tecting significant increases in antibody level in rubella virus infections. Individuals showing significant rises in antibody titer to myxovirus antigens or *Mycoplasma pneumoniae* did not show antibody rises with the rubella complement-fixing antigen.

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Antibacterial Action of PMN Lysosomal Cationic Proteins Resolved By Density Gradient Electrophoresis.* (30749)

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Granules (lysosomes) from polymorphonuclear leucocytes (PMN) contain not only a variety of hydrolytic enzymes, but also highly cationic proteins that have antibacterial activity as well as the ability to produce inflammation and tissue injury(1,2,3,4).

Previous studies using paper electrophoresis and ethanol precipitation have shown that highly cationic proteins can be separated from the lysosomal enzyme activities(1). The ability of the cationic proteins to kill bacteria and invoke tissue damage may be a consequence of their strong electropositivity whereby they can interact with and disorganize membrane structure and destroy permeability barriers. They may also combine with and inactivate anionic constituents like nucleic acid, bacterial mucopolysaccharides and enzyme systems of the cells.

Because of the varied biological activities and electrophoretic heterogeneity of the cationic protein fraction, a preparative scale resolution of granule proteins by sucrose density gradient electrophoresis has been developed which provides further elucidation of lysosome constituents.

Materials and methods. A lysosomal fraction of rabbit peritoneal inflammatory PMN was prepared as previously described(1). The lysosome suspension was extracted with 0.01 N HCl and dialyzed against acetate buffer pH 4.0, ionic strength 0.01.

Ascending electrophoresis was carried out in a sucrose gradient column with acetate buffer pH 4.0, ionic strength 0.01, at 700

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volts, 16 m amp, 22°C, using an LKB‡ apparatus(5). The right electrode vessel (anode) was filled with 50% sucrose (500 g reagent grade sucrose in buffer to total volume of 1 l) up to 2 cm into the center column. A concentration gradient was formed in the center column by gravity flow from the mixing vessels (LKB) with 50% sucrose in the bottom and buffer in the upper reservoir. Both electrode vessels were then filled with buffer. Eight to 10 mg of lysosomal protein in 5.0 ml buffer was brought to a density equal to that of the sucrose buffer at the capillary tip in the electrophoresis column, by addition of solid sucrose(5). This sample was introduced through the capillary tube into the center column by gravity flow, rinsing the tubing with sucrose of the same density. A sharp layer about 8 mm high was observed at the capillary tip. Electrophoresis was started immediately and continued for either 4 or 19 hours. At the conclusion of the run the column contents were collected by gravity flow through the capillary tubing into a fraction collector. Three ml or 5 ml fractions were collected at a flow rate of 1.5 ml per minute.

The enzymatic assays for lysozyme(6), ribonuclease(7), deoxyribonuclease(7), acid phosphatase(8) and β glucuronidase(9) were done according to the standard methods. Protein content of the fractions was estimated by ultraviolet absorption at 280 mμ. Antibacterial activity was determined as previously described(11). *Escherichia coli* labeled with ^{32}P were exposed to 0.1 ml of different fractions for 1 hour in citrate phosphate buffer pH 5.6. After centrifugation at 6000 *g* for 10 minutes, the released radioactivity in the supernatant fluid was measured in a scintillation counter. Zone electrophoresis on cellulose acetate paper was carried out according to a method described elsewhere(1).

Results. Resolution of antibacterial and enzymatic activities was achieved after 4 hours electrophoresis (Fig. 1A, 1B). Under the described conditions the components migrated towards the cathode. Most of the antibacterial activity moved well ahead of lysozyme and other enzymes, although a minor peak of antibacterial activity stayed at the

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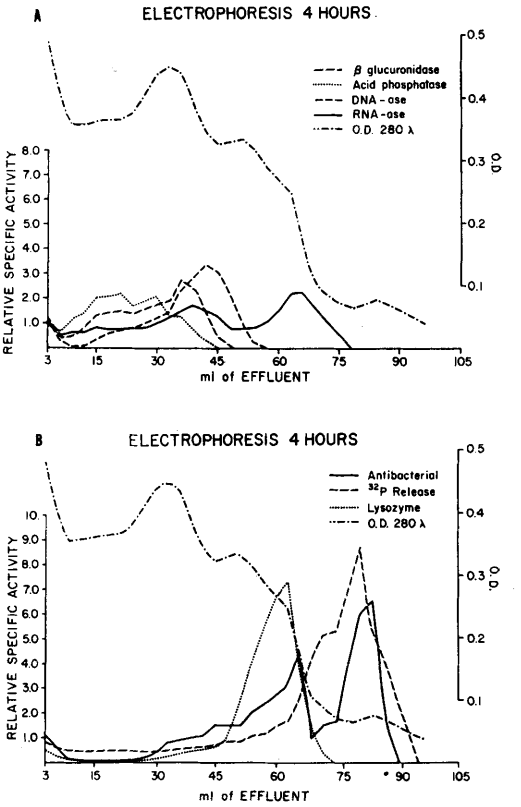

FIG. 1. Sucrose density gradient column electrophoresis of PMN lysosome acid-extract after 4 hr. Migration to cathode at right. (A) Distribution of enzyme activities; (B) Distribution of lysozyme and antibacterial activities.

level of lysozyme. The ^{32}P releasing activity was closely associated with the antibacterial activity. Lysozyme moved ahead of all the assayed enzymes except ribonuclease and was demonstrable as a single peak between 45-70 ml of effluent. The ribonuclease activity was discernible in most of the effluent fractions with two major peaks, one at the level of lysozyme and the other at the level of DNAase. DNAase and β glucuronidase were separated although considerable overlapping occurred. Acid phosphatase stayed nearer to the origin than the others.

Samples from the effluents at various levels of the column were compared with whole granules by electrophoresis on cellulose acetate paper (Fig. 2). An aliquot from the 84 ml effluent fraction that contained antibacterial and ^{32}P releasing activities resolved into bands corresponding to the most cationic

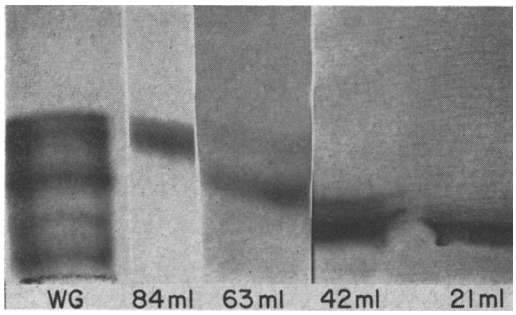

FIG. 2. Cellulose acetate electrophoresis of effluent fractions obtained after 4 hr electrophoresis in density gradient column (Fig. 1). WG, whole granules. Cathode at top.

components of intact granules. The pherogram showed that the fraction was essentially free of lysosomal enzymes. However, the presence of two bands in the fraction suggested that it was still heterogeneous. The electrophoretic pattern of the sample taken from the 63 ml effluent level coincided with components of intact granules that have been found to correspond electrophoretically with lysozyme and ribonuclease bands of guinea pig PMN granules(1). The pherogram also revealed that this fraction was contaminated with the fast moving antibacterial (82 ml effluent) cationic components. This could account for the minor peak of antibacterial activity observed in this fraction. The last two pherograms (42 ml and 21 ml effluent) showed that enzymes, DNAase, β glucuronidase, and acid phosphatase, were located in the slow components of the pherogram of the whole granule.

In another experiment, 10 mg of PMN granule proteins were subjected to 19 hours of electrophoresis. As represented in Fig. 3, the resolution of enzymatic activities was better than that achieved by 4 hours electrophoresis. The major peak of ribonuclease activity that coincided with lysozyme at 4 hours was clearly separated from it by 19 hours. DNAase moved further from β glucuronidase towards the cathode, whereas the relative position of the minor peaks of ribonuclease and DNAase remained unaltered. Contrary to the results at 4 hours, neither the antibacterial activity nor the ^{32}P releasing activity could be recovered from the fractions between 5-350 ml.

Discussion. Previous experiments with cellulose acetate electrophoresis of guinea pig granules have shown that the granule components migrate in pH 4 buffer towards the cathode with antibacterial cationic components moving ahead of lysosomal enzymes. The mobilities towards the cathode have seemed to be directly proportional to the isoelectric points of the known proteins(1,10). In the present work with density gradient electrophoresis a similar migration of granule components took place.

After 4 hours electrophoresis the antibacterial components had migrated towards the cathode fast enough to be adequately separated from lysosomal enzymes, although the fractions on cellulose acetate still showed some evidence of electrophoretic heterogeneity. Enzymatic activities in turn showed a considerable degree of trailing and overlapping. After a period of 19 hours, though the resolution of enzymes was much improved, the antibacterial activity could not be recovered. Because of rapid cathodal migration the antibacterial components moved into the cathode vessel of the apparatus. The presence in the 63 ml effluent of components elec-

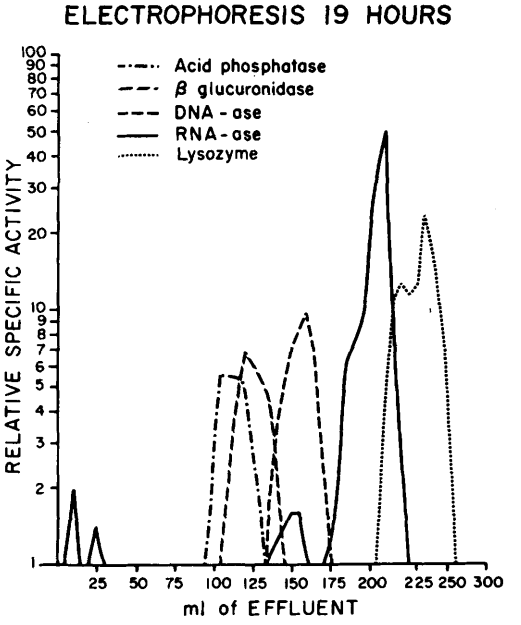

FIG. 3. Sucrose density gradient column electrophoresis of PMN lysosomes acid-extract after 19 hr. Migration to cathode at right.

trophoretically identical to those of 84 ml effluent could well account for the slight antibacterial activity in this fraction, in which lysozyme and ribonuclease may have exerted a synergistic effect. Nevertheless, in view of the heterogeneity of the lysosomal cationic proteins, the possibility of the existence of additional antibacterial components cannot be excluded.

The heterogeneity of ribonuclease from bovine pancreas and other sources is amply documented(12). The isoelectric points of some of these ribonucleases have been found to vary between 3 to 9.5(13,14). In our experiments the appearance of ribonuclease activity at various levels of the column indicated that the rabbit lysosomal RNAase is also heterogeneous.

The close proximity of ^{32}P releasing activity with antibacterial activity shows that the two activities may be identical. The concomitant disappearance of the two activities during a 19-hour electrophoretic run is another indication of their possible identity. It is significant that enzymes *per se* were incapable of invoking release of ^{32}P from bacterial cells, which would suggest that membrane damaging activity of cationic proteins is prerequisite for the *in vivo* degradation of phagocytized bacterial cells by lysosomal enzymes.

Summary. The antibacterial and enzymatic cationic proteins of acid extracts of PMN lysosomes were resolved on a preparative scale by density gradient electrophoresis. The ^{32}P releasing activity was closely associated with antibacterial action. Lysosomal ribonuclease was electrophoretically heterogeneous.

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Metabolism of Isomers of 3-Hydroxykynurenine-C^{14} to Quinolinic Acid, Niacin Metabolites and Carbon Dioxide.* (30750)

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Hydroxykynurenine, first identified in biological sources by Butenandt *et al*(1), has since been found in a variety of sources(2,3). The enzymatic synthesis in mammals of this key intermediate in the pathway from tryptophan to niacin was first shown by De Castro *et al*(4) to occur in mitochondria of liver and kidney and to have a requirement of NADPH and O_2. The conversion of hydroxykynurenine to pyridine nucleotides was implied by its niacin-replacing activity in rats (5) and Neurospora(6).

The present experiments were done to study the effectiveness of keto-labeled hydroxykynurenine as a precursor of respira-

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