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From the Department of Microbiology and Immunology and the Department of Food Hygiene, Veterinary College of Norway, Oslo.

FACTORS NEUTRALIZING THE BACTERIOCIDAL EFFECT OF LYSOLECITHIN

By

Olav Sandvik and Tore Høyem

A marked growth-inhibiting action of lysolecithin on certain strains of bacteria has been reported (*Sandvik & Høyem* 1969). Great differences occurred with respect to the sensitivity of various organisms to this compound. However, the inhibiting effect was very dependent on the composition of the growth medium. Thus, the presence of blood components reduced the growth-inhibiting ability of lysolecithin dramatically.

The aim of the present work was to obtain further knowledge of the factors and mechanisms which are antagonistic to the growth-inhibiting effect of lysolecithin.

MATERIALS AND METHODS

Strains. Staphylococcus aureus (NVH* 310), an extremely lysolecithin-sensitive organism, was used for the demonstration of growth-inhibiting effect. The unselected strain of this organism will be referred to as "wild type".

In addition, the following organisms were used: Escherichia coli (ATCC** 14948), Serratia marcescens (NVH 2672), Listeria monocytogenes (NVH 2673), and Bacillus cereus (NVH 2665).

Media. Nutrient agar (Difco) and nutrient broth (Difco) were used. Blood agar was made by the addition of defibrinated bovine blood (final concentration 8 %) to melted and cooled nutrient agar at 50° C. Lysolecithin agar was prepared by adding lysolecithin to nutrient agar (final concentration 0.09 mg/ml).

* The Culture Collection of the Veterinary College of Norway, Oslo, Norway.

** American Type Culture Collection, Rockville, Maryland, USA.

Chemicals. Lysolecithin (Sigma* Grade II, from egg lecithin) was used in all experiments. Other bioorganic compounds used are listed in Table 1.

The chemicals used for analyses, and in chromatography, were ordinary pro analysi reagents from various manufacturers.

Viable count. Dilution of cultures and assay of colony-forming cells by agar surface spreading were carried out according to *Clowes & Hayes* (1968). All counts were made from duplicated dilution series and the results recorded as the mean of the two figures.

Inoculation and growth conditions. The test strain of S. aureus was always picked from a fresh blood agar culture by touching a single colony with a loop, followed by transfer to a tube with 5 ml of nutrient broth. After 1½ hrs.' incubation at 37° C, inoculations were made onto the different agar media used. Similar inoculation procedures were also generally used for the other organisms. Initial selection of mutants was made by touching the colonies with a loop.

All cultures were incubated at 37° C, and the plates were read after 24, 48 and 72 hrs.

Sterile filtration. Millipore** filters (HA 0.45 μ) were used. Sterility of the filtrates was checked by inoculation into nutrient broth followed by incubation at 37° C for 48 hrs.

Assay of growth-promoting or -inhibiting effect. One tenth ml (about 10^8 cells) of a 1½ hrs. broth culture of the test organism was spread on the agar surface in Petri dishes and dried. The solutions to be tested were applied in a volume of 0.05 ml into wells cut with a 6 mm cork borer in the different agar plates. Distilled water was used as diluent for the compounds tested.

Thin-layer chromatography. Lysolecithin was demonstrated by thin-layer chromatography on silica gel coated plates (Eastman Chromagram*** 6061). Chloroform-methanol-water (60:50:15) was used as solvent, and the developed plates sprayed with a solution of 40 mg bromothymol blue in 100 ml of 0.01 N sodium hydroxide (*Jatzkewitz & Mehl* 1960) to stain the lysolecithin spots (average R_f value 0.35).

The chromatograms were performed in duplicate, and while the first one was stained as described, the second was used for the microbiological location of lysolecithin as described by *Sandvik & Høyem* (1969).

Gel filtration. Gel filtration of lysolecithin-neutralizing culture filtrates was performed on a Sephadex† G 200 column (42 × 2.5 cm) at an elution rate of 12–15 ml/hr., using 0.05 M acetate buffer (pH 5.6) + 0.1 M-NaCl as eluant. The eluate was collected in 3 ml fractions by means of an LKB†† Fraction Collector. Each fraction was read at 280 nm in a Beckman DU spectrophotometer†††, and tested for lysolecithin-neutralizing activity as described above.

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* Sigma Chemical Company, St. Louis, Missouri, USA.

** Millipore Filter Corporation, Bedford, Massachusetts, USA.

*** Eastman Kodak, Rochester, N.Y., USA.

† Pharmacia Fine Chemicals, Uppsala, Sweden.

†† LKB-Produkter, Stockholm, Sweden.

††† Beckman Instruments, Inc., Fullerton, California, USA.

Table 1. Compounds neutralizing the growth inhibiting effect of lysolecithin in an agar medium.

Solution	Satellite growth of Staphylococcus aureus (NVH 310) wild type, around wells containing the solutions in lysolecithin agar
Hemoglobin (Sigma[1]), ovine, Type III, 9 × crystallized, dialyzed and lyophilized, 1 %	+
Hemoglobin (Sigma), bovine, Type I, 2 × crystallized, dialyzed and lyophilized 1 %	+
Albumin (NBC[2]), bovine, V, 4 × crystallized, 1 %	+
β-globulin (NBC), bovine, fraction III, 1 %	+
Sodium caseinate (Eastman Kodak[3]) P 914, 1 %	+
Bacto-peptone (Difco[4]) B 118, 1 %	+
Proteose pepton (Difco) B 120, 1 %	+
Neopeptone (Difco) B 119, 1 %	—
Bacteriological peptone (Oxoid[5]) L 37, 1 %	—
Soy protein, promine D[6] with 0.1 % titanium dioxide, 1 %	+
2 % solutions of each of 20 amino acids[7] (NBC)	—
Cytochrome C (NBC), 1 %	—
Folic acid, crystalline (NBC), 1 %	—
Flavin adenine dinucleotide (NBC), 1 %	—
Flavin mono nucleotide (NBC), 1 %	—

+ = Satellite growth.

[1] Sigma Chemical Company, St. Louis, Missouri, USA.

[2] Nutritional Biochemical Corporation, Cleveland, Ohio, USA.

[3] Eastman Kodak, Rochester, N.Y., USA.

[4] Difco Laboratories Inc., Detroit, Michigan, USA.

[5] Oxoid Ltd., London, England.

[6] Central Soya, Chicago, Illinois, USA.

[7] Including L alanine, L arginine HCl, L asparagine, L aspartic acid, L cysteine HCl, L cystine, L glutamic acid, glycine, L histidine HCl, L isoleucine, L leucine, L lycine HCl, L methionine, L phenylalanine, L proline, L serine, L threonine, L tryptophane, L tyrosine, L valine.

Hydrolysis of proteins. Proteins were hydrolyzed to amino acids by heating to 120° C in 6 N-HCl (sealed ampoules). The hydrolyzates were diluted 1:2 with distilled water before application onto filter paper for chromatography of the amino acids.

Analytical methods. Amino acids were demonstrated by descending paper chromatography (Whatman paper No. 1), using *n*-butanol-acetic acid-water (2:1:1) as solvent, and the spots visualized by spraying with a solution of 0.2 g ninhydrin in 95 ml *n*-butanol and 5 ml 2 N acetic acid.

Carbohydrate was tested qualitatively by the Molisch reaction, and by ascending paper chromatography (Whatman paper No. 4) in *n*-butanol-acetic acid-water (60:30:30), using 1 % *p*-toluidine in 96 % ethanol as staining reagent (*Høyem* 1963).

The presence of phosphates was tested by ascending paper chromatography (Schleicher & Schüll 2043 b Mgl paper), using a mixture of trichloroacetic acid (5 g), isopropanol (75 ml), 20 % ammonia (0.3 ml) and water (25 ml) as solvent. The spots were stained according to *Hanes & Isherwood* (1949).

Protein was determined by ultraviolet spectrophotometry at 280 nm, by the Folin-Lowry method (*Lowry et al.* 1951) and by the biuret method (*Gornall et al.* 1949).

RESULTS

Assay of lysolecithin-neutralizing compounds

Sterile filtered solutions of a number of organic compounds were applied into wells in lysolecithin agar with Staphylococcus aureus "wild type" as the test organism. A possible neutralization of the lysolecithin effect was indicated by the occurrence, around the well, of a distinct satellite growth of the otherwise inhibited test organism (Fig. 1 a). The compounds tested and the results of the examinations are presented in Table 1. It can be seen that all the neutralizing solutions contained proteins or peptides.

Solutions of hemoglobin, serum albumin, soy protein and sodium caseinate were dialyzed against a great excess of tap water, followed by distilled water. In all cases, the major part of the lysolecithin-neutralizing activity remained within the dialysis bag.

Lysolecithin-resistant mutants in cultures of Staphylococcus aureus

Comparable viable counts were made from 1½ hrs. broth cultures of the lysolecithin sensitive strain S. aureus wild type, on (a) nutrient agar and (b) lysolecithin agar plates. The results of a representative experiment are shown in Table 2.

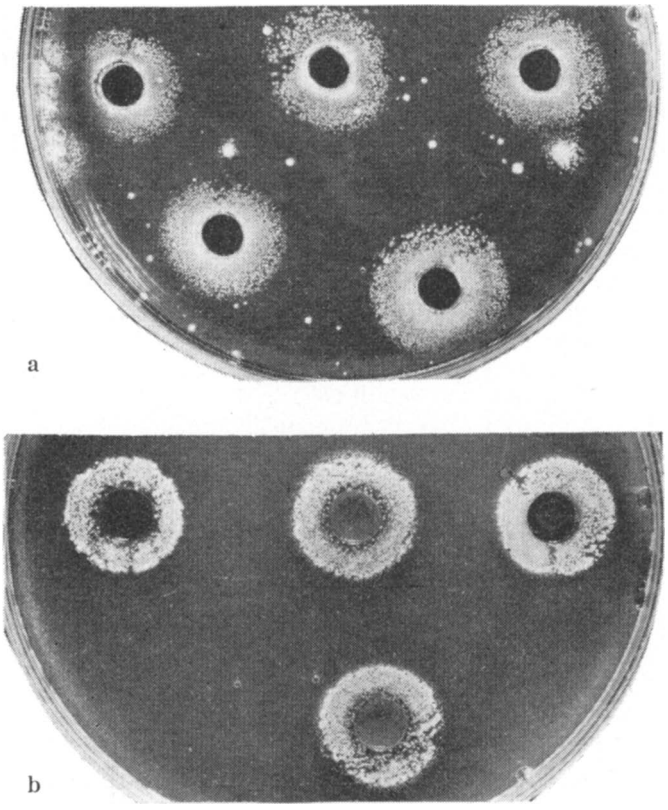

Figure 1. Growth of lysolecithin sensitive colonies of Staphylococcus aureus around wells containg hemoglobin (a) and Escherichia coli culture filtrate (b) in lysolecithin agar.

The relationship between the number of lysolecithin resistant mutant colonies, and the total viable count for the wild type was between 10^{-3} and $10^{-4}\left(\frac{0.0035 \times 10^7}{11.4 \times 10^7}\right)$. The resistant mutants developed full size colonies in 15–48 hrs. By this time, a striking satellite growth occurred, as a great number of secondary colonies grew up within a zone around most mutant colonies (Fig. 2). While rapidly developing primary colonies generally induced an abundant satellite growth, such growth was poor or absent around the slow-growing primary colonies. Corresponding viable counts from broth cultures of two fast growing mutants (M_1 and M_2) on nutrient agar and lysolecithin agar, are shown in Table 2.

Table 2. Viable counts from broth cultures of Staphylococcus aureus (NVH 310) wild type, and lysolecithin resistant mutants (M_1 and M_2), on nutrient agar and lysolecithin agar.

		Viable counts on	
		nutrient agar	lysolecithin agar (primary colonies)
Staphylococcus aureus	wild type	11.4×10^7	0.0035×10^7
„	mutant M_1, first passage	9.1×10^7	5.0×10^7
„	mutant M_2, first passage	9.2×10^7	4.9×10^7
„	mutant M_1, after 10 passages	9.6×10^7	9.2×10^7
„	mutant M_2, after 10 passages	9.1×10^7	13.5×10^7

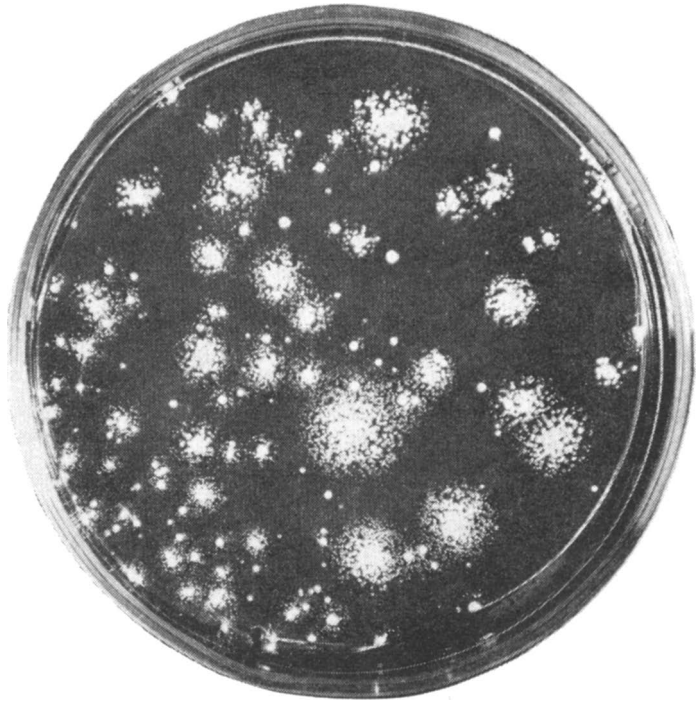

Figure 2. Satellite growth of lysolecithin sensitive colonies around resistant mutants of Staphylococcus aureus on lysolecithin agar. Some resistant colonies have not induced satellite growth.

The number of colonies was of the same order on both media, and the lysolecithin resistance remained stable, also after 10 weekly passages on blood agar before the next counts were made.

Different bacteria showing lysolecithin-neutralizing effect

Lysolecithin resistant strains of Escherichia coli, Serratia marcescens, Listeria monocytogenes and Bacillus cereus were point-inoculated onto lysolecithin agar with S. aureus wild type, spread on the surface. After incubation for 24–28 hrs. satellite colonies of S. aureus occurred around the inocula of all the strains.

Lysolecithin-neutralizing effect of culture filtrates

S. aureus wild type and mutant M_1, E. coli, S. marcescens and L. monocytogenes were grown in nutrient broth, and small samples were taken after 4 hrs. and once a day for up to seven days. The samples were filtered through Millipore filters and the filtrates applied in wells in lysolecithin agar with S. aureus wild type, spread on the agar surface. A distinct satellite growth of this test organism occurred around the wells after incubation (Table 3 and Fig. 1 b). The filtrate could usually be diluted up to 1:8–1:16 without eliminating the satellite growth promoting

Table 3. Lysolecithin neutralizing ability of culture filtrates of some organisms.

Organism	Satellite growth on lysolecithin agar of Staphylococcus aureus (NVH 310) wild type, around wells containing broth culture filtrate of the organisms harvested after incubation for		
	4 hrs.	24 hrs.	up to a week
Staphylococcus aureus (NVH 310) wild type	+	+	+
Staphylococcus aureus mutant M_1	+	+	+
Escherichia coli (ATCC 14948)	—	+	+
Serratia marcescens (NVH 2672)	+	+	+
Listeria monocytogenes (NVH 2673)	—	—	—

activity. It should be emphasized that no lysolecithin-neutralizing effect could be demonstrated with filtrates of L. monocytogenes. On the other hand a distinct satellite growth occcurred when unfiltered broth culture or heat-killed cultures (90° C for 10 min.) of this organism were applied in the wells instead of culture filtrate.

Lysolecithin-neutralizing filtrate of E. coli was made from 0.5 l broth culture after four days' incubation. When the filtrate was dialyzed against running tap water overnight and subsequently against several changes of distilled water, the neutralizing factor remained predominantly within the dialysis bag. The dialyzed material was flash evaporated to $^1/_6$ of its original volume and gel-filtered on a Sephadex G 200 column. The result of a representative experiment is shown in Fig. 3. Two lysolecithin-neutralizing fractions, well separated, were found in front of the ultraviolet absorbing peak, in tubes 11–14 and 20–25, respectively. Tubes 11–14 and 20–25 were combined (LNF 1 and 2), and lyo-

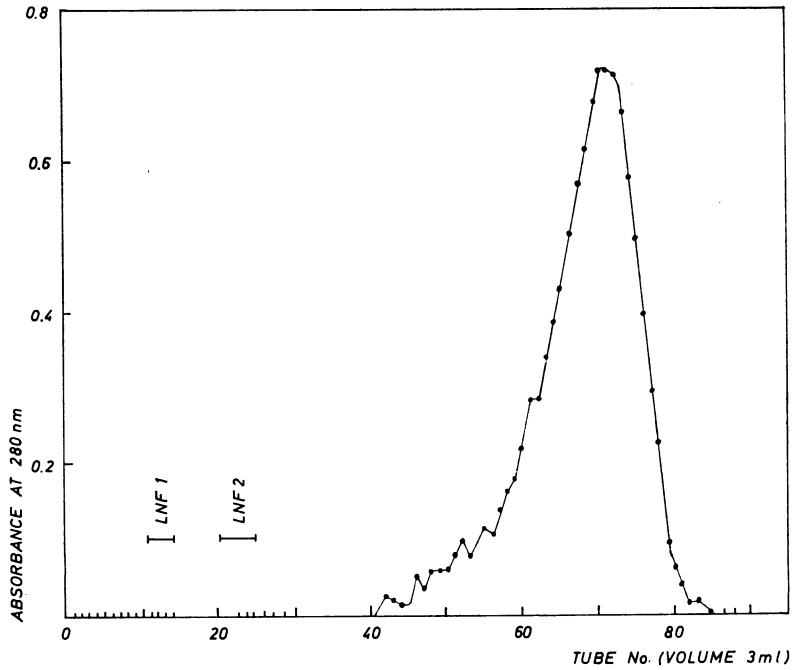

Figure 3. Elution diagram showing two lysolecithin-neutralizing fractions (LNF 1 and 2) in front of the ultraviolet absorbing peak, obtained by gel filtration of Escherichia coli culture filtrate on a Sephadex G 200 column. For experimental conditions, see text.

philized. The lyophilized material was dissolved in 1.0 ml of distilled water and subjected to analysis for carbohydrate, protein, amino acids (before and after hydrolysis in 6 N-HCl), phosphate and ultraviolet absorbing material. Negative results were obtained for all the compounds tested, when using 25–100 µl aliquots of the concentrate, except for chromatography of LNF 2 after acid hydrolysis which revealed five ninhydrin positive spots. Three of these were tentatively identified as alanine, glutamic acid and glycine, while the other two remained unidentified.

Incubation experiments with mixtures of lysolecithin and neutralizing materials

The following experiments were performed:

1. *Lysolecithin-hemoglobin-system.* Further details are given in Table 4. Both the test system (a) containing a mixture of lysolecithin and hemoglobin solution, and the controls (b) and (c) were prepared in triplicate. One was frozen immediately at −20° C, the two remaining were incubated at 37° C for 4 hrs. and 24 hrs., respectively, and then frozen until further analyses were made.

2. *Lysolecithin-culture filtrate-system.* This experiment corresponded to the former except that a dialyzed culture filtrate from a four days' broth culture of E. coli was substituted for the hemoglobin solution (Table 4). The experiments were carried out aseptically, and with inhibitor solutions sterilized by filtration.

All mixtures in the two experiments were tested for growth-inhibiting ability towards S. aureus wild type, on nutrient agar. Table 4 shows that only the lysolecithin control (b) inhibited the test organism. Furthermore, all mixtures were assayed for the presence of lysolecithin by thin layer chromatography. It can be seen from Table 4 that by direct application of the (a)-mixtures on nutrient agar, with S. aureus as test organism, it was impossible to demonstrate bacteriocidal effect despite the content of 500 p.p.m. lysolecithin in the solution. After thin layer chromatography, however, the presence of lysolecithin was demonstrated in these mixtures, both by the staining procedure, and by biological localisation using S. aureus wild type. Similar results were obtained both with hemoglobin (experiment 1) and E. coli culture filtrate (experiment 2) as lysolecithin-neutralizing agents.

Table 4. Assay of lysolecithin in mixtures with hemoglobin or Escherichia coli culture filtrate after different times of incubation.

Mixtures	Growth inhibition when applying the mixtures in wells in nutrient agar spread with Staphylococcus aureus after			Thin layer chromatography					
				lysolecithin demonstrated by biological localization after			lysolecithin demonstrated by staining after		
	no incubation	4 hrs. at 37°C	24 hrs. at 37°C	no incubation	4 hrs. at 37°C	24 hrs. at 37°C	no incubation	4 hrs. at 37°C	24 hrs. at 37°C
1 a 500 p.p.m. lysolecithin in 0.5 % hemoglobin and 0.1 M phosphate buffer, pH 7.0	—	—	—	+	+	+	+	+	+
b 500 p.p.m. lysolecithin in 0.1 M phosphate buffer, pH 7.0	+	+	+	+	+	+	+	+	+
c 0.5 % hemoglobin in in 0.1 M phosphate buffer, pH 7.0	—	—	—	—	—	—	—	—	—
2 a 500 p.p.m lysolecithin in 50 % Eschericia coli culture filtrate and 0.1 M phosphate buffer, pH 7.0	—	—	—	+	+	+	+	+	+
b 500 p.p.m. lysolecithin in 0.1 M phosphate buffer, pH 7.0	+	+	+	+	+	+	+	+	+
c 50 % Escherichia coli culture filtrate in 0.1 M phosphate buffer, pH 7.0	—	—	—	—	—	—	—	—	—

Heat stability of lysolecithin-inhibiting factors

These investigations were performed with different lysolecithin-neutralizing agents at pH 6.5. The results are summarized in Table 5.

The hemoglobin precipitated as soon as the temperature reached 70° C, and the precipitation seemed to correlate with the inactivation of the neutralizing activity.

DISCUSSION

The investigations indicate that a variety of non-dialyzable compounds of polypeptide or protein nature are able to neutralize

Table 5. Heat stability of different lysolecithin neutralizing compounds.

Inhibitor (origin)	Satellite growth of Staphylococcus aureus (NVH 310) wild type, when applying the compound in wells in lysolecithin agar after heating at				
	60°C for 5 min.	70°C for 5 min.	75°C for 5 min.	100°C for 30 min.	115°C for 15 min.
Ovine hemoglobin, 1 %	+	+	—	—	—
Bacto-peptone (Difco) B 118, 1 %	+	+	+	+	+
Soya protein, Promine D, 1 %	+	+	+	+	+
Escherichia coli filtrate, undiluted	+	+	+	+	+
Staphylococcus aureus filtrate, undiluted	+	+	+	+	—
Serratia marcescens filtrate, undiluted	+	+	+	+	—

the bacteriocidal effect of lysolecithin. Thus, all the examined proteins and some hydrolyzed proteins (peptones) showed this activity. No such ability could be demonstrated for the other organic compounds examined.

Of special interest are the lysolecithin-neutralizing effects indicated by the satellite growth of the test organism around the resistant "mother colonies", and the corresponding ability caused by cell-free filtrates of broth cultures of certain organisms. In most cases the neutralizing factors could be demonstrated in broth culture filtrates of the organisms that stimulated satellite growth of the test organism when grown on lysolecithin agar. L. monocytogenes, however, was an exception in that no neutralizing factor could be found in its culture filtrate, although the organism markedly stimulated satellite growth when grown on lysolecithin agar. Heat-killed unfiltered broth culture of this organism also exhibited lysolecithin-neutralizing activity: The neutralizing factor in L. monocytogenes seems to be inactivated or retained by filtration. The fact that filtrable lysolecithin-neutralizing factors of some organisms were demonstrable already after 4 hrs.' incubation (Table 3) indicates that they may be rapidly liberated extracellularly.

The mechanism of the lysolecithin-neutralizing ability of the organisms mentioned may be a matter for discussion. A primary possibility was that extracellular lysolecithin-decomposing enzymes, such as a phospholipase B, are formed in cultures of these organisms. This type of phospholipase is known to exist in cul-

tures of moulds of the genus Penicillium (*Fairbairn* 1948 and *Dawson* 1958), and of Serratia plymuthicum (*Hayaishi & Kornberg* 1954). However, the high degree of heat resistance observed for the neutralizing factors of bacterial origin indicates that they are probably not of high molecular weight. In addition, the incubation experiments with mixtures of lysolecithin and hemoglobin or E. coli culture-filtrate (Table 4) seem to exclude an enzymatic decomposition of the lysolecithin. Thus, even after incubation active lysolecithin could be demonstrated by thin layer chromatography of the mixtures, despite the fact that the unseparated mixtures did not show bacteriocidal effect when tested immediately after mixing.

Gel filtration of culture filtrate of E. coli on Sephadex G 200 yielded two separate fractions (LNF 1 and 2) possessing neutralizing power. Although concentrated to $^1/_{15}$—$^1/_{20}$ of their original volumes, compounds such as carbohydrate, protein, or phosphate could not be detected by the micro methods employed. Amino acids were demonstrated after hydrolysis in fraction 2 (LNF 2), indicating that this fraction, at least, contained protein or peptide material, although in very small amounts. This points to the fact that the neutralizing factors have a rather strong inhibiting power towards lysolecithin. The observations made seem to indicate that the lysolecithin-neutralizing ability of the various compounds studied is mainly due to an "unspecific" effect of polypeptides or proteins. The neutralizing factors in bacterial culture filtrates seem to be metabolites formed during the growth period. However, it should be emphasized that it cannot be excluded that adaptive lysolecithin-splitting enzymes under certain conditions may also be produced by the organisms in question. Filtrates of lysolecithin-containing broth cultures were not assayed for the presence of splitting enzymes.

The observation that hemoglobin, in contrast to the other proteins and peptides tested, lost its neutralizing ability after heating to above 70° C may be due to the rapid clotting of hemoglobin at high temperatures.

It is interesting that a variety of proteins are able to combine with lysolecithin. This observation is, however, well known in other connections. Thus it has been observed that lysolecithin is bound to albumin (*Switzer & Eder* 1965). Microcalorimetric experiments have demonstrated that this binding is exothermal (18.000 cal./mole), and in a 1:1 ratio (*Klopfenstein* 1969). *Kli-*

bansky & de Vries (1963) studied the erythrocyte-lysolecithin interaction, which, in vivo and in vitro, leads to a non-hemolytic erythrocyte sphering. According to these authors, the attachment of lysolecithin to the erythrocyte can be reversed by albumin.

The fact that S. aureus wild type, which is found to be very sensitive to lysolecithin (*Sandvik & Høyem* 1969), produced considerable amounts of a lysolecithin-neutralizing factor was unexpected. The explanation of the phenomenon may be the comparatively high frequency of lysolecithin-resistant mutants occurring in cultures of this organism. Many of these mutants stimulated abundant satellite growth of sensitive organisms on lysolecithin agar. It seems possible that these mutants may cause a considerable production of the neutralizing factor in nutrient broth cultures of S. aureus wild type.

From an ecological point of view it is difficult to consider the significance of the bacteriocidal effect of lysolecithin in relation to the neutralizing factors. However, some kind of neutralizing factor is probably included in most lysolecithin-containing ecosystems. These factors may be partly of microbial, and partly of other biological origin. Although the present investigations have been performed with egg lysolecithin, it is possible that organisms infecting mammals can, paradoxically, be protected against the host lysolecithin through the neutralizing activity of the proteins of the host. On the other hand, the possibility that the effect of the neutralizing factors may be eliminated or reduced in the course of an infection, cannot be excluded.

Altogether, the role of lysolecithin and corresponding inhibitors in microbial ecosystems seems to be very complicated and many problems remain to be elucidated.

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SUMMARY

The investigations indicate that a variety of non-dialyzable proteins and peptides, including hemoglobins, blood serum proteins, casein, soy protein and hydrolyzed proteins (peptones) are able to neutralize the bacteriocidal effect of lysolecithin.

A number of lysolecithin-resistant bacteria are shown to produce lysolecithin-inhibiting metabolites that also promote growth of sensitive organisms in lysolecithin-containing media. On lysolecithin-containing agar this can result in a characteristic satellite growth of sensitive organisms around resistant "mother colonies". Stable resistant mutants were easily selected from a wild type of Staphylococcus aureus after heavy inoculation on lysolecithin-containing nutrient agar.

The bacterial lysolecithin-neutralizing factors examined are not considered to be of enzymatic nature. The factors in culture filtrate of Escherichia coli were separated into two active fractions by gel filtration. Due to extremely small amounts of the substances responsible for the neutralizing activity, chemical analyses of these fractions proved problematic, and only a few amino acids could be demonstrated.

The neutralizing activity of the bacterial factors, and some of the proteins and peptides, resisted 100° C, or more, for several min.

Some aspects of the lysolecithin-inhibitor-interaction are discussed.

SAMMENDRAG

Faktorer som virker nøytraliserende på den bakteriocide effekt av lysolecithin.

Undersøkelsene viser at forskjellige ikke-dialyserbare proteiner og peptider som hemoglobiner, serumproteiner, kasein, soyaprotein og hydrolyserte proteiner (peptoner) er i stand til å nøytralisere den bakteriocide effekt av lysolecithin.

En rekke lysolecithinresistente bakterier ble funnet å kunne produsere lysolecithin-hemmende metabolitter som også induserer vekst av lysolecithin-følsomme mikroorganismer i lysolecithin-holdige medier. På lysolecithin-holdig agar kan dette resultere i en karakteristisk satellittvekst av følsomme organismer omkring resistente "morkolonier". Stabile, resistente mutanter kunne lett selekteres fra en "vill type" av Staphylococcus aureus etter rikelig inokulasjon av stammen på lysolecithin-holdig næringsagar.

De undersøkte lysolecithin-nøytraliserende faktorer i kulturfiltrat av bakteriell opprinnelse synes ikke å være av enzymatisk natur. Ved hjelp av gel-filtrering kunne slike faktorer i kulturfiltrat av Escherichia coli separeres i to aktive fraksjoner. På grunn av at de substanser som var ansvarlig for den nøytraliserende aktivitet bare forekom i meget små vektmengder, ble det vanskelig å foreta kjemiske analyser, og det var bare mulig å påvise et fåtall aminosyrer i den ene av fraksjonene.

Den nøytraliserende aktivitet knyttet til de bakterielle fraksjoner og noen av de undersøkte proteiner og peptider motsto temperaturer på 100° C eller mer i flere minutter.

Forskjellige problemer når det gjelder forholdet mellom lysolecithin og lysolecithin-inhibitorer er diskutert.

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