may prove helpful in investigating reactions of organisms to light which are mediated by non-optic receptors, such as the dermal light sense (*10*).

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References and Notes

1. K. T. Brown and M. Murakami, *Nature* **201**, 626 (1964); K. T. Brown, K. Watanabe, M. Murakami, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 457 (1965).
2. R. A. Cone, *Nature* **204**, 736 (1964); *Cold Spring Harbor Symp. Quant. Biol.* **30**, 483 (1965).
3. K. R. Brown, *Nature* **207**, 1249 (1965).
4. T. G. Ebrey and R. A. Cone, *Nature*, in press.
5. Some observations of the early response in frog skin are included in a report by G. B. Arden, H. Ikeda, and I. M. Siegel [*Vision Res.* **6**, 373 (1966)] which appeared after this report was accepted for publication. These authors observed the early response in the iris as well as in skin, and suggest melanin as the photopigment because they observed no responses in albino tissue. We find, instead, that similar early responses occur in both albino and pigmented skin; however, the albino's response is smaller and requires more intense stimulus flashes to be observed clearly.
6. *Rana pipiens, Cavia porcellus, Rattus rattus, Ambystoma mexicanus, Mollienesia lattipinnia.*
7. J. T. Bagnara and M. Obika, *Comp. Biochem. Physiol.* **15**, 33 (1965); A. Zettner, *Z. Biol.* **108**, 210 (1955).
8. All albino, pink-eyed animals used in these experiments were carefully examined for the presence of melanin, and no black pigment could be detected visually.
9. W. L. Pak, in preparation.
10. D. M. Steven, *Biol. Rev. Cam. Phil. Soc.* **38**, 204 (1963); G. H. Parker, *Amer. J. Physiol.* **10**, 28 (1903).
11. Supported in part by grants NB-06108 (R. A. Cone) and NB-03333 (T. H. Goldsmith) from the Institute of Neurological Diseases and Blindness, PHS. H. E. Becker is a predoctoral fellow of the U.S. Public Health Service and an R. C. Bates junior fellow of Jonathan Edwards College, Yale University. H. E. Becker thanks Dr. T. H. Goldsmith, in whose laboratory the initial experiments were performed, and Dr. M. J. Averner for their advice.

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Vesicular Stomatitis Virus Replication in Human Leukocyte Cultures: Enhancement by Phytohemagglutinin

Abstract. *The replication of vesicular stomatitis virus in human leukocyte cultures shows that virus yields can be enhanced 6- to 180-fold by treating the leukocyte cultures with phytohemagglutinin prior to virus inoculation. Data suggest that a substance is produced in phytohemagglutinin-treated leukocyte cultures which is capable, on transfer to fresh leukocytes, of inducing blast cell formation and enhancing virus replication.*

A variety of viruses have been demonstrated to replicate to low titer in human leukocyte cultures (*1*). As an approach to the investigation of the role of the human leukocyte in the host response to viral infections, we have developed in the laboratory a model system consisting of vesicular stomatitis virus (VSV) and human leukocyte cultures. Vesicular stomatitis virus produces a mild disease in animals and man (*2*). It was chosen because it replicates in a wide range of cell culture systems (*3*, *4*) and, though interferon-sensitive, does not itself induce interferon in infected cells (*4*). Phytohemagglutinin (PHA), an extract of the red kidney bean, *Phaseolus vulgaris*, was employed in these studies because it has been demonstrated to induce an interferon in human leukocytes (*5*) and thus might have a potential role in augmenting host resistance to the virus infection. We thought that PHA might also stimulate VSV replication because of its stimulatory effect on macromolecular synthesis in normal leukocytes (*6*) and its ability to induce replication of herpes simplex (*7*) and mumps (*8*) viruses in human leukocyte cultures.

Whole blood was obtained by venapuncture from healthy adults and placed in sterile tubes containing 0.2 ml of heparin per 12 ml of blood. Leukocytes were obtained by incubating the blood at 37°C for 1 to 2 hours, permitting sedimentation of cellular elements. The leukocyte-rich plasma supernatant was aspirated and centrifuged at 1000 rev/min for 10 minutes. The plasma was removed and the pellet, consisting of leukocytes and a small number of red cells, was resuspended in warm growth medium consisting of Eagle's minimum essential medium supplemented with tryptose phosphate broth (4 percent), unheated fetal calf serum (10 percent), sodium bicarbonate (1.75 g/liter), penicillin, and streptomycin. All cell culture vessels were gassed with 5 percent CO_2 in air and incubated at 37°C. Immediately after being placed in culture, the leukocytes were counted in a hemocytometer, employing an erythrosin B vital dye exclusion technique (*9*) which showed cell viability consistently greater than 99 percent. The procedures employed in obtaining leukocyte cultures did not significantly alter the cell differential count from that of the donor's whole blood. The leukocytes were then diluted in growth medium to a final concentration of approximately 1×10^6 cells per milliliter.

Lyophilized commercial phytohemagglutinin-P (Difco Co.) was dissolved in phosphate-buffered saline (10 mg/ml) at room temperature, sterilized by filtration, and stored at 4°C. Each fluid portion of PHA was discarded after 3 weeks because there was a gradual depletion of activity with longer storage. Fifty μg of PHA in a 0.05-ml volume was added to each milliliter of cultured leukocyte suspensions containing 1×10^6 cells per milliliter; control cultures received identical volumes of phosphate-buffered saline. The PHA-treated and control cultures were then incubated either stationary or in a roller drum at 37°C for intervals varying between 1 minute and 3 days before VSV inoculation.

Vesicular stomatitis virus, Indiana strain, was added to screw-cap tubes containing leukocyte cultures at a virus-to-cell multiplicity ratio of approximately 1 : 1. The culture tubes were placed in a roller drum at 37°C for 1 hour to allow cellular adsorption of virus. The leukocytes were then washed by one cycle of centrifugation, and the cell pellet and leukocytes adhering to the glass were resuspended by pipetting in growth medium at the original volume. Portions of each culture were quickly dispensed into screw-cap culture tubes, 1 ml per tube, gassed, and rolled at 37°C. Cultures collected at intervals thereafter were frozen and thawed rapidly three times to disrupt the cells, and the virus yield in each tube was assayed in monolayers of mouse L cells. Virus titers were calculated