Release of Enzyme from Human Leukocytes on Damage by Bacterial Derivatives.* (19883)

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Bacterial polysaccharides are well known for their pyrogenic effect. The need for knowledge concerning their effect on cellular metabolism is apparent. Detailed studies on the alteration in the metabolic processes of human leukocytes on exposure of the cells to these substances have been carried out in this laboratory by Martin, McKinney, Chaudhuri, and Green(1). Such studies are time-consuming and require large quantities of cells for their completion. A screening method has been reported elsewhere(2) whereby less detailed information concerning adverse metabolic effects can be obtained more rapidly and with relatively small quantities of leukocytes. The method depends on the release from injured cells of cellular contents; the released substance measured is an enzyme with the properties of lysozyme. The data obtained by this method have correlated well with those obtained by the more detailed methods of metabolic study. Although little information is derived by the screening method as to the nature of the adverse metabolic effects, the simplicity of the method makes possible a wider range of comparative observations. The present study compares the injurious effect of a variety of bacterial polysaccharides and other bacterial derivatives on human leukocytes. The relative effect of the substances varies from individual to individual donor. The possible significance of this variation is discussed.

Materials and methods. Subjects. Patients with no evidence of organic disease or with non-infectious diseases excluding primary metabolic or hematologic disorders. All subjects were only mildly ill, clinically. *Leukocyte suspension.* Sedimented leukocytes prepared by a method described previously(2) were resuspended in a buffered balanced physiologic salt solution plus 186 mg % dextrose to a final count of 5500-6500 leukocytes per cmm. *Bacterial derivatives.* PV and PS Piromen; concentrates of *E. coli* and *B. subtilis;* glucosamine (Baxter Laboratories, Inc.). Friedlander B polysaccharide (Dr. Harold Ginsberg). Pneumococcus type III SSS (Dr. O. T. Avery). Shwartzman-potent meningococcal filtrate (Dr. B. S. Black-Schaffer). Staphylococcus toxin (Lederle Laboratories). Tuberculin PPD (Dr. Florence Siebert). Appropriate dilutions were made in physiologic unbuffered saline for the experiments tabulated in I and in balanced salt solution plus glucose for II, to the final concentrations noted in the tables. *Lysozyme substrate.* Bacto Lysozyme Substrate (Difco Laboratories, Inc.) stock suspension was prepared and diluted for use by a method previously described. *Experimental procedure.* 0.7 ml of the diluent or of the test substance (in concentration 5 times the desired final concentration) was added to 2.8 ml of leukocyte suspension in 25 ml sterile siliconed flasks. The mixtures were incubated at 37°C in a Warburg shaker for 1 hour before use. In each instance, 1.0 ml of the test suspension was added to 1.5 ml of lysozyme substrate, and readings were obtained at zero time and at 20 minutes at 540 mμ in a Coleman Junior Spectrophotometer, as previously described (2), 100% transmission being set by use of a blank tube containing 1.0 ml of the test suspension and 1.5 ml of buffer. All tests were run in duplicate. Percent lysis of the substrate was calculated by the formula 100 minus (the optical density at 20 minutes divided by the optical density at 0, times 100). A correction was then made for the initial speed of the enzymatic reaction, since the significance of differences between control and test observations is considerably altered by

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TABLE I. Effect of Varying Concentrations of Bacterial Polysaccharides on Human Leucocytes as Indicated by Enzyme Release from the Cell.

Leucocytes from various human subjects, plus:	Index of injury of leucocytes by added bacterial polysaccharides* —Conc. of polysaccharide added/ml— 10 γ	5 γ	.5 γ	.05 γ
PS Piromen		155	111	71
PV "		108	76	66
PV "		136	98	95
E. coli concentrate		188	102	77
B. subtilis "		135	73	43
Friedlander B. polysaccharide		64	59	55
Pneumococcus III SSS	70	61	11	

* See text for derivation of index which relates each test reading to the control. The larger the index, the greater the injurious effect on the cells as compared to control leucocytes. An index of ± 24 or more is significantly altered (calculated using 2 S.D. of the method).

TABLE II. Comparison of Indices of Injury* to Human Leucocytes from 4 Subjects on Exposure of the Cells to Various Bacterial Polysaccharides.

Bacterial derivative added:		Human leucocytes from subject No. 1	No. 2	No. 3	No. 4
PS Piromen	4 γ/ml	137	107	99	69
PV "		205	172	143	123
E. coli concentrate		107	94	92	81
B. subtilis "		137	84	130	83
Friedlander B polysaccharide		77	56	53	60
Pneumococcus III SSS		83	19	83	67
Glucosamine		−11	4	3	−17
Staph. toxin	1:25	67	136	119	96
Meningococcal filtrate	1×10^{-5}	152	83	83	84
PPD	.02 mg/ml	77	11	69	36

* See footnote, Table I.

this factor (2); the following index was determined: (% lysis in the presence of leukocytes exposed to test substance) minus (% lysis in the presence of control leukocytes), divided by (% lysis in the presence of control leukocytes), times 100. The larger the index, the greater the lysis of substrate in the presence of test leukocytes as compared to control leukocytes. Control experiments on the method, using 2 S.D. in the calculations, indicate that an index of ±24 is significantly altered.

Results. The design and results of experiments are summarized in tabular form. Table I indicates the rough quantitative difference in injurious effect with varying concentrations of bacterial polysaccharide. The leukocytes used in each polysaccharide series were obtained from a different subject.

Table II tabulates comparative data for all the bacterial derivatives studied, each of the 4 series of 10 derivatives being run on a leukocyte suspension from a single subject.

Discussion. The injurious effect of various bacterial derivatives on human leukocytes is demonstrated by a method which depends upon the release of cellular contents when the cell is injured. The method is sensitive and may find useful application in the detection of pyrogen-contamination in solutions which are not in themselves injurious to leukocytes. Rough quantitation of the amount of pyrogen present in such solutions would be possible only if the pyrogen concentration were low, the effect of the test solution being compared with the effect of known pyrogen concentrations on the same leukocyte suspension. Determination of pyrogen levels in plasma or serum has not seemed feasible because of the presence in the test samples of significant amounts of the lysozyme-like enzyme measured.

When leukocytes from a single individual are exposed to the entire series of bacterial derivatives used in the present study, it is noted that the injurious effect of PV Piromen is relatively large and that the effects of

Friedlander B and of Pneumococcus III SSS are relatively small. Of further interest is the variability from individual to individual on comparison of the effect of the various bacterial derivatives on their leukocytes. It seems reasonable to postulate that this may reflect the previous experience of the donor individual with the various bacteria concerned. Detailed studies are in progress to test this hypothesis.

Summary. By a method which detects cell injury by measurement of a lysozyme-like enzyme released from the cell, the adverse effect of various bacterial derivatives on human leukocytes is demonstrated. Variation in degree of effect from individual to individual is evident.

1. Martin, S. P., McKinney, G. R., Chaudhuri, S. N., and Green, R., in mss.

2. Kerby, G. P., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 129.

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Erythrocyte Potassium Levels in Pernicious Anemia and Non-Tropical Sprue. (19884)

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Taylor and coworkers(1) have shown that compounds known to inhibit cholinesterase activity interfere with the maintenance of the *in vitro* potassium concentration gradient of erythrocytes in normal human subjects. In these systems, the amount of inhibitor needed to effect the gradient was more than enough to bring cholinesterase activity to zero. In pernicious anemia, this enzyme activity is depressed during relapse, elevated during the period of reticulocytosis, and normal during remission(2). Although red cell cholinesterase never reaches zero in pernicious anemia in relapse, the degree of variation seen in the course of this disease made it of interest to determine whether erythrocyte potassium levels were abnormal in relapse, and whether any significant change took place during the period of reticulocytosis.

Methods. This study included one patient with non-tropical sprue and 5 persons with typical Addisonian pernicious anemia in relapse. Each case in the latter group showed hyperchromic macrocytic anemia, histamine-fast achlorhydria, and megaloblastic bone marrow. Roentgen examinations of the gastrointestinal tract, urinalyses and blood chemistry determinations disclosed no abnormalities. All of the patients were treated with intramuscular injections of liver extract. The patient with non-tropical sprue showed hyperchromic macrocytic anemia, histamine-fast achlorhydria, megaloblastic bone marrow and flat oral glucose tolerance curve. There were frequent liquid stools containing abundant fatty acid crystals. Roentgen evidence of spasm, segmentation and "puddling" of the barium column in the small intestines were reported. He was treated with citrovorum factor,* intravenously. The results of this study will be presented elsewhere(3). In all of the patients hemoglobin, red blood cell, hematocrit, plasma and whole blood potassium determinations were made three times a week, leukocyte counts once a week, and reticulocyte estimations daily. Potassium analyses were made with a flame photometer with lithium as an internal standard. Erythrocyte potassium values per liter were calculated by subtracting plasma potassium from whole blood values using the hematocrit, and correcting by a factor of 0.95 for trapped plasma.

Results. All of the patients showed satis-

* Citrovorum factor supplied by Dr. J. M. Ruegsegger, Lederle Laboratories Division, American Cyanamid Co., Pearl River, N. Y.