present in the cell membrane since such particles appear to be artifacts of preparation (*2*, *4*).

Wild-type cells of *R. rubrum* (*5*) were grown anaerobically in 2.5-liter flasks illuminated by tungsten lamps (75 to 95 lu/m^2 at 30° ± 2°C). Flasks were filled to the neck with the G3X medium of Kohlmiller and Gest (*6*), the surface of which was overlayered with sterile light paraffin. Cells were harvested after 3 to 4 days of growth, centrifuged at 0°C, and resuspended in 0.01*M*, *p*H 6.8 phosphate buffer containing 10 percent sucrose. The cells were then treated with lysozyme according to the method of Karunairatnam *et al.* (*7*) to obtain a spheroplast suspension free of walls. Microscopic examination showed practically complete conversion to wall-free spheroplasts. After osmotic lysing of spheroplasts with water the lysed material was centrifuged and washed once with water. The sedimented cell membranes or ghosts were then lyophilized until they were dry. Lyophilized materials (cells or cell membranes) were extracted with *n*-heptane, the extract was evaporated to dryness, and the residue taken up in ethanol and run on a thin-layer silica gel G chromatogram in a benzene : iso-octane : acetone (25 : 25 : 1.5 by volume) system (*8*). Coenzyme Q^{10} was estimated spectrophotometrically (*9*) by the decline in extinction with sodium borohydride at 275 mμ.

Separate analyses were made on three different batches of cells and the results are presented in Table 1. Coenzyme Q was quantitatively recovered in the cell membranes. Bishop and King (*10*) have shown that in the nonphotosynthetic bacteria *Escherichia coli* and *Micrococcus lysodeikticus*, coenzyme Q and vitamin K are localized in the cell membrane. Since coenzyme Q participates in photosynthesis (*11*) our data support the view that the photosynthetic apparatus of *R. rubrum* is built into a continuous membrane which also serves as the cytoplasmic membrane (*2*, *4*).

Table 1. Analyses of cells and ghosts of *Rhodospirillum rubrum*. The results for the whole cells are expressed as milligrams of coenzyme Q per gram of dry weight, and for the ghosts as milligrams of coenzyme Q per gram dry weight of ghosts (A), or per gram dry weight of whole cells (B).

Expt. No.	Whole cells	Ghosts	
		A	B
1	1.54	2.75	1.94
2	1.98	2.32	1.64
3	1.78	2.32	1.64
Average	1.77	2.46	1.74

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Adhesion and Emigration of Leukocytes Produced by Cationic Proteins of Lysosomes

Abstract. *A cationic protein fraction isolated from rabbit polymorphonuclear leukocyte lysosomes causes adhesion and emigration of leukocytes and petechial hemorrhage in the microcirculation of the rat and rabbit mesentery.*

In studies of lysosomes de Duve (*1*) suggested that, under pathological conditions, these subcellular particles may liberate hydrolytic enzymes capable of injuring living cells and their surrounding tissue components. In support of this, Thomas and Weissmann have recently reported the production of inflammation and necrosis in several tissues after local injection of intact lysosomal granules combined with systemic administration of bacterial endotoxin (*2*), or the local injection of dissolved contents of frozen thawed lysosomes alone (*3*). In addition, Golub and Spitznagel have recently published (*4*) observations on dermal lesions induced in rabbits by homologous polymorphonuclear leukocyte (PMN) lysosomes, and they suggest that such lysosomes are potent sources of tissue damage in the Arthus reaction.

One mechanism for the tissue injury attributed to these granules implicates the action of cathepsins and other of the acid hydrolases contained within the particles (such as phosphatases, nucleases, polysaccharidases, and lysozyme). A precedent for this assumption is the observed degradation of cartilage matrix by acid cathepsins released from lysosomes in vivo under certain experimental conditions (*5*). However, in addition to the enzymes known to be present within lysosomes, a basic protein fraction possessing marked bactericidal and agglutinating properties has been extracted from PMN lysosomes of the guinea pig by Zeya and Spitznagel (*6*). Moreover, Frimmer and Hegner (*7*) recently reported that histone-like, basic polypeptides isolated from calf thymus nuclei had leukotactic and permeability-increasing effects upon the microcirculation of the rat mesentery. In view of these findings and because adhesion and emigration of leukocytes through the walls of capillaries and venules is a cardinal feature of the inflammatory process, we tested rabbit polymorphonuclear leukocyte lysosomes and especially the cationic proteins of these particles for their capacity to induce leukocyte sticking and emigration in homologous and heterologous tissues.

Rabbit peritoneal leukocytes were obtained in large numbers from exudates induced by administration of 0.1 percent glycogen. Total and differential white cell counts were made on each exudate, and the cell suspensions collected from all animals were then pooled. After washing in sucrose, the leukocytes (95 to 100 percent polymorphonuclear cells) were lysed in 0.34*M* sucrose by the technique of Cohn (*8*). The lysate was centrifuged at low speed (400*g* for 10 minutes), and the sediment, consisting largely of nuclei and unbroken cells, was discarded. Leukocyte granules were then collected by centrifuging at 8500g for 15 minutes. The particles were re-

suspended in buffered saline at *p*H 7.0 and then ruptured by repeated rapid freezing and thawing. The suspension of lysed granules was clarified by high-speed centrifugation (17,000*g* for 20 minutes) and the supernatant was dialyzed extensively in the cold against buffered saline at *p*H 7.0. A small sample of the dialyzed material (approximately 50 μg of protein) was then tested on the mesoappendix of the anesthetized rat according to techniques previously described (*9*). The preparation was maintained under constant microscopic observation (× 120) for 30 minutes. Within 2 to 3 minutes after the material had been applied, sticking of leukocytes became evident along the endothelial surface of capillaries (8 to 12 microns wide) as well as of the venules (25 to 40 microns wide). In the venules adhesion of white cells gradually increased in degree until a thick layer of cells imbedded in a clear, amorphous material was present over the entire inner surface of the vessels. At 10 minutes, emigration of leukocytes into perivascular tissue could be observed. In many instances, palisading of cells was so extensive that the blood flow was greatly reduced, resulting even in complete stasis. After 20 to 30 minutes, occasional petechial hemorrhages were observed, marked by extravasation of red cells through defects in the walls of capillaries and collecting venules. When intact leukocyte granules were applied to the surface of the mesoappendix, a similar but significantly milder and more slowly developing set of reactions was observed. On the other hand, the granule-free fraction (post-8500*g* supernatant) of the lysed polymorphonuclear leukocytes, after freezing, thawing and dialysis, caused no significant changes in the microcirculation when applied onto the rat mesentery. The foregoing observations were repeatedly confirmed in the course of 40 trials with 10 separate leukocyte preparations.

The leukocytes of rabbits repeatedly primed with glycogen might conceivably contain antibody to this polysaccharide and such antibody could cross-react with glycogen present in endothelial cells of rat microvessels and lead to inflammatory reactions falsely attributed to nonspecific lysosomal components. To test for this, experiments were carried out with granules of leukocytes collected from rabbits never before treated with glycogen. The lysates of these granules were also fully reactive in the rat mesentery preparation.

Table 1. Properties of ethanol-precipitated protein fractions of rabbit PMN lysosomes. The lysed granule supernatant was obtained from frozen-thawed leukocyte granules. The ethanol fractions were precipitated from 0.2*N* H_2SO_4 extracts of leukocyte granules by the concentrations of ethanol shown.

Protein		Enzymes					Physiologic Action*		
Recovery of total granule protein (%)	Concn. (mg/ml)	Acid-phosph. (μg)†	Beta-gluc. (μg)†	Acid-RNA-ase (O.D.)‡	Lysozyme (μg)§	Catalase	Leukocyte adhesion	Leukocyte emigration	Petechial hemorrhage
Lysed granule supernate									
25	4.4	2.9	2.5	0.065	2100	0	++	++	+
20 percent ethanol fraction (I)									
9	2.2	0	0	0	30	0	++++	++++	+
45 percent ethanol fraction (II)									
8	2.3	0.1	0.005	0.068	500	0	0	0	0

* Rat mesoappendix assay. † Acid-phosphatase and beta-glucuronidase activities expressed as micrograms of phenolphthalein released per 100 μg of protein per hour at 37°C. ‡ Acid ribonuclease activity expressed as increment in optical density measured at 2600 Å per 100 μg protein per hour at 37°C. § Lysozyme activity expressed as microgram equivalents of crystalline egg white lysozyme per milliliter of fraction.

To test for the possibility that the effect of lysed granules on the mesenteric vessels might represent a reaction to foreign protein, a similar set of tests was carried out on the mesenteries of anesthetized rabbits. The same degree of injury could be produced in mesenteric vessels of a rabbit with lysates of granules collected from the animal's own leukocytes.

Having demonstrated the presence of a component or components in PMN lysosomes capable of producing a sticking reaction in microvessels, similar experiments were then undertaken with a fraction of the granules composed entirely of the basic proteins described by Zeya and Spitznagel (*6*). The fraction was prepared according to the technique of Ui (*10*) as modified by Zeya and Spitznagel (*6*) by extraction of the granules with 0.2*N* H_2SO_4 and precipitation of proteins with ethanol. Two separate protein fractions were obtained. Fraction I was precipitated by 20 percent ethanol; it was partly insoluble above *p*H 4.0 (but could be completely dissolved at neutral *p*H in the presence of gelatin); and it contained essentially zero activity of the lysosomal enzymes for which it was assayed (including lysozyme). On the basis of these properties, this fraction in all probability corresponds to the antibacterial, polycationic protein described by Zeya and Spitznagel (*6*). This basic protein, after dialysis and titration to *p*H 7.0 in gelatin solution, produced all of the aforementioned injury reactions (leukocytic sticking, emigration, and stasis) in the rat mesoappendix at a rate and to a degree equal to that previously described for lysate from whole granules. The biologic activity of this material was such that 20 μg of protein from this fraction produced reactions equivalent to those induced by 50 μg of whole granule lysate protein. On the other hand, fraction II (precipitated by 45 percent ethanol) and containing appreciable quantities of lysozyme and ribonuclease was completely inactive in the mesoappendix assay. These tests were repeated with three separate preparations from the ethanol fractionation (Table 1).

The leukocyte-sticking reaction produced by ethanol fraction I also occurred in rats treated beforehand with an antihistamine drug (15 mg of Phenergan Hydrochloride per kilogram of body weight, administred intravenously 15 minutes before the test). Indeed, sticking and emigration of leukocytes occurred even more rapidly in animals treated with Phenergan, probably as a result of the reduced vasodilation and correspondingly slower rate of blood flow through the vessels.

Attempts to obtain quantitative yields of single, pure proteins from the 20 percent ethanol fraction have not yet proved successful. However, preliminary work has shown that component(s) of this fraction remaining in solution at *p*H 10.5 constitute the most active elements of the fraction in the rat mesoappendix test (as little as 4 μg producing a 2 to 3+ reaction). This material contains less than 3 percent contamination with nucleotides (ribonucleic

acid equivalents as determined by the orcinol reaction) and has an absorbance ratio of 1.40 at 2800 to 2600 Å (measured at a concentration of 0.5 mg/ml in 0.15*M* saline at *p*H 3.7).

Thus, the cationic protein fraction of rabbit PMN granules originally described by Zeya and Spitznagel (*6*) is capable, by itself, of producing an inflammatory reaction in rat and rabbit mesentery. This component of the granules is likely to be responsible for the adhesion and emigration of leukocytes produced by the complete granule lysate.

Besides sticking and emigration of leukocytes, another frequent event in the delayed-phase reaction to tissue insult (infection and thermal injury) is the participation of capillary vessels (in addition to venules) in the affected area. In view of the fact that this capillary phase of the reaction is not often observed after the introduction of the standard mediators of inflammation into tissues (*11*), the production of a capillary-phase reaction by cationic proteins extracted from leukocyte granules is of special significance. The presence of these substances in lysosomes (at least of leukocytes) provides further evidence of an important role of these cytoplasmic particles in tissue-injury reactions. The production, by rabbit PMN lysosomes, of leukocyte sticking and emigration in autologous tissue (donor mesentery) also supports the view that these particles take part in the pathophysiology of inflammation. Whether the sticking reaction produced by cationic protein involves an alteration of surface charges or a more complex mechanism is unknown.

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Phenotypic Repair of RNA-Bacteriophage Mutants by Streptomycin

Abstract. *A class of host-dependent mutants of RNA-phage f2 are dependent on streptomycin for growth in certain bacterial hosts. Maximum response to streptomycin occurred late in the growth cycle of phage, indicating phenotypic "repair" of a late phage function.*

Gorini and Kataja (*1*) have recently described a class of mutants of *Escherichia coli* which show a conditional dependence for streptomycin. One of the mutants, an arginine auxotroph, which possessed a defective structural gene for the enzyme ornithine transcarbamylase, synthesized functional enzyme when streptomycin (SM) was added to the growth medium; that is, the arginine auxotroph grew in a medium without arginine provided streptomycin was present. Because other amino-acid auxotrophs showed a similar response to streptomycin (*1*), Gorini and Kataja postulated a general streptomycin-activated suppression mechanism which might function to repair (correct) phenotypically mutations in a wide variety of cistrons.

Suppression of many different mutants by particular bacterial suppressor genes has been described by Benzer and Champe (*2*) for bacteriophage T4 rII mutants, by Garen and Siddiqi (*3*) for alkaline phosphatase mutants, by Campbell (*4*) for the class of bacteriophage lambda hd mutants, and by Epstein, *et al.*, (*5*) for the class of bacteriophage T4 amber mutants. Some of these suppressor-sensitive mutants have been shown to be phenotypically reverted by 5′-fluorouracil (*6*).

Using the Garen and Campbell strains, Zinder and Cooper (*7*) isolated a set of host-dependent mutants of the RNA-containing bacteriophage, f2. We are now reporting that one of the classes of these mutants is partially restored by streptomycin, and that the time during the phage's latent period in which the reagent was most effective can be determined. This serves to indicate the time when the gene represented by the mutation plays its most critical role.

Bacteriophage mutants of the class su-3 (*7*) are host-dependent mutants which produce plaques on strain K37 of *E. coli* (permissive for phosphatase mutants) but do not produce plaques on strain K19, (a derivative of C600, permissive for hd mutants of lambda bacteriophage) or on K38 (nonpermissive parent of K37). Our strain K19 was resistant to high concentrations of streptomycin. The genetic lesion in su-3 has not yet been completely defined; it is, however, a single-step mutation. Details of the characterization of su-3 are presented elsewhere. Infection of strain K38 by su-3 elicits no detectible response, implying an early block in virus synthesis. Infection of K19 with su-3 (multiple infection) results in a several-fold increment in the viral-induced RNA polymerase without significant production of phage particles, the lack implying a block in a late function. These two aspects of the su-3 mutation are not yet reconcilable. In-

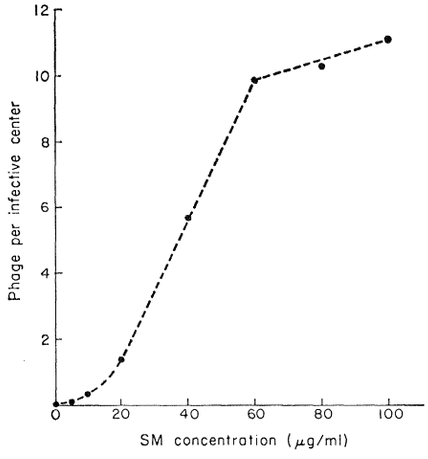

Fig. 1. Effect of streptomycin concentration on the growth of su-3 on K19. To K19 growing exponentially in tryptone broth (1.7×10^8 cells per milliliter) was added su-3 at 0.5 particles per bacterium. After 10 minutes of adsorption at 36°C, the cells were centrifuged and antiserum to bacteriophage f-2 was added to eliminate unadsorbed phage. The infected cells were diluted with tryptone broth (10^4 cells per milliliter) with different amounts of streptomycin (0 to 200 μg/ml) and incubated at 36°C for 50 minutes to allow development of phage. Chloroform and lysozyme were added to terminate growth, and the solution containing phage was plated on permissive and nonpermissive hosts.