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Effect of Type A *Pasteurella multocida* Fractions on Bovine Polymorphonuclear Leukocyte Functions

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The effect of various *Pasteurella multocida* fractions on bovine polymorphonuclear leukocyte (PMN) functions was examined in vitro by using two encapsulated strains, P-2383 and P-1062 (both are Carter capsular type A and of bovine origin). The ability of PMNs to ingest *Staphylococcus aureus* and iodinate protein was significantly inhibited in the presence of live cells, heat-killed whole cells, or saline-extracted capsules but not in the presence of the decapsulated heat-killed cells. None of the fractions of the two strains inhibited nitroblue tetrazolium reduction by PMNs. The saline extract did not inhibit the binding of iodine to protein by a reaction involving xanthine, xanthine oxidase, and horseradish peroxidase. The PMN inhibitory factor was further characterized as a heat-stable capsular material of greater than 300,000 molecular weight.

Pasteurella multocida is a major etiological agent in bovine respiratory disease (3, 6), with capsular type A strains being most frequently isolated in North America. Some encapsulated strains of *P. multocida* are known to be highly pathogenic in experimental animals, and the presence of the mucoid capsule is important for virulence (3, 7). The capsule of type A strains is primarily composed of hyaluronic acid (4, 5), which serves as a framework for the attachment of polysaccharides, proteins, and lipids (3).

The importance of pasteurellae in bovine respiratory disease has led to the development and use of various bacterial products for immunization. The efficacy and safety of most of these products still remain in question (2, 13, 17, 19). There is evidence that the use of bacterins that are currently available may, in fact, be detrimental to the health of the animal (2, 19).

Recent studies on type A *P. multocida* capsular materials indicated that a KSCN extract (12, 20) and a saline extract (16, 25) were protective against experimental challenge in mice, chickens, and turkeys. The capsule of a type A *P. multocida* has also been demonstrated to inhibit the phagocytic activity of bovine neutrophils (18). Therefore, the capsule may contain not only a protective antigen but also a component which interferes with phagocytic cell function. It is not unusual for bacterial surface material to contain both a protective antigen and a virulence factor (8, 9, 14).

Phagocytosis of invading microorganisms by polymorphonuclear leukocytes (PMNs) can be one of the major cellular defense mechanisms in protecting animals from microbial infection. Maheswaran and Thies (18) reported that an encapsulated type A *P. multocida* (NA77) inhibited the phagocytic activity of neutrophils (PMNS). When they measured the uptake of [^{3}H]thymidine-labeled bacteria by PMNs, only 3.8% of the encapsulated organisms were ingested. When the encapsulated bacteria were treated with bovine testicular hyaluronidase, however, 90% of the decapsulated organisms were ingested. They concluded that the factor which inhibited the phagocytic activity of PMNs was probably hyaluronic acid, which is a major component of the capsule of type A *P. multocida*.

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The purpose of the present experiment was to further characterize an inhibitory factor present in type A *P. multocida* capsule and determine its effects on specific aspects of PMN function.

MATERIALS AND METHODS

Organisms. Two strains of *P. multocida* were used throughout this study (P-2383 and P-1062). Strain P-2383 was isolated from a case of bovine pneumonia presented to the Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa. This isolate was a typical *P. multocida* strain; subsequent typing confirmed it to be Carter capsular type A. Strain P-1062, also a type A strain of bovine origin, is a challenge strain (IRP-198, National Veterinary Services Laboratory, U.S. Department of Agriculture, Ames, Iowa). Each strain was inoculated into the yolk sac of 6-day-old embryonated chicken eggs. After incubation at 37°C for 18 h, the yolk material was aseptically removed and frozen in aliquots at −70°C. These aliquots were used as inoculum for preparation of bacterial fractions used in this study.

Bacterial fractions. The preparation of bacterial fractions is illustrated in Fig. 1. Infected yolk material was thawed, inoculated on a 5% bovine blood agar plate and incubated at 37°C for 24 h. One colony was transferred to 5 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) containing 0.5% yeast extract (Difco) and 5% sterile bovine serum (BHISY) and incubated at 37°C for 4 h. This culture was then added to 100 ml of BHISY and incubated an additional 4 h. Roux bottles containing dextrose-starch agar (Difco) were inoculated with 2.0 ml of BHISY culture and incubated at 37°C for 36 h. Cells were harvested by washing the agar surface with sterile phosphate-buffered saline solution (PBS, pH. 7.2), and cell concentration was adjusted so that a 10-fold dilution had an optical density (OD) of 0.4 at 540 nm (approximately 10^{10} cells per ml). The cells were washed three times in PBS, and an aliquot was saved for the live-cell fraction (LCF). The remaining cells were washed one more time in PBS. Cells packed by centrifugation were suspended to the original volume in 2.5% (wt/vol) sodium chloride solution and placed in a water bath at 56°C for 1 h. After this saline extraction, an aliquot was saved for the preparation of a heat-killed whole-cell fraction (KCF). The

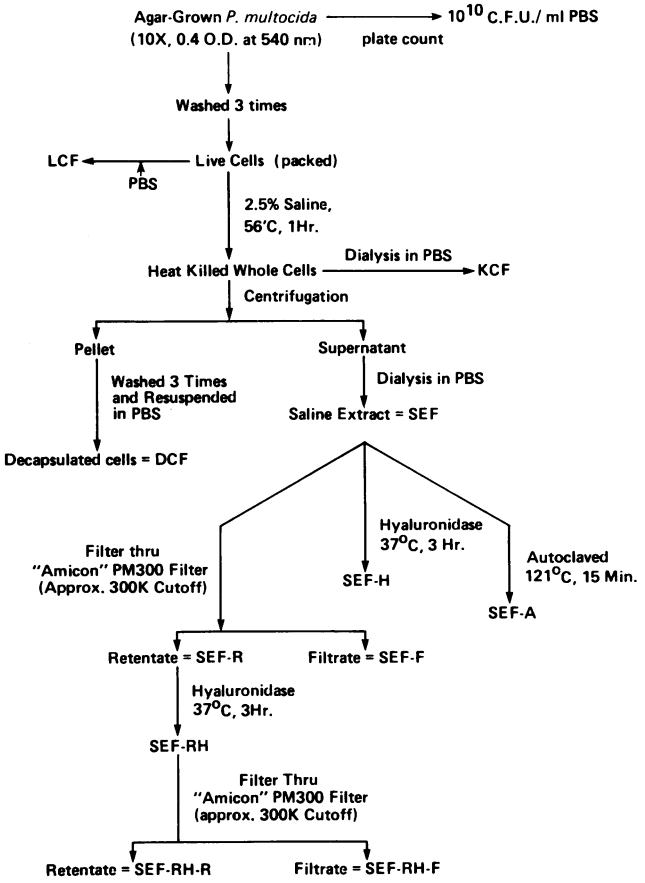

FIG. 1. Preparation of bacterial fractions.

remainder was centrifuged at 17,300 × *g* for 20 min at 4°C. The supernatant was saved, and the pelleted cells were washed three times and suspended with an equal volume of sterile PBS. This preparation was designated the decapsulated heat-killed cell fraction (DCF). The KCF and supernatant fraction were dialyzed against PBS for 3 days to remove excess salt and used as the KCF and saline extract fraction (SEF) (1.06 mg [dry weight]/ml for strain P-2383 and 0.91 mg/ml for strain P-1062). Aliquots of SEF were treated in various ways including: (i) treatment with bovine testicular hyaluronidase (Sigma Chemical Co., St. Louis, Mo.; 1 mg of hyaluronidase per mg [dry weight] of SEF) by incubating in a water bath at 37°C for 3 h (SEF-H); (ii) autoclaving at 121°C for 15 min (SEF-A); and (iii) filtration with a 300,000-molecular-weight cutoff filter (Amicon Corp., Lexington, Mass.). The filtrate was designated SEF-F (0.20 mg [dry weight]/ml for strain P-2383 and 0.25 mg/ml for strain P-1062) and utilized directly. The retentate was washed three times on the filtration membrane with PBS and designated as SEF-R (0.94 mg [dry weight]/ml for strain P-2383 and 0.80 mg/ml for strain P-1062). Control hyaluronic acid (human umbilical cord) (Sigma) and hyaluronidase (Sigma) at a concentration of 1 mg/ml in PBS were prepared. The SEF-R of strain P-2383 was treated with bovine testicular hyaluronidase (1 mg of hyaluronidase per mg [dry weight] of SEF-R of strain P-2383) (Sigma) and then filtered with a 300,000-molecular-weight cutoff filter (Amicon). The filtrate was designated SEF-RH-F (0.80 mg [dry weight]/ml for strain P-2383 and 0.62 mg/ml for strain P-1062) and utilized directly. The retentate was washed three times as previously described and designated SEF-RH-R (1.4 mg [dry weight]/ml for strain P-2383 and 0.98 mg/ml for strain P-1062).

PMN preparation. PMNs were isolated as previously described (23). Briefly, peripheral blood from healthy adult cattle was collected in acid-citrate-dextrose solution and centrifuged, and the plasma and buffy coat layer were discarded. Erythrocytes in the packed-cell fraction were lysed with distilled water, and the remaining cells, which generally consisted of greater than 90% granulocytes, were washed and suspended in PBS to a concentration of 5.0×10^7 PMNs/ml. The cells were held at room temperature and were used in all three PMN function tests.

PMN function tests. The procedures for evaluating PMN functions have been described in detail before (23). All PMN function tests were conducted in duplicate, and the average of duplicate values was used for calculation.

(i) *Staphylococcus aureus* ingestion. Heat-killed [^{125}I]iododeoxyuridine ([^{125}I]UdR, Amersham Corp., Arlington Heights, Ill.)-labeled *S. aureus* was used to evaluate ingestion by PMNs. The test was conducted in plastic tubes (12 by 75 mm), and the standard reaction mixture contained 0.1 ml of [^{125}I]UdR-labeled *S. aureus*, 0.05 ml of PMNs (2.5×10^6 PMNs, bacteria-to-PMN ratio = 60:1), 0.05 ml of a 1:10 dilution of bovine anti-*S. aureus* serum, and 0.3 ml of Earle balanced salt solution (EBSS, GIBCO, Grand Island, N.Y.). To determine the effect of bacterial fractions on ingestion by PMNs, 0.05 ml of a *P. multocida* fraction (5.0×10^8 cells or equivalent solution) or 0.05 ml of PBS as a control was added to the standard reaction mixture. The reaction was allowed to proceed for 10 min at 37°C, and the extracellular *S. aureus* was removed by lysostaphin (Sigma) treatment. The PMNs were washed by centrifugation, and the amount of PMN-associated radioactivity was determined. The results were expressed as the percentage of [^{125}I]UdR-labeled *S. aureus* that was ingested.

(ii) NBT reduction. This test was conducted in silicon-coated glass tubes (15 by 100 mm), and the standard reaction mixture contained 0.2 ml of nitroblue tetrazolium (NBT) solution (2 mg/ml), 5.0×10^6 PMNs, 0.1 ml of preopsonized zymosan preparation (10 mg/ml), and 0.6 ml of EBSS. To determine the effect of bacterial fractions on NBT reduction by PMNs, 0.1 ml of a *P. multocida* fraction (10^9 cells or equivalent solution) or 0.1 ml of PBS as a control was added to the standard reaction mixture. After 10 min of incubation at 37°C, the reaction was stopped. The purple formazan formed by the reduction of NBT was extracted with pyridine, and the OD at 580 nm was determined. The results are expressed as OD per 5.0×10^6 PMNs per 10 min in 5.0 ml of pyridine.

(iii) Iodination. The iodination test was conducted in polystyrene snap-cap tubes (12 by 75 mm) (model 2058, Falcon, Oxnard, Calif.). The standard reaction mixture contained 2.5×10^6 PMNs, 0.05 μCi of ^{125}I (Carrier Free, Amersham Corp., Arlington Heights, Ill.), 40 nmol of NaI, 0.05 ml of opsonized zymosan (10 mg/ml), and 0.3 ml of EBSS. To determine the effect of bacterial fractions on iodination by PMNs, 0.05 ml of *P. multocida* fraction (5.0×10^8 cells or equivalent solution) or 0.05 ml of PBS as a control was added to the standard reaction mixture. The reaction was allowed to proceed for 20 min at 37°C, and the amount of trichloroacetic acid-precipitable radioactivity was determined. The results are expressed as nanomoles of NaI per 10^7 PMNs per hour.

Data analysis of PMN function test. To determine the effect of bacterial fractions on each PMN function, the value

obtained when a bacterial fraction was added to the PMNs was compared with the value obtained with control (PBS-treated) PMNs. An analysis-of-variance procedure was used to determine significance of the differences in PMN function. For the graphic presentation of the data, all treatment values were expressed as a percentage of the control.

Chemical analysis of bacterial fractions. Total carbohydrate was determined with a phenol-sulfuric acid procedure (11), using glucose as a standard. Hyaluronic acid was determined by the reaction of hexuronic acid with carbazole (Sigma) and H_2SO_4 (10), using D-glucuronic acid (Sigma) as a standard. Protein content was determined colorimetrically from the reaction of protein with Serva blue G dye (Serva fine Chemicals, Inc., Long Island, N.Y.), using bovine serum albumin (Sigma) as a standard (21).

Xanthine-xanthine oxidase-horseradish peroxidase-mediated iodination. A chemical iodination procedure was formulated by following the basic principle of PMN iodination (15, 22). Xanthine (Sigma) was used as a substrate, and xanthine oxidase (Sigma) served as the enzyme for the production of superoxide anion. Horseradish peroxidase (Sigma) was used to catalyze the iodination reaction. The standard mixture contained 40 nmol of NaI, 0.05 μCi of ^{125}I, 0.3 ml of EBSS containing 0.1% bovine serum albumin, 0.5 mg of xanthine, 0.5 U of horseradish peroxidase, and 0.05 ml of SEF or PBS as a control. The reaction was started by the addition of 0.02 U of xanthine oxidase. The mixture was incubated and processed by the same procedures as that used for PMN iodination. A blank containing all components except xanthine oxidase was run with each experiment. Results are expressed in counts per minute.

RESULTS

Effect of *P. multocida* fractions on *S. aureus* ingestion. To determine the effect of *P. multocida* fractions on phagocytic activity, PMNs were added to a standard suspension of opsonized *S. aureus* in the presence or absence of *P. multocida* fractions. Control PMNs ingested 28.6 ± 2.3% (mean ± standard error) (n = 22) of the *S. aureus* in the reaction mixture. In the presence of LCF, KCF, and SEF of the two strains of *P. multocida*, *S. aureus* ingestion was inhibited by 42 to 51% (Fig. 2). DCF had no effect on *S. aureus* ingestion by PMNs. To further characterize the inhibitory factor, SEF was treated with hyaluronidase, autoclaved, or filtered through a 300,000-molecular-weight cutoff filter. Neither autoclaving nor treatment with hyaluronidase destroyed the inhibitory activity of SEF on *S. aureus* ingestion by PMNs (Fig. 3). SEF-R did inhibit *S. aureus* ingestion by PMNs by 41 to 60%, but SEF-F had no effect on the PMN ability to ingest *S. aureus*. In a separate experiment to further characterize SEF-R of strain P-2383, the control PMNs ingested 36.1 ± 1.4% (mean ± standard error) (n = 8) of the *S. aureus* in the reaction mixture. SEF-RH-R inhibited *S. aureus* ingestion by PMNs by 59%, but SEF-RH-F had no effect on the PMNs ability to ingest *S. aureus* (see Fig. 7). *S. aureus* ingestion by PMNs was not inhibited by either hyaluronic acid or hyaluronidase. To determine whether the inhibitory substance was binding to the *S. aureus* or the PMNs, the *S. aureus* ingestion assay was performed by using *S. aureus* and PMNs which had been separately treated with strain P-2383 SEF-RH-R by incubation for 20 min at 37°C in a shaking water bath and then washed three times with PBS. The results (Table 1) indicate that the inhibitory substance was removed by washing and did not bind to either the PMNs or the *S. aureus*.

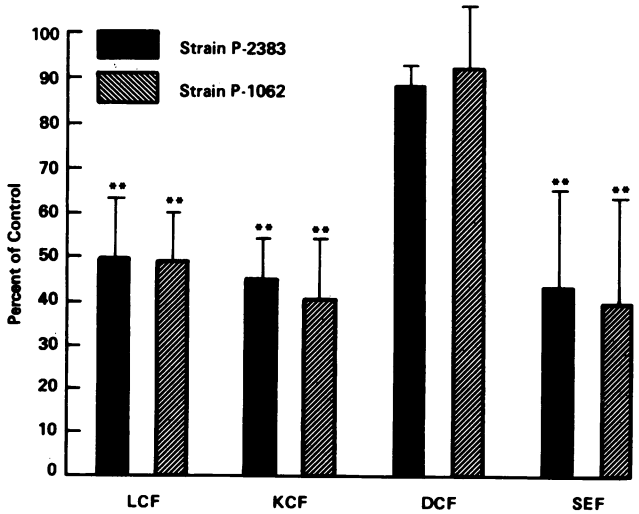

FIG. 2. Effect of type A *P. multocida* fractions on *S. aureus* ingestion by bovine PMNs. Values represent mean percentages (±standard deviation) of the control value. Statistically significant differences from the control value are as indicated: **, $P < 0.01$; n = 16 for the SEF and n = 6 for all other fractions.

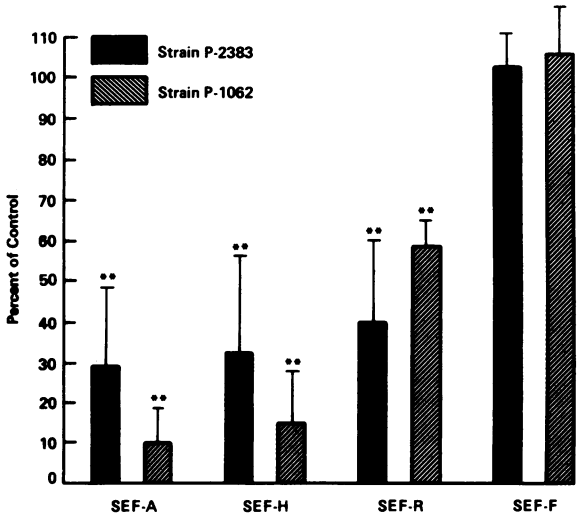

FIG. 3. Characterization of the inhibitory activity in the SEF on *S. aureus* ingestion by bovine PMNs. Values represent mean percentages (±standard deviation) of the control value. Statistically significant differences from the control value are as indicated: **, $P < 0.01$; n = 6 for all fractions.

Effect of *P. multocida* fractions on NBT reduction. To study the effect of *P. multocida* fractions on oxidative metabolism of PMNs, the ability of PMNs to reduce NBT by the production of superoxide anion in the presence or absence of the bacterial fractions was determined. NBT reduction by control PMNs was 0.47 ± 0.01 OD at 580 nm (mean ± standard error) (n = 15). None of the fractions of the two type A strains significantly ($P > 0.05$) affected NBT reduction by PMNs (Fig. 4).

Effect of *P. multocida* fractions on iodination. To study the effect of *P. multocida* fractions on the myeloperoxidase-H_2O_2-halide antibacterial system of the PMNs, the ability of PMNs to iodinate protein in the presence or absence of the

TABLE 1. Effect of preincubation of PMNs and *S. aureus* with strain P-2383 SEF-RH-R on the ingestion of *S. aureus* by PMNs

Pretreatment (followed by washing)	% ingested *S. aureus*[a] in bacterial fraction added to reaction mixture:	
	None	SEF-RH-R
None	29.8 ± 3.3	19.9 ± 1.8
S. aureus preincubated with SEF-RH-R	27.1 ± 2.1	17.6 ± 2.4
PMNs preincubated with SEF-RH-R	31.8 ± 4.4	21.2 ± 4.8
Both *S. aureus* and PMNs preincubated with SEF-RH-R	29.8 ± 3.1	23.4 ± 2.4

[a] Mean ± standard error (n = 6).

bacterial fractions was determined. The value for iodination by control PMNs was 42.3 ± 2.2 nmol of NaI per 10^7 PMNs per h (mean ± standard error) (n = 22). The ability of PMNs to iodinate protein in the presence of LCF, KCF, and SEF of the two strains was inhibited 30 to 36%, but DCF had no effect on iodination by PMNs (Fig. 5). When SEF was processed for further characterization of the inhibitory factor as described for the *S. aureus* ingestion assay, neither autoclaving nor treatment with hyaluronidase destroyed the inhibitory activity of SEF on iodination by PMNs (Fig. 6). SEF-R inhibited iodination by PMNs by 35 to 49%, and SEF-F did not. In a separate experiment to further characterize SEF-R of strain P-2383, the value for iodination by control PMNs was 37.8 ± 1.6 nmol of NaI per 10^7 PMNs per h (mean ± standard error) (n = 8). SEF-RH-R inhibited iodination by 70%, and SEF-RH-F had no effect (Fig. 7). Iodination by PMNs was not inhibited by either hyaluronic acid or hyaluronidase.

Titration of the inhibitory activity of the bacterial fraction. Strain P-2383 SEF was used to evaluate the concentration effect of the bacterial fraction on *S. aureus* ingestion and iodination by PMNs. The inhibitory activities were concentration dependent, but neither was completely inhibited even at the highest concentration of the bacterial fraction (Fig. 8).

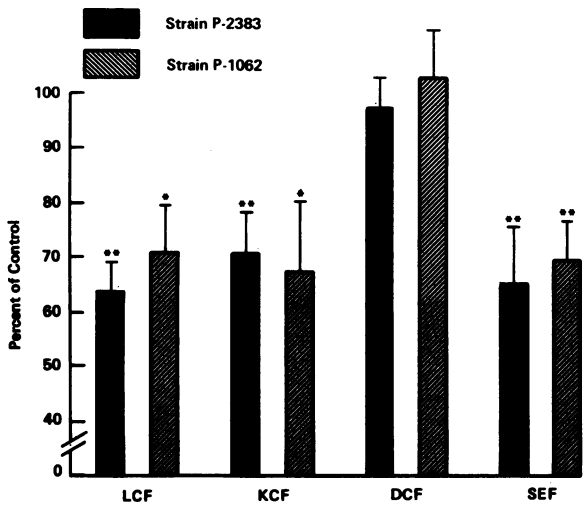

FIG. 5. Effect of type A *P. multocida* fractions on iodination by bovine PMNs stimulated with opsonized zymosan. Values represent mean percentages (±standard deviation) of the control value. Statistically significant differences from the control values are as indicated: **, $P < 0.01$; *, $0.01 < P < 0.05$; n = 16 for SEF and n = 6 for all other fractions.

Chemical analysis. The results of chemical analysis of the bacterial fractions are shown in Table 2.

Effect of the saline-extracted capsule on xanthine-xanthine oxidase-horseradish peroxidase-mediated iodination. To further study the effect of SEF on the iodination reaction, a chemically mediated iodination reaction was used which did not involve PMNs. Xanthine and xanthine oxidase were used to generate superoxide anion, which spontaneously forms H_2O_2. Horseradish peroxidase was used to catalyze the hydrogen peroxide-halide reaction. SEF did not inhibit the xanthine-xanthine oxidase-horseradish peroxidase iodination system (Table 3).

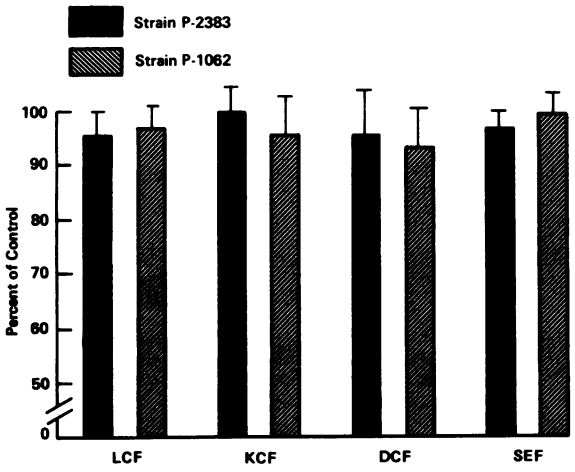

FIG. 4. Effect of type A *P. multocida* fractions on NBT reduction by bovine PMNs stimulated with opsonized zymosan. Values represent mean percentages (±standard deviation) (n = 6) of the control value. None of the differences was statistically significant ($P > 0.05$).

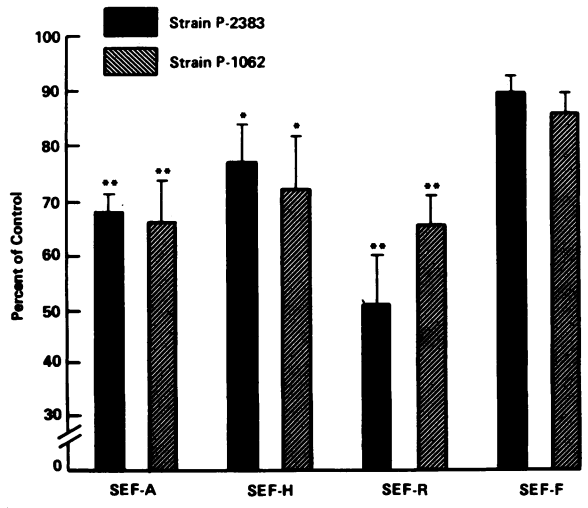

FIG. 6. Characterization of the inhibitory activity of the SEF on iodination by bovine PMNs. Values represent mean percentages (±standard deviation) (n = 6) of the control value. Statistically significant differences from the control value are as indicated: **, $P < 0.01$; *, $0.01 < P < 0.05$.

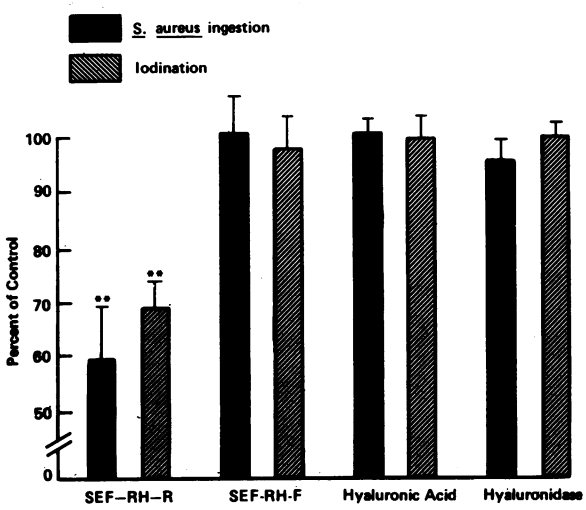

FIG. 7. Characterization of the inhibitory activity of the SEF of strain P-2383 on *S. aureus* ingestion and iodination by bovine PMNs. Values represent mean percentages (±standard deviation) of the control value. Statistically significant differences from the control value are as indicated: **, $P < 0.01$; $n = 8$ for all fractions.

DISCUSSION

PMNs are important in protecting animals from microbial infection by their phagocytic activities. According to Root and Cohen (22), two major microbicidal events occur inside a PMN when a microorganism is ingested: (i) the generation of highly toxic products of oxygen by the oxidative metabolism, and (ii) the enzymatic destruction and digestion of the microorganism by the lysosomal enzymes present in the intracellular granules which fuse with the phagocytic vacuoles. However, many pathogenic microorganisms have developed mehanisms to inhibit phagocytic cell activity to facilitate their survival in the host (9).

The ingestion of bacteria is the first step in the bactericidal activity of the PMN. The results of the *S. aureus* ingestion assay indicate that the capsule of type A *P. multocida*

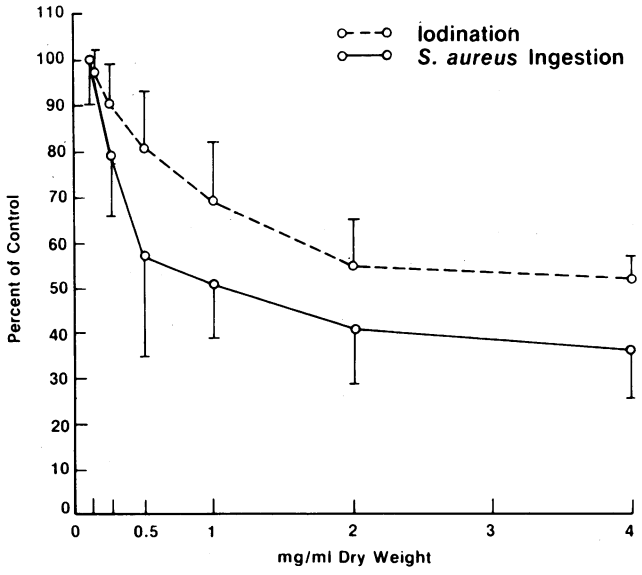

FIG. 8. Effect of various concentrations of strain P-2383 SEF on *S. aureus* ingestion and iodination by PMNs ($n = 6$).

TABLE 2. Chemical analysis of strain P-2383 bacterial fractions

Fraction	Concn (mg [dry weight]/ml)	Carbohydrate (mg/ml)	Hyaluronic acid (mg/ml)	Protein (mg/ml)
SEF	1.06	0.38	0.17	0.07
SEF-R	0.94	0.33	0.17	0.06
SEF-RH-R	1.40	0.35	0.12	0.25
SEF-RH-F	0.80	0.26	0.04	0.22

inhibits the ability of PMNs to ingest particles. When bacterial fractions containing whole organisms were used, the inhibition of ingestion of *S. aureus* may have been due to competition between the two bacterial species. However, when soluble bacterial fractions were used competition for ingestion should not have been a factor. In addition, the results shown in Table 1 indicate that the inhibitory activity was not retained after washing of pretreated PMNs or bacteria. Apparently, this substance has an effect on PMN metabolism which is reversible and is not mediated through binding to a high-affinity receptor. The mechanism of action of this material is not known and is the subject of further investigation. Maheswaran and Thies (18) reported that removal of the capsule from type A *P. multocida* (the same capsular type used in this experimentation) with hyaluronidase enabled PMNs to ingest the decapsulated organisms. Maheswaran and Thies speculated that the inhibitory substance was hyaluronic acid. The results reported here, however, indicate that hyaluronic acid per se is not responsible for the inhibition of PMN function. Hyaluronic acid isolated from human umbilical cord did not suppress PMN function. Thus, the repeating disaccharide (D-glucuronic acid and *N*-acetyl-D-glucosamine) backbone structure of hyaluronic acid, which would be common between the hyaluronic acids of human and bacterial origins, was not responsible for the inhibition of PMN function. It is possible that the PMN inhibitory substance is structurally linked to the bacterial hyaluronic acid. The treatment of the capsular material with hyaluronidase did not destroy the inhibitory factor. The hyaluronidase treatment did destroy the mucoid viscous nature of the SEF. Even after hyaluronidase treatment, there was still some glucuronic acid (a component of hyaluronic acid) which did not pass through the 300,000-molecular-weight cutoff membrane. Since hyaluronic acid of the *P. multocida* capsule serves as a framework for other molecules (3), there may be a variety of chemical components attached to hyaluronic acid which would not be degraded by the hyaluronidase. These molecules may be large enough to retain associated glucuronic acid from passing through the 300,000-molecular-weight cutoff membrane. The hyaluronic acid fragments which did pass through the membrane (SEF-RH-F) did not inhibit PMN function.

TABLE 3. Effect of SEF of type A *P. multocida* on xanthine-xanthine oxidase-horseradish peroxidase-mediated iodination

Bacterial fraction	Iodination value (cpm)[a]
Blank (no xanthine oxidase)	245 ± 18
Control (PBS)	1,466 ± 129
SEF of strain P-2383	1,453 ± 78
SEF of strain P-1062	1,502 ± 89

[a] Mean ± standard error of eight experiments. The differences from the control value are not statistically significant ($P > 0.05$).

Oxidative metabolism of the PMN is an important aspect of its bactericidal activity (22). When a PMN receives the proper stimulus, an oxidase enzyme on the surface of the plasma membrane or phagosomal membrane will catalyze the conversion of oxygen to superoxide anion. Superoxide anion spontaneously dismutates to hydrogen peroxide. NBT is directly reduced by the superoxide anion to an insoluble purple formazan (26). NBT reduction is therefore a measure of superoxide anion generation by the PMN. Since NBT reduction was not inhibited by whole bacteria or bacterial fractions (Fig. 4), type A *P. multocida* apparently does not inhibit the production of superoxide anion by PMNs.

The iodination reaction is a measure of the ability of PMNs to convert inorganic iodide to a trichloroacetic acid-precipitable (protein-bound) form and occurs inside the phagocytic vacuole via the action of hydrogen peroxide and myeloperoxidase. This system has been found to exhibit a marked toxic activity toward bacteria, fungi, and viruses (1, 24). The iodination reaction by PMNs is dependent upon the generation of hydrogen peroxide, degranulation to release myeloperoxidase, the presence of iodine, the unimpaired ability of myeloperoxidase to catalyze the reaction, and the presence of tyrosine to bind iodine. Hydrogen peroxide is formed spontaneously from superoxide anion. Since SEF did not inhibit NBT reduction, superoxide anion generation by PMNs is apparently not affected by the inhibitory factor. Since the xanthine-xanthine oxidase-horseradish peroxidase-mediated iodination was not inhibited by SEF, the rate of formation of hydrogen peroxide from superoxide anion and the rate of hydrogen peroxide destruction were apparently not affected. In addition, the ability of the peroxidase enzyme to catalyze the reaction was apparently not impaired. It must be kept in mind that horseradish peroxidase and myeloperoxidase are different enzymes. It is possible that the inhibitory factor could inhibit myeloperoxidase directly without inhibiting horseradish peroxidase, but this does not seem likely.

This experimentation demonstrated that phagocytosis and protein iodination by PMNs were inhibited in the presence of whole *P. multocida* organisms and bacterial fractions. Since the removal of the capsule removed the inhibitory capability, the inhibitory activity apparently resides in the capsule or surface structure of the bacterial cell. The inhibitory factor is a heat-stable, saline (2.5%, wt/vol)-extractable capsular material of greater than 300,000 molecular weight. The inhibitory activity cannot be attributed to hyaluronic acid, but it may be structurally associated with it.

ACKNOWLEDGMENTS

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