cooled again to −196°C. The glow curves were made by heating with a water bath at 95°C.

The glow curve for the illumination at −196°C consists of two spikes, one at about 4 seconds and one at 53 seconds after starting the heating. The 4-second spike is not due to light absorbed by chlorophyll, since it is not seen after illumination with red light. Furthermore, the charging curve for this spike is quite different from that shown in Fig. 3. For illumination at temperatures higher than −32°C, the spike at 53 seconds becomes smaller and a new spike appears at about 43 seconds. At still higher temperatures, as shown by the curve for −6°C, the spike at 53 seconds has almost disappeared. The maximum light intensity now is at 43 seconds, with a new spike showing at about 30 seconds.

These results show that there is structure to the glow curves; more than one activation energy must be involved. It would be interesting to use the activation energy found from the glow curve to calculate the level of trap A and compare it with various redox potentials. Unfortunately, the fast glow curves are not yet good enough.

A very rough calculation of the activation energies can be made. We use the differential equation for a glow curve given by Randall and Wilkins (*9*), and the frequency factor of 2.5×10^9 found for dried chloroplasts (*8*). We know that 46 seconds after the start of the heating the copper plug is at 0°C, and, if we assume that the heat flow into the plug is proportional to the difference in temperature between the plug and the water at 95°C and that the heat capacity is constant, then we can calculate the temperature of the sample at any time. The activation energies of the three spikes shown in Fig. 7 are 0.57, 0.52, and 0.47 ev. These values are in rough agreement with the 0.63 ev that we expected.

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Antimicrobial Specificity of Leukocyte Lysosomal Cationic Proteins

Abstract. *Differences in antimicrobial specificities against* Staphylococcus aureus, Streptococcus faecalis *(group D), and* Proteus vulgaris *exist among the electrophoretically separable components of lysosomal cationic proteins of polymorphonuclear leukocytes.*

In previous reports we have described the isolation from polymorphonuclear leukocytes of a group of cationic proteins that possessed antimicrobial activity (*1*). The proteins were distinct from and more basic than lysozyme and ribonuclease. Fractions of such proteins have also been shown to account for a considerable part of the tissue-damaging activity (*2*) and pyrogenicity (*3*) of polymorphonuclear lysosomes.

The lysosomal cationic proteins exhibited electrophoretic heterogeneity that varied from species to species (*4*). This heterogeneity might be attributed to: (i) various degrees of aggregation of single protein species (in this case it could be expected that the biological activities of the electrophoretic components might be essentially similar); or (ii) true chemical heterogeneity corresponding to a mixture of different molecular species of proteins (in this case it would be expected that on resolution these protein species would differ substantially in biological activities).

By means of sucrose density gradient electrophoresis we have resolved the constituents of lysosomal protein to a degree of homogeneity that has made possible comparative studies of the antibacterial specificities of the resolvable components.

This report presents evidence that the components of the polymorphonuclear lysosomal cationic proteins thus resolved manifest a form of specificity in their antibacterial activity against *Staphylococcus aureus, Streptococcus faecalis* (group D), and *Proteus vulgaris*, suggesting the existence of a true chemical heterogeneity of the cationic components.

Polymorphonuclear leukocytes were obtained from aseptic peritoneal exudates of rabbits. The lysosomal fraction was prepared by homogenization and differential centrifugation of polymorphonuclear cells in 0.25*M* sucrose (*5*). The lysosomal fraction was extracted with 0.01*N* HCl, dialyzed against distilled water, and lyophilized. Fifty milligrams of lysosomal proteins were subjected to ascending electrophoresis in a sucrose density gradient column (LKB Instruments, Washington, D.C.) at 25°C for 10 hours (acetate buffer *p*H 4, ionic strength 0.01, 700 volts, 16 ma) according to the procedure described elsewhere (*6*). At the end of the run, 5-ml fractions were collected. Protein in the fractions was estimated by Lowry's method (*7*). Lysozyme was determined according to a standard method (*8*). For antibacterial assay the photometric method of Muschel (*9*) was modified. Cultures of *Streptococcus faecalis* (group D), *Staphylococcus aureus*, and *P. vulgaris* were grown overnight in 40 ml of trypticase soy broth. On the day of the test the bacteria were washed twice with distilled water and resuspended in 5 ml of distilled water. A portion (0.25 ml) of the suspension was transferred into 10 ml of trypticase soy broth and cells were grown in a shaker bath at 37°C for 4 hours. At the end of the period a 1 : 10 dilution of the culture was prepared in fresh trypticase soy broth and was further incubated for 1 hour. This culture was finally adjusted with appropriate dilutions in trypticase soy broth to give an optical density of 0.15 ± 0.01 at 650 mμ. The cells were then centrifuged to remove trypticase soy broth and resuspended in citrate-phosphate buffer (*p*H 5.6) to the original volume. The standardized bacterial suspension (0.3 ml) was added to 1.7 ml of citrate-phosphate buffer (*p*H 5.6) containing known quantities of sample from each lysosomal fraction. After the tubes were incubated for 1 hour for maximum inhibitory effect, 5 ml of trypticase soy broth was added to the incubation mixture and the tubes were returned to the water bath at 37°C. Incubation was carried out until the optical density in the control tubes reached the range of 0.4 to 0.45. A measure of the antibacterial activity was obtained by dividing the optical density of each tube by the optical density of the control and multiplying by 100.

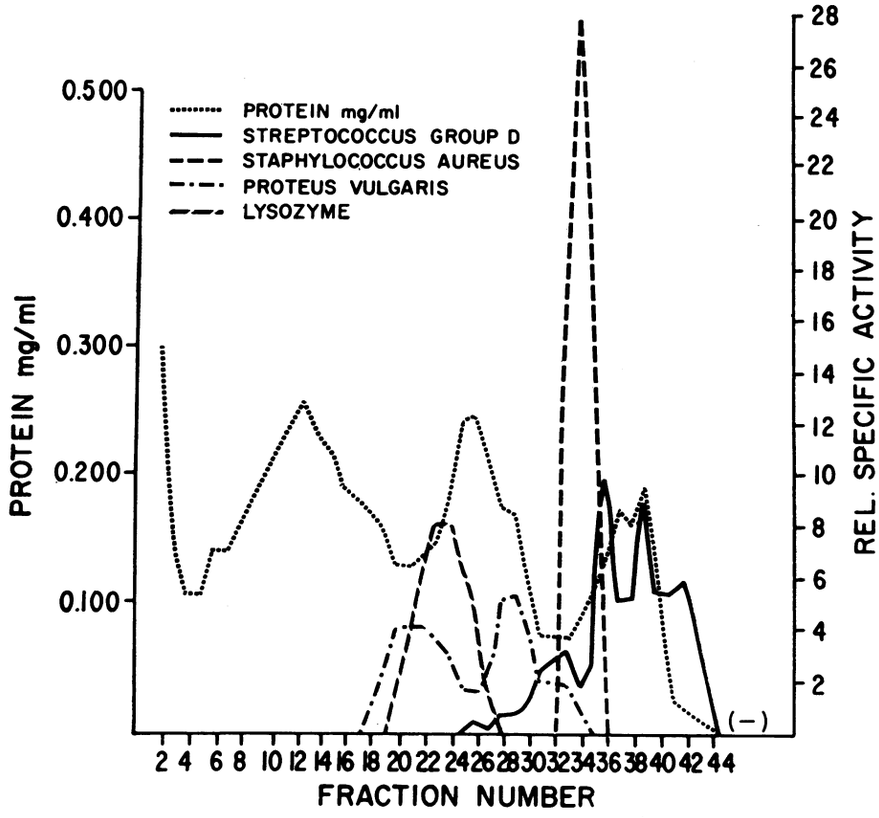

Fig. 1. Sucrose density gradient electrophoresis of acid extract of polymorphonuclear lysosomes. Cathode on the right. Distribution of the antibacterial activities of the effluent fractions measured against *Streptococcus faecalis* (group D), *Staphylococcus aureus*, and *Proteus vulgaris*.

The 50 percent inhibition end point (IEP_{50}) for each fraction was calculated by plotting graphically (on logarithmic probability paper) the percentage optical densities obtained in the presence of twofold serial dilutions of the fraction. The quantity of the fraction giving a 50-percent inhibitory point was taken as 1 IEP_{50} unit. Cellulose acetate paper electrophoresis (*1*) was used to estimate the homogeneity of proteins in individual fractions as well as for the correlation of the components with the antibacterial activities. In order to relate the composition of fractions to that of whole granules, the electrophoretic mobility of components from individual sugar density gradient electrophoresis fractions was compared with that of whole granules.

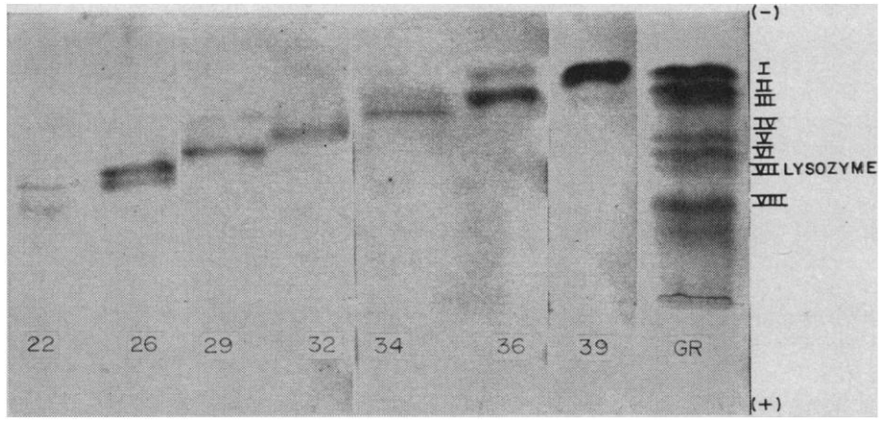

Fig. 2. Cellulose acetate electrophoresis of effluent fractions obtained from density gradient column (as described in Fig. 1). GR, whole granules; cathode at top. Anti *S. faecalis* activity in fraction Nos. 39, 36, and 32; anti *S. aureus* activity in fraction No. 34; anti *P. vulgaris* activity in fraction Nos. 29 and 22; lysozyme, fraction No. 26.

The results of sucrose density gradient electrophoresis are shown in Fig. 1. Under the described conditions the lysosomal components migrated toward the cathode. Analysis of the column effluents showed that the antibacterial activities against *S. faecalis*, *S. aureus*, and *P. vulgaris* were distributed in different regions of the column, and were (with one exception, see below) associated with components that migrated toward the cathode ahead of lysozyme. The activities for hydrolytic enzymes (ribonuclease, deoxyribonuclease, β glucuronidase, and acid phosphatase) were distributed in fractions between lysozyme and the origin (*6*). The antibacterial activity against *S. faecalis* was associated with the most cathodal components from the column, and two major peaks appeared in tubes No. 39 and No. 36. Electrophoretic analysis on paper, of samples from tube 39, showed the presence of a single component that corresponded to the most cathodal component (I) of the whole granule (Fig. 2). Paper electrophoresis of the sample from tube No. 36 revealed the emergence of a second, slower component (II) as the major constituent, with traces of component I, thus indicating that the components I and II either singly or in combination were active against *S. faecalis*. A specific activity of 1000 to 1500 IEP_{50} units per milligram of protein was obtained, showing the exceptional susceptibility of the organisms to these components.

Staphylococcus aureus was not inhibited by the antistreptococcal components. The activity against *S. aureus* was confined to a narrow zone that showed a peak in tube No. 34, a point where the activity against *S. faecalis* dropped sharply. Paper electrophoresis of a sample from tube No. 34 showed a third component (III) in this fraction. Though the fraction also contained traces of component II, the inability of component II in higher concentrations in the preceding tubes to inhibit *S. aureus* would suggest that component III is specific for *S. aureus*. The specific activity of component III for *S. aureus* (400 IEP_{50} units/mg of protein) was lower than that of components I and II for the *S. faecalis*.

Activity inhibitory for *P. vulgaris* appeared in two well-separated fractions in the region of, but different from, lysozyme (Fig. 1). *Proteus vulgaris* was not inhibited by components

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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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