Wattieux, R., Appelmans, F., Biochem. J., 1955, v60, 604.

5. Goldfischer, S., Arias, I. M., Essner, E., Novikoff, A. B., J. Exp. Med., 1962, v115, 467.

6. Popper, H., Schaffner, F., Rubin, E., Barka, T., Paronetto, F., Ann. N. Y. Acad. Sci., 1963, v104, 988.

Received May 15, 1967. P.S.E.B.M., 1967, v125.

Opsonic Property of Multiple Myeloma Serum.* (32339)

ROBERT P. MAY,† JACK A. BARNETT, JAMES W. SMITH,†
EUGENE P. FRENKEL, AND JAY P. SANFORD
Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas

Bacterial infections are a frequent and often fatal occurrence in patients with multiple myeloma(1). Despite a number of studies of host defense mechanisms, disagreement still exists as to specific defects. Three broad host factors have been considered. First, multiple myeloma may be associated with a suppression of antibody synthesis. Second, there may develop a diminution in the degree of delayed hypersensitivity. Third, the hyperglobulinemia may result in alterations in the phagocytic capabilities of leucocytes. Both Ingram and Penny and Galton utilizing iron granules or heat-inactivated yeast cells as particles have demonstrated inhibition of phagocytosis(2,3). This defect was related to the abnormal amounts of globulin present in the serum.

The following studies were directed toward assessment of opsonic activity as a possible additional serum defect. By employing a bacterial strain which is both ingested and killed by leucocytes in the presence of a heat-labile serum factor, the opsonic activity of serum from patients with multiple myeloma can be measured.

Materials and methods. Seven patients with multiple myeloma were selected from the hematology service at Parkland Memorial Hospital. The definitive diagnosis of multiple myeloma was based on the classical clinical features and morphological criteria of sheets of malignant-appearing plasma cells in the bone marrow.

Blood was drawn from the patients with multiple myeloma and from normal controls, the serum was separated and stored at −5°C and was used within 2 weeks to maintain its natural opsonins. Neither patients nor controls were on antibiotics at the time that the specimens were obtained.

The *in vitro* phagocytosis technique was basically that described by Hirsch and Strauss, substituting human leucocytes and a strain of *E. coli* 0-4 in their test procedure(4). Blood was obtained from normal antepartum patients and leucocytes were separated by a modification of the technique of Rogers(5). Siliconized glassware was used throughout the experiment. Twenty milliliters of blood were added to 10 ml of heparinized 3% dextran in saline. Blood was allowed to settle for 30 minutes at 37°C. The white cell rich supernatant was aspirated with a Pasteur pipette, transferred to a conical centrifuge tube and centrifuged at 1000 rpm for 7 minutes. The supernatant was discarded, and the white cell sediment washed twice in heparinized saline. The cells were then resuspended in Hanks' balanced salt solution containing barbital buffer (0.1 M, pH 8.6) and 0.1% gelatin to a concentration of approximately 2×10^7 cells per ml.

E. coli 0-4 were grown for 18 hours in trypticase soy broth (BBL), centrifuged at 8000 rpm for 10 minutes and washed twice in Hanks' solution. They were then resuspended in Hanks' solution to an optical density of 0.6 at 650 λ in a Bausch and Lomb spectronic

* These studies were supported in part by Research Grant HD-00851 from National Inst. of Child Health & Human Development, Nat. Inst. Health, USPHS.

† Studies performed during the tenure of a postdoctoral traineeship under Training Grant T1-AI-30 from Nat. Inst. of Allergy & Infect. Dis., Nat. Inst. Health, USPHS.

20 colorimeter. When this suspension was diluted 1:50 in Hanks' solution, 0.1 ml contained approximately 10^6 organisms.

Studies were performed in 15 × 125 mm siliconized test tubes with Parafilm-covered rubber stoppers. The basic phagocytic systems included: bacterial suspension 0.1 ml (10^6 organisms), test serum (concentrations studied included 10, 40 and 90%), normal leucocyte suspension 0.5 ml (10^7 leucocytes). In each study, both multiple myeloma and normal serum were used in the test and controls. Tubes were finally diluted to a total volume of 1.0 ml with Hanks' solution. Thus the total leucocyte:bacteria ratio was approximately 10:1, while the polymorphonuclear leucocyte: bacteria ratio was 5-8:1. In instances when 90% serum was used in the presence of leucocytes, a volume of leucocytes was centrifuged and resuspended in an equal volume of serum. 0.5 ml of this suspension was used. Additional controls included: 1) bacterial suspension in Hanks' solution, 2) bacterial suspension and unheated serum, 3) bacterial suspension and heat-inactivated serum, 4) bacterial suspension and leucocytes without serum, and 5) bacterial suspension, heat-inactivated serum and leucocytes. The pH of the 90% serum mixtures was noted to be 8.2 to 8.6, which inhibited the ability of leucocytes to kill *E. coli* 0-4, so the pH was adjusted into the 7.0 to 7.5 range by passing 10% CO_2:90% O_2 into the tightly capped tubes.

All tubes were then rotated at 18 rpm for 2 hours in a tissue culture Rollordrum‡ at 37°C. At the end of incubation, samples containing leucocytes were diluted in Hanks' solution in Pyrex tissue grinders, Tenbroeck type,§ and homogenized, following which 10-fold serial dilutions were done. Dillutions of the control set were made in saline. Aliquots of the 10-fold serial dilutions were pipetted into disposable Petri dishes into which MacConkey's agar was poured. After 18 hours' incubation at 37°C, discrete colonies of *E. coli* 0-4 were enumerated. A 2.0 log (99%) or greater difference in bacterial count between the containing leucocytes and the sets from which they were omitted was considered "optimum phagocytosis"(4).

‡ New Brunswick Scientific Co., New Brunswick, N. J.

§ W. H. Curtin Co., Dallas, Texas.

TABLE I. Serum Protein Characteristics of Patients with Multiple Myeloma.

	Total serum proteins, g %	Total globulin, g %	Presence of M spike	Type of M protein
M1	7.6	3.34	0	—
M2	8.3	6.95	+	γG
M3	8.7	4.80	+	γG
M4	7.5	3.75	0	—
M5	7.6	4.00	+	γA
M6	8.7	4.80	+	γG
M7	8.6	4.90	+	γG

Results. The serum protein studies in the patients demonstrated mild to moderate hyperglobulinemia in 6 of the 7 (Table I). An M spike on paper electrophoresis was present in 5 of the sera. Immunoelectrophoretic study of serum from these 5 patients revealed the abnormal protein to be gamma G in 4 patients and gamma A in 1 patient.

Characteristics of this phagocytic system are depicted in Chart 1. Bacterial multiplication of almost 1.0 $\log_{10}$ during the 2 hours of incubation occurred in Hanks' solution alone, with leucocytes alone in Hanks' solution or with 10% fresh serum without leucocytes. When 90% fresh serum without leucocytes was studied, less bacterial growth occurred; however, serum alone was not bactericidal for this strain of *E. coli*. When the system contained both leucocytes and serum at either a 10% or 90% concentration, a 2.0 log decrease (99%) in bacterial counts occurred. When heat-inactivated serum was substituted for the fresh serum, despite the presence of leucocytes, no bacterial killing was observed. Thus, this human leucocyte-*E. coli* 0-4 phagocytic system requires a heat-labile serum factor to promote opsonization and killing of the *E. coli*. A 10% concentration of normal serum was sufficient to supply this natural opsonin.

Representative studies on serum which contained a gamma G homogeneous spike (serum globulin 6.95 g%) obtained from a patient with multiple myeloma (M-2) demonstrated the same findings (Chart 2). It is apparent that the myeloma serum contained an adequate quantity of natural opsonin and that presence of the abnormal globulin did not in-

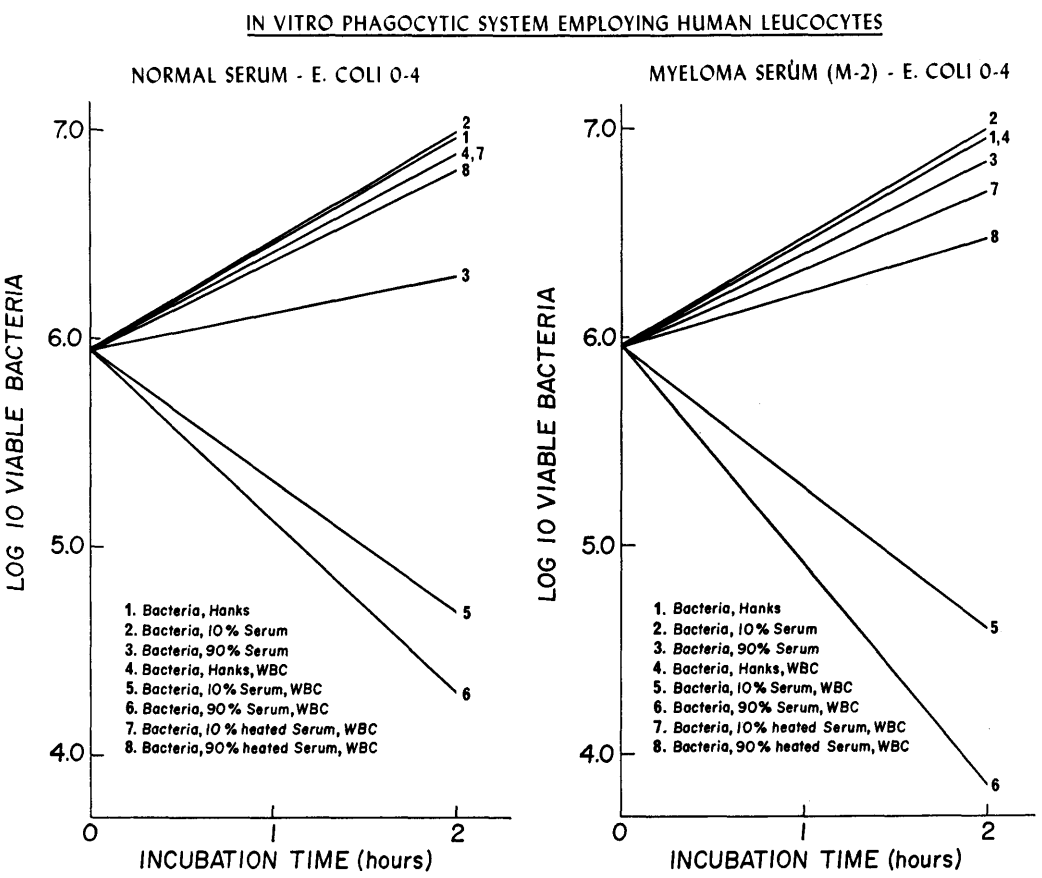

FIG. 1 (left): Characteristics of *in vitro* phagocytic system employing normal human serum.
FIG. 2. (right): Characteristics of *in vitro* phagocytic system employing myeloma serum.

terfere with phagocytosis and killing of the test organism.

Observations in the 7 patients revealed "optimum phagocytosis" could be achieved with serum obtained from each (Table II).

Discussion. Elucidation of the mechanism of the increased susceptibility of patients with multiple myeloma to bacterial infections is as yet incomplete. Several defects have been observed which might account for the inability of these patients to handle bacterial infections. The first may be the relative inability of the multiple myeloma patient to produce antibody. Zinneman and Hall immunized myeloma patients with pneumococcal polysaccharide, *Brucella abortus* and typhoid-paratyphoid

TABLE II. Opsonization of *E. coli* 0-4 for Human Leucocytes by Normal and Myeloma Serum.

Serum concentration	Normal	M1	M2	M3	M4	M5	M6	M7
0 (Hank's)	.2*	0 *	.1*	.2*	.1*	.2*	.1*	.3*
10% Fresh	2.5*	2.7*	2.4*	2.1*	3.3*	3.7*	2.8*	2.3*
40% Fresh	1.9*	1.0	2.7	2.3	2.0*	—	2.1*	2.8*
90% Fresh	2.0*	2.2*	3.0*	2.6	2.1*	3.3*	1.9*	2.3*
10% Heated	.2*	—	.4	.3	.1*	—	.1*	.2*
90% Heated	0 *	—	.7	0 *	0 *	—	.1*	.2*

* Average of 2 or more separate experiments employing different serum.
Log_{10} decrease in number of bacteria after 2 hr incubation at 37°C in systems containing leucocytes.

vaccines and found that the patients had poor responses to these antigens(1). They hypothesized that these patients represented a form of functional hypogammaglobulinemia in which the myeloma protein was non-functional and that the amount of abnormal gamma globulin was inversely related to the ability to make antibody. In a group of patients with multiple myeloma, Fahey *et al* compared the frequency of occurrence of infections with both the abnormal globulin levels and the antibody response to various antigens (typhoid H, diphtheria, mumps and influenza) (6). Although neither the type nor the quantity of abnormal globulin correlated with the incidence of infection, it was shown that those patients with fewer than 0.13 infections per month had greater ability to respond to antigens than did those with more than 0.13 infections per month. However, even in the former group, the number of responses was less than in controls. Cone and Uhr have shown that patients with multiple myeloma show a normal secondary antibody response to diphtheria toxoid and a reduced but nonetheless primary antibody response to bacteriophage(7).

The second defect may relate to impaired delayed hypersensitivity. Cone and Uhr demonstrated normal delayed hypersensitivity reactions to commonly encountered antigens, *e.g.*, oidiomycin, trichophyton, histoplasmin, purified protein derivative, coccidioidin, mumps, diphtheria toxoid and streptokinase-streptodornase. However, the response to primary development of delayed hypersensitivity upon exposure to dinitrofluorobenzene was diminished(7).

While a third possible defect occurred in patients with multiple myeloma, it did not appear specific but was the consequence of the hyperglobulinemic state interfering with phagocytosis by leucocytes. Ingram in a phagocytosis system utilizing iron granules in heparinized whole blood has shown that hypergammaglobulinemia, whether in serum from myeloma patients or produced by the addition of immune serum globulin to normal serum, inhibited the uptake of iron granules by leucocytes(2). In a phagocytic system using heat-inactivated yeast cells and human leucocytes, Penny and Galton have shown that plasma from patients with multiple myeloma and Waldenstrom's macroglobulinemia supported phagocytosis to a lesser degree than did normal plasma(3).

The phenomenon of phagocytosis may be examined in terms of several biological functions. The studies of Hirsch and Strauss have clearly defined organisms which require both antibody and a normal labile serum factor for phagocytosis, whereas other organisms require only the labile factor or no serum factors at all(4). The studies of Cohn and Morse demonstrated that phagocytosis might be considered as a two-stage phenomenon, the ingestion of particles by leucocytes and the subsequent intracellular killing of the ingested organisms, this latter phenomenon also being partially determined by serum factors(8). Our studies were designed to test two phases of phagocytosis which have not heretofore been studied in relation to the host defect in multiple myeloma: the effectiveness of labile serum factor (opsonin) from patients with multiple myeloma in enhancement of phagocytosis and the capability of the serum factor to enable subsequent intracellular killing of ingested organisms by leucocytes. The data indicate that patients with multiple myeloma have sufficient heat-labile opsonin to promote phagocytosis and intracellular killing and that the presence of myeloma protein does not interfere with this property.

Summary. The opsonic activity of serum from 7 patients with multiple myeloma was studied in a phagocytic system employing human leucocytes and a strain of *E. coli* 0-4. It was demonstrated that these patients have sufficient heat-labile opsonin to promote phagocytosis and intracellular killing of the ingested organisms. The presence of myeloma protein did not interfere with this property.

1. Zinneman, H. H., Hall, W. H., Ann. Int. Med., 1954, v41, 1152.

2. Ingram, M., Proc. Soc. Exp. Biol. & Med., 1960, v105, 404.

3. Penny, R., Galton, D. A. G., Clin. Res., 1966, v14, 360.

4. Hirsch, J. G., Strauss, B., J. Immunol., 1964, v92, 145.

5. Rogers, D. E., Melby, M. A., J. Exp. Med., 1960, v111, 533.

6. Fahey, J. L., Scoggins, R., Utz, J. P., Szved, C. F., Am. J. Med., 1963, v35, 698.
7. Cone, L., Uhr, J. W., J. Clin. Invest., 1964, v43, 2241.
8. Cohn, Z. A., Morse, S. I., J. Exp. Med., 1959, v110, 419.

Received May 17, 1967. P.S.E.B.M., 1967, v125.

Influence of Hydrocortisone Acetate on the Chemical Composition of Experimental Granulomas.* (32340)

A. L. ORONSKY AND M. R. NOCENTI
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

Although it is well established that certain adrenocortical hormones inhibit granuloma formation (1-6), the mechanism by which this inhibition occurs remains unknown. Many investigators have been concerned with the inhibition of collagen synthesis (7-10), but little attention has been given to the possibility that the observed effects on collagen and other substances are secondary to an alteration in some basic process of protein synthesis. Eichhorn and Sniffen(11) reported a reduced total DNA and protein in cortisol treated granulomas. Yet they found that the amount of labeled amino acids incorporated *in vitro* by the granulomatous cells was not altered and, therefore, concluded that protein biosynthesis was not significantly influenced by *in vivo* administration of cortisol. However, the results were variable, since significant decreases in total amino acid incorporation were obtained in a number of experiments(11). Other investigators have found that certain corticoids have either an inhibitory or stimulatory influence on protein metabolism in various tissues other than granulomas(12-15). In order to explore further the possibility of an effect of cortisol on protein metabolism in granulomatous tissue, studies on the temporal sequence of changes in collagen, DNA, RNA, or protein content and in the incorporation of labeled amino acids in steroid treated granulomas were undertaken.

Methods. Two granulomas were induced in each animal by bilateral implantation of weighed, sterilized cotton sponges (30 ± 1 mg) subcutaneously in the dorsum of male Sherman rats (175-200 g). Penicillin G (2,000 units) was injected intramuscularly. The treated sponges were impregnated with 300 μg of hydrocortisone acetate (Hydrocortone, Merck) and allowed to dry prior to implantation.

On days 1, 3, 5 or 7 after implantation, the granulomas were removed by carefully dissecting the surrounding connective tissue, blotted on filter paper and immediately weighed to the nearest 0.1 mg. Those implants used to determine water content were dried at 98-99°C to constant weight.

For the chemical studies, the granuloma from one side was weighed immediately and used for estimating the total hydroxyproline content by the method of Neuman and Logan (16). The second granuloma was weighed, and total protein and nucleic acids were separated by the Schneider technique(17). Total protein content was determined by the method of Lowry *et al*(18). The diphenylamine and orcinol reactions were used for DNA and RNA estimations, respectively(19,20).

For the study of labeled amino acid uptake, the cotton sponge implanted on one side was impregnated with 300 μg of hydrocortisone acetate while the other was untreated. C^{14}-L-proline (35 μc, uniformly labeled) was injected intraperitoneally at the time of sponge implantation. The implants were removed at 12 or 24 hours. After weighing and homogenization, the total protein was isolated by the method of Schneider(17). An aliquot was then counted in a Packard Tri-Carb Liquid

* Supported in part by USPHS Training Grant 5T01-GM-00257 and Merck, Sharp and Dohme Research Gift.