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CONTRIBUTION TO KNOWLEDGE OF FACTORS PARTICIPATING IN VIRULENCE OF LISTERIA MONOCYTOGENES

II. Study of the Chemical Nature of Complex E_i

M. MÁRA, F. PATOČKA, J. JULÁK, B. POTUŽNÍKOVÁ

Laboratory for Special Medical Microbiology, Faculty of Medicine, Charles University, Prague

In the previous report (9), we described the isolation and biological properties of complex E_i obtained from the surface of washed *Listeria monocytogenes* bodies. A critical discussion of all the heretofore attempts to isolate the individual constituent factors of *L. monocytogenes* virulence was presented, and it was stated that the problem of pathogenicity of this organism has not yet been completely resolved. For this reason it was undertaken to isolate the E_i complex by a modification of the method for preparing G-bacterial endotoxin (12). In the present work, the substance obtained was subjected to chemical analysis and attempts were made to fractionate it and determine the effectiveness of the fractions. In addition, E_i preparations isolated from two different strains described in the previous paper were compared for their chemical composition and effect on experimental animals.

MATERIAL AND METHODS

Strains, media, cultivation and treatment of broth cultures *Listeria monocytogenes* strains Brat. 1 (K_1) and Brat. 2 (K_2) were described in the previous paper (9). The media employed, modes of cultivation and broth culture treatment were also as previously.

Preparation of biologically active complex

The initial phase of the preparation was as in the method for G-bacterial endotoxin isolation developed by Ribi et al. (12) and described in the previous paper (9). Washed bacterial suspension from 1 litre of broth culture obtained by static cultivation or 2 g of half-wet weight from submerged cultivation was suspended in 200 ml of physiological saline, agitated with 400 ml of ethyl ether for 1 min and held at laboratory temperature for 24 h. With this proportion of solvents and the immediate addition of the whole ether

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volume, good separation of both phases was obtained. If a lower dilution of bacterial suspension was used and especially if ether was added gradually, a gel-like interphase formed that was difficult to separate.

The water phase was drawn off in a separator and spun in an MSE or Janetzki K 24 centrifuge at 12 000 rpm for 40 min. The supernatant fluid was either dialyzed against water and freeze-dried (preparation E_{dl}) or acidified with 0.1 N HCl to pH 3.6. At pH 5.0 a flocculent, readily sedimenting precipitate began to form. At pH 3.6 the precipitation was practically quantitative, without denaturation occurring. After 10 min the precipitate was spun on an MSE centrifuge at 12 000 rpm for 20 min and the sediment immediately dissolved in 10—15 ml of 0.15 M phosphate buffer, pH 7.2; the insoluble, denatured portion, if any, was removed by centrifugation (E_i preparations). In the text, individual batches of this preparation are denoted with arabic numerals.

The sediment after ether extraction and centrifugation, containing dead listeria bodies, was immediately suspended in a further volume of physiological saline (10—25 ml), the slimy suspension aerated with a pipette, centrifuged at 12 000 rpm for 20 min, and the supernatant (preparation E_p) used for experiments.

The ether portion was dried with anhydrous Na_2SO_4 and evaporated dry (preparation E_t). It was suspended in physiological saline for administration to the rabbit.

The same procedure was applied to mechanically disrupted bacterial bodies and to the sediment obtained by centrifuging this cell debris at 10 000 rpm (cell walls).

A general scheme of the preparation is presented in Fig. 1.

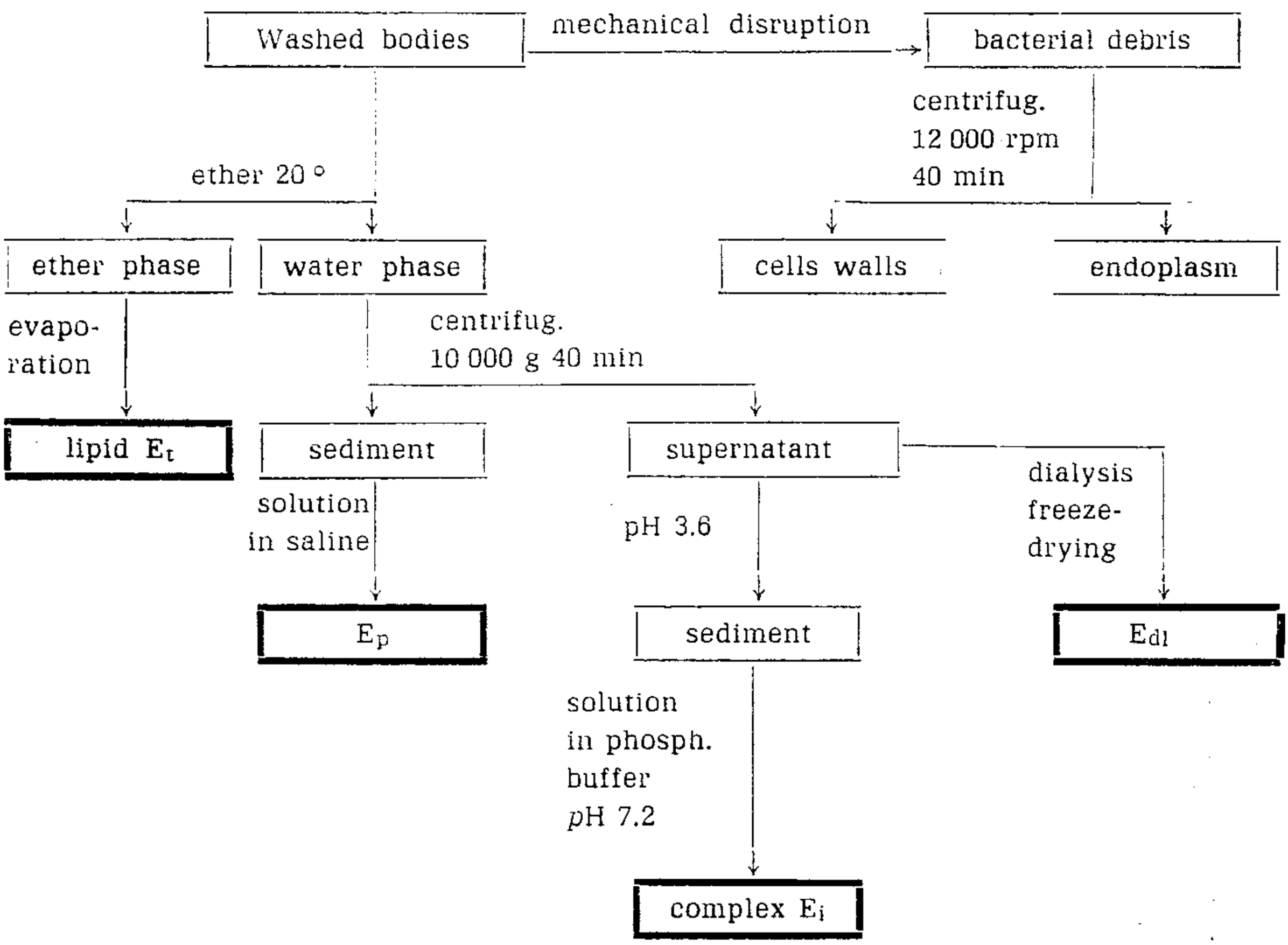

Fig. 1. Scheme of the preparation of components E_i, E_{dl}, E_p and E_t from washed *L. monocytogenes* bodies.

Table 1. Protein-to-polysaccharide ratios in preparations E_i and E_p. Mean values for all batches analyzed are given. Preparations E_iI and E_pI were obtained from strain K_i, E_iII from strain K_2

Preparation	No. batches analyzed	Protein-to-polysaccharide ratio
E_i I	19	83:17
E_i II	3	71:29
Ep I	11	63:37

Chemical analysis

Proteins were determined by the folin-phenol method of Lowry (5).

Polysaccharides were determined by the orcinol method.

Qualitative assays for nucleic acids were performed by spectral measurements in the range of 220—300 nm a Unicam spectrophotometer SP 8000 on the basis of absorption at 260 nm.

Lipids were determined by cold chloroform-methanol (2:1) extraction for 3 days, and after evaporation of solvents and drying over silicagel, by gravimetry (2).

Fatty acids were determined from the hydrolysates of the lipids extracted as outlined. Hydrolysis was performed with a boiling saturated KOH solution in methanol for 8 h. After precipitation into ether, drying the Na_2SO_4 extract and evaporating the ether, fatty acids were esterified with a methanol solution of BF_3. Analyses were performed by gas chromatography on a Chrom 4 apparatus using a stainless column 3700×3 mm packed with 15 % of neopentylglycolsuccinate on Chromosorb W-AW 60/80, column temperature 190 °C, carrier gas nitrogen, gas-flowrate 20 ml/min, or a column 250×3 mm packed with 4 % SE-40 on Chromosorb W-AW 80/100 mesh, column temperature 190 °C, nitrogen flow 10 ml/min. Quantitative results were calculated after direct calibration of the response of the flame-ionizing detector with standard methylester solutions and recalculation for free acids; relative weight percentages were calculated by normalizing peak areas.

NAD^+ase was determined after Kaplan et al. (4).

Serological methods

Haemolysis was determined by the classical method using 1 % of cystein and 1 % of albumin in basic phosphate buffer, pH 7.5, and 5 % sheep erythrocytes.

Haemolysin was also determined by the method for determining streptolysin-O MHD. Half a millilitre of complex E_i was diluted in a 2-step series of phosphate buffer, pH 6.4, containing albumin and 0.14 % Na_2SO_4. After adding 0.5 ml of a 6 % rabbit-erythrocyte suspension the mixture was incubated in a water bath of 37 °C for 1 h and following centrifugation haemolysis was read in the supernatant.

Passive haemagglutination was performed with rabbit erythrocytes nonsensitized or tannin-sensitized after Boyden (1), as described in the previous work (9). Precipitation in agar was performed by the Ouchterlony method (8), similarly as in the previous paper (9).

Gel-filtration

Complex E_i was fractionated on Sephadex G-100 and G-200 (Pharmacia, Uppsala, Sweden). Sephadex swelling was carried out with boiling liquid, fine particles were removed by decantation. Column packing was performed in distilled water. After 24-h

Table 2. Composition of fatty acids of starting *L. monocytogenes* biomass and preparation E_i18 (in relative-weight percentages)

Fatty acids	Biomass, %	E_i18, %
10:0[a]	—	tr
11:0	tr	tr
12:0	0.5	1
iso 13:0[b]	—	tr
ante 13:0[b]	tr	tr
13:0	tr	0.5
iso 14:0	2	2
14:0	4	4
iso 15:0	8	6
ante 15:0	48	31
15:0	1	2
15:1	—	1
iso 16:0	4	3
16:0	3	14
16:1	—	2
iso 17:0	2	1
ante 17:0	27	22
17:0	—	1
18:0	—	7
18:1	—	2
ante 19:0	0.5	0.5

a) Number of carbon atoms: number of double bonds
b) Iso and anteiso isomers of branched acids

rest the column was stabilized with phosphate buffer, pH 7.2. Column height was 50 cm, diameter 2.5 cm, the flow rate 30 m/h. Dead volume was determined by means of the blue-dextran (Pharmacia, Uppsala, Sweden). The sample was applied in a 5-ml volume in 1-M sucrose under the buffer surface, and the presence of proteins in the fractions was determined by spectrophotometry at 280 nm a Unicam SP 500 apparatus. Combined fractions corresponding to the individual peaks were concentrated by precipitation at pH 3.6, centrifugation and solution in phosphate buffer, pH 7.2, in a volume corresponding to the amount of sample originally applied. These concentrated fractions were analyzed and administered to the animals. For serological reactions and biological-activity assays, the fractions were adjusted by diluting with phoshate buffer to an equal content of organic matter.

Thermoresistance

The thermoresistance of preparation E_i was tested by heating concentrated specimens (batches E_i10, E_i11, E_i18, E_i24) in test tubes at 56, 60, 80, and 100 °C for 30 min. After heating at 80 °C and 100 °C, a weak and a massive precipitate, respectively, appeared. Prior to administration to animals the precipitate was removed by centrifugation.

Experiments in animals

The thermoresistance of preparation E_i and the biological activities of the individual fractions from Sephadex were determined by administering 0.1-ml volumes of specimen, concentrated and in dilutions of 1:10 and 1:100, to white mice (20 g) burdened by 10 μg of actinomycin D given simultaneously (10, 13). The death rate of the mice was followed up to the 7th day after the administration; the LD_{50} was calculated according to Read and Muench (11).

The activity of preparation E_i and its fractions was also investigated via i.d. administration of 2-step-diluted samples to rabbit and calculation of the so-called minimal reactive dose (MRD). This is defined as the minimal amount of material, in terms of the quantity of organic matter, capable of eliciting a palpable reaction of 3-mm diameter in the rabbit's skin.

RESULTS

Different batches of preparation E_i were obtained in the form of an opalescent, foaming and readily dissolvable solution. They were analyzed for the protein and polysaccharide content. It was thus possible to determine the ratio of these main E_i-complex components. A comparison of several batches isolated from strains K_1 (E_i I) and K_2 (E_i II) with E_p preparations isolated from strain K_1 is presented in Table 1.

For the exact determination of the chemical composition of the E_i complex, the E_i18 preparation was used, which had been dialyzed against water and freeze-dried. The analyses were performed in a weighed, freeze-dried sample and expressed in percent of organic-matter dry weight. The sample was shown to contain 59 % of protein, 18.2 % of polysaccharide and 8.0 % of lipid.

Measurement of the UV spectrum in the range of 220—300 nm showed a clear-cut peak at 260 nm in preparation E_i23, proving thus the presence of nucleic acids.

The content of lower fatty acids (up to C_{20}) in preparation E_i18 was determined by gas-chromatography. The hydrolysate of lipids from 483.0 mg of this preparation yielded 4.6 mg of fatty acids, i.e. 0.88 %.

Comparison of the content of individual fatty acids in the total lipid isolated from *L. monocytogenes* biomass and in the lipid of preparation E_i18 obtained from this biomass is shown in Table 2. The table gives the relative weight per-

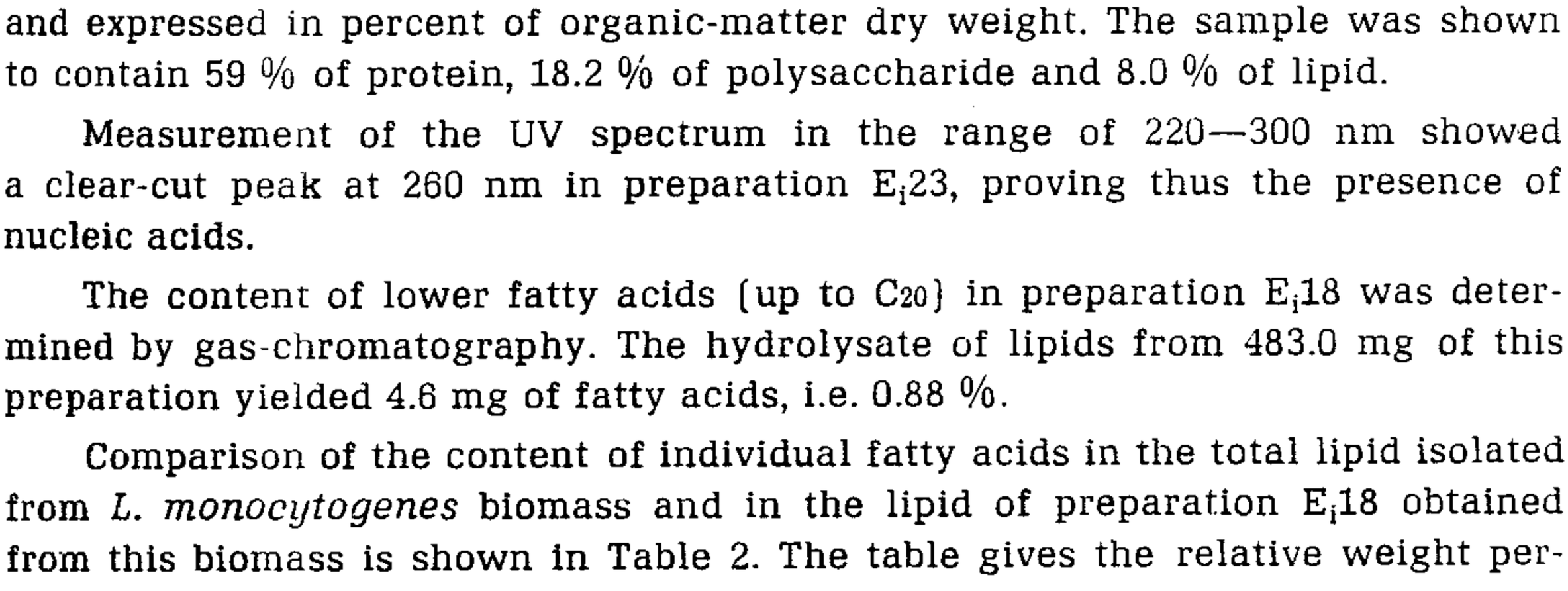

Fig. 2. Gas chromatography of fatty acids from preparation E_i. Feed: 3 μl of methylated acids from 483.0 mg of E_i in 0.5 ml of hexane; stationary phase neopentylglycolsuccinate. See text for assay parameters. Beneath the record proper is a skeleton chromatogram characterizing the column employed.

Table 3. Biological activity of E_i and E_p complexes after i.d. administration to rabbit

Preparation	No. batches adm.	Applied amount of E_i, (mg organic matter)			MRD, (mg organic matter)		
		mean	min.	max.	mean	min.	max.
E_i I	23	1.10	0.40	2.03	0.24	0.02	0.73
E_i II	7	0.86	0.47	1.33	0.20	0.02	0.45
Ep I	12	0.26	0.05	0.54		negative	

Mean values for all batches analyzed are given. E_iI and E_pI were obtained from strain K_1, E_iII from strain K_2.

centages of fatty acids C_{10}—C_{20} as obtained on a polar column packed with neopentylglycolsuccinate. A chromatogram of the methyl esters of the fatty acids from preparation E_i18 upon this column is presented in Fig. 2. The identification of the fatty acids found was verified on a nonpolar column packed with SE-30.

Table 1 gives the protein-to-polysaccharide rations in preparations E_i and E_p isolated from strains K_1 and K_2. The biological activities of a large number of batches of these preparations for the rabbit are demonstrated in Table 3. In order to make possible comparison of the activities of these preparations it was necessary to express the i.d. test on the rabbit in a quantitative manner. Determination of the so-called minimal reactive dose (MRD) as described in the Methods section was considered the most adequate procedure. According to these results, the E_i preparations isolated from both strains do not differ substantially, whereas the E_p preparation was negative in all experiments.

The lipids obtained by cold ether extraction of the microbial bodies (E_t) did not either give a positive reaction after intradermal administration to the rabbit.

Preparations of E_i isolated from *L. monocytogenes* cell walls displayed, in preliminary experiments, equal activity to those which had repeatedly been isolated from washed microbial bodies.

Table 4 demonstrates the thermoresistence of preparation E_i, utilizing the above-mentioned quantitative method of determining biological activity in the

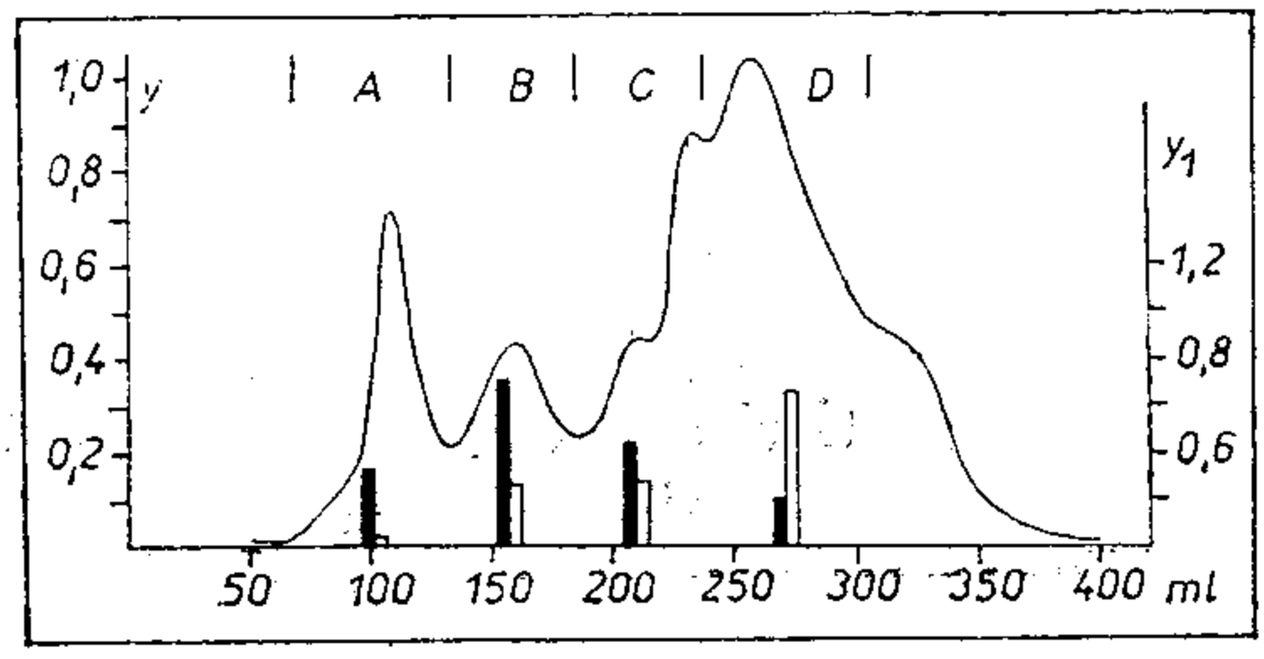

Fig. 3. $E_i$franctionation on Sephadex G-200. The left-hand-side scale (y) gives optical density at 280 nm (curve), the one on the right (y_1) mg of protein (full columns) and mg of polysaccharide (empty columns) per 1 ml of preparation.

rabbit and LD_{50} as established in white mice burdened with a sublethal dose of actinomycin D.

Complex E_i is thermostable up to 80 °C. Heating to 100 °C for 30 min destroyed maximum of the activity.

The nonhomogeneity of preparation E_i was verified by gel filtration on Sephadex G-100 and G-200. Simple fractionation upon Sephadex G-200 yielded the graph shown in Fig. 3. The volumes corresponding to the four main peaks (A, B, C and D) were united, concentrated by means of adjusting pH to 3.6, and then analyzed; their biological activity was determined by the two quantitative tests used and antigenic capacity by means of passive haemagglutination (Table 5).

Table 4. Effect of temperature on the biological activity of complex E_i. The biological activity is expressed in terms of LD_{50} for mice burdened with a sublethal dose of actinomycin D (AD 10 μg/mouse) and the minimal reactive dose (MRD) for the rabbit after i.d. administration. Preparation E_i24 was used

Temp.	Time, min.	LD_{50} for mice under AD	MRD (μg org. matter/rabbit)
		μg org. matter/mouse	
20	—	15	70
56	30	—	70
60	30	86	280
80	30	50	140
100	30	263	560

Many of the complex-E_i batches were tested for the presence of haemolysin by both of the above-mentioned methods. The results were negative. Since NAD^+-ase had been shown to be present in listeriolysin preparations (14), the presence of this enzymic activity was likewise tested for in some batches of freeze-dried and nonfreeze-dried preparation E_i. The results were also negative. This excludes the possibility of an admixture of these virulence factors in the material isolated.

In conclusion, a comparison of two E_i specimens prepared in parallel (from strains K_1 and K_2) is presented. While the quantity of isolated substance, chemical composition and biological activity in the rabbit are in good agreement, the two preparations exhibit differences in antigenic capacity (Table 6).

DISCUSSION

Ribi's original method (12) developed for the isolation of crude endotoxin from G-bacteria was applied to the isolation of a biologically active substance from the surface of *L. monocytogenes.* Repeatedly, an opalescent solution containing substances released by ether into the water phase was obtained.

Table 5. Biological activity of complex-E_i (E_i23) fractions obtained by gel filtration on Sephadex G-200. The biological activity is expressed in terms of LD_{50} for mice burdened with a sublethal dose of actinomycin D (10 μg/mouse) and the minimal reactive dose (MRD) for the rabbit after i.d. administration. Preparation E_i23 contained 1.375 mg of protein and 0.61 mg of polysaccharide per 1 ml. The separation of E_i23 on Sephadex G-200 and the protein and polysaccharide content in the individual fractions is demonstrated in Fig. 2. For passive haemagglutination, the organic-matter content in these fraction samples was adjusted to an equal value (0.36 mg/ml)

Fraction	Protein : polysaccharide	LD_{50} for mice	MRD for rabbits	Passive-haemagglut. titre	
				serum 1	serum 4
A	87:13	118	22	160	2560
B	73:27	145	359	160	1280
C	62:38	neg.	166	—	—
D	23:77	114	51	—	—

It was kept in mind that the water-soluble substances contain a certain amount of endoplasmic material liberated from cells at autolysis, which was verified by a control experiment without using ether. The nucleic-acid admixture detected in specimen E_i23 undoubtedly came from this autolysis-liberated endoplasmic material. However, the preparation obtained in this way only contained a minor amount of this liberated material and was substantially less active. Apart from this, the isolation of active substance from *L. monocytogenes* cell walls in the manner described testifies to the importance of ether in releasing the substance into the water solution.

These considerations will have to be supplemented by an elimination experiment with material obtained from *L. monocytogenes* bodies by mechanical disruption and differential centrifugation of structural cell particles, study of their biological activity and preparation of factor E_i from these cell fractions.

Dialysis against distilled water and freeze drying of the water phase yielded an effective component, E_{dl}, a part of which, however, would not dissolve in phosphate buffer. It was therefore attempted to find a more sparing preparation

Table 6. Comparison of chemical composition, biological activity and antigenic properties of preparations E_i isolated from strains K_1 and K_2. Preparations E_iI, batch 26 and E_iII, batch 25 were used in the experiment

Preparation	Strain	I. d. dose given to rabbit			MRD (μg org. matter)	Passive-haemagglut. titre		Precipitation in agar	
		mg org. matter per 1 ml	protein mg	polysaccharide mg		serum 1	serum 4	serum 1	serum 4
E_i 26	K_1	3.15	2.00	0.52	236	80	2560	+	+
E_i 25	K_2	3.23	2.04	0.54	242	320	1280	—	+

procedure via precipitating the substance in its isoelectric point. On lowering the pH to 5.0, an opalescence began to appear which passed into precipitation and sedimentation of a flocculent substance. The precipitation was optimal and practically quantitative at pH 3.6. By applying immediate centrifugation, it was possible to dissolve the sediment obtained quantitatively in phosphate buffer of pH 7.2 (E_i). Maintenance of the preparation in the cold throughout the purification procedure was practically without denaturation.

Since the pH value employed is very close to that at which some authors (14) had precipitated listeriolysin from the supernatant of a listeria broth culture, tests for haemolytic activity in the E_i batches isolated were performed using both of the methods described. This activity was negative in all the preparations, as was also NAD^+-ase activity, likewise demonstrated by the authors cited (15) as an admixture of listeriolysin. It was thus verified that our preparations did not contain any admixture of biologically active components demonstrated by other authors in the supernatant fluid of listeria broth culture that could influence the biological activity of factor E_i.

Repeated isolations of E_i (altogether 25 batches) were undertaken to investigate whether standard figures would be obtained for the quantity, chemical composition and biological activity of the preparations. As the tables presented show, complex E_i was standardly isolated with approximately equal, only minimally variable chemical composition. Its activity, in view of the possible variability of rabbit reactivity as discussed in the previous work (9), also displayed a high degree of standard in terms of the MRD for the rabbit.

Current check on the batches isolated was performed by assay for their protein and polysaccharide, which with the small specimen volumes analyzed was a more precise procedure than gravimetric determination. The small quantities of isolate did not allow the usual gravimetric determination of lipid following extraction with organic solvents in each batch. Analysis of a freeze-dried specimen confirmed that the complex contained a predominance of proteins and roughly one fifth polysaccharides. The percentage of lipids in this specimen was higher than in whole listeria bodies when cultivated in glucose-containing medium (6, 7). This high percentage could be an indication that lipids are an important constituent of the complex, linked to the proteins, and not merely an admixture captured in the course of its preparation. However, analysis of the fatty acids in freeze-dried specimen E_i18 after its hydrolysis yielded approximately only 1 % of fatty acids in dry matter. Since fatty acids amount to about one half of the total listeria lipids, which was also indicated by our experiments with different listeria strains cultured under varying conditions (6, 7), this difference may be explained by the presence of fatty acids higher than C_{20} in preparation-E_i lipid. Paper chromatography of the hydrolysate of the lipid from some *L. monocytogenes* strains showed spots corresponding to fatty acids with a longer carbon chain, perhaps similar to the mycolic acids of mycobacteria and corynebacteria (6, 7); gas chromatography detected small amounts of C_{20}—C_{26} in whole body extract (3). The fatty acid spectrum of the lipids isolated from E_i does not differ from that in total listeria lipids qualitatively but differ

quantitatively. The whole problem of the nature of lipids in E_i requires further study, whichm is a difficult matter owing to their very small content.

Accordingly, the results of chemical analysis indicate that the crude complex E_i is a glycoprotein with an admixture of lipids.

Apparently, 24-h extraction liberates the active component from the cell-body surface into the water phase quantitatively, since the further precipitation portion (E_p) is biologically inactive both for rabbits and mice, and is also distinct chemically from E_i having a larger percentage of polysaccharides.

An attempt was made to fractionate complex E_i, of whose homogeneity we were not convinced for this if no other reason that an admixture of endoplasmic material had been detected in it. Nonhomogeneity appeared already upon Sephadex G-100. Upon a Sephadex G-200 column the complex separated into four main fractions, which were identified spectrophotometrically by determining protein at 280 nm. This fractionation, apart from demonstrating the complexity of preparation E_i, is also the first step toward purification of the active component. A striking feature of the chemical composition of the individual fractions is that their decreasing molecular weight was matched by a decrease of the protein-to-polysaccharide ratio. As regards biological activity, this is by far the most pronounced in the first fraction A, of the highest molecular weight and relative protein content. Correspondingly, when fixed at the blood-cell surface, this fraction has the greatest ability to bind antibodies against the E_i complex. The weaker reaction of fraction B may be explained by the shortcomings of preparative separation on Sephadex G-200, familiar from the separation of protein mixtures by gel filtration. Fraction C was negative in the experiment on mice, displaying but low activity on rabbits, and was, similarly as fraction D, quite negative as to the ability of binding antibody. Surprising is only the activity of fraction D observed in both of the experimental animals. Nevertheless, the fractionation indicated that the individual fractions differed in their biological activity and hence that it was not a nonspecific reaction of organic matter but the effect of a factor isolated thus far in impure form that was involved. The activity of two nonneighbouring fractions, which excludes preparatory admixture, is evidence either of two molecular forms of the factor or of preparatory aggregation into a single component. The purification will naturally be continued.

On the basis of these results the complex E_i may be claimed to be a glycoprotein or possibly glycolipoprotein having the isoelectric point at pH 3.6, a relatively high molecular weight and biological properties as described previously (9), of which its antigenicity and neutralization in the experiment on mice lend further supports to its being of macromolecular nature.

Factor E_i can be isolated with identical composition, biological activity and in equal amounts from both of the *L. monocytogenes* strains studied, which are mutually distinct in terms of virulence for the mouse, haemolysin production and serotype. The preparations only differ in antigenic properties: E_i isolated from strain K_2 (E_iII) does not precipitate with serum I obtained by immunization with live and killed bodies of strain K_1. This is evidence of a relationship

of complex E_i to the antigenic components of the listeria surface that account for the serotype of the strain. In view of the demonstrated heterogeneity of the complex, however, this need not signify that this precipitating component stands in a direct relation to the biologically active fraction.

The preparation procedure and some biological properties of complex E_i are the same as with the endotoxin of G-bacteria. On the other hand, the chemical composition and, in particular, the lipid composition of the complex do not correspond completely to the endotoxin. So far a conclusive standpoint on this question cannot be taken.

SUMMARY

1. By a modification of the method of preparing G-bacterial endotoxin, a procedure for isolating a biological active complex, E_i, from *L. monocytogenes* was developed.

2. Analysis of this complex showed that it is composed of protein up to 60 %, polysaccharide up to 18 % and lipid up to 8 %. The protein-to-polysaccharide ratio as verified on a large number of batches is approx. 4:1.

3. Using the methods of determining the minimal reactive dose (MRD) after i.d. administration to the rabbit and the LD_{50} for white mice burdened with actinomycin D, the mean activity of individual batches was studied. The mean MRD was 0.2 mg of E_i, the MRD of the most effective batch being 0.02 mg of E_i. The same methods established that the effectiveness of E_i is abolished by heating at 100 °C for 30 min.

4. Preparation E_i yielded 0.9 % of fatty acids C_{10}—C_{20}. The fatty-acid spectrum of the lipids isolated from E_i does not differ from that in total listeria lipids qualitatively but differ quantitatively.

5. No substantial difference was found between the biological activity and chemical composition of E_i preparations isolated from strains K_1 and K_2, mutually distinct in serotype, virulence for the mouse and haemolysin production. In some serological reactions differences were observed that were associated with the different serotypes of the two strains.

6. Complexity of factor E_i was demonstrated by gel filtration. Separation on Sephadex G-200 yielded four main fractions mutually distinct in the protein-to-polysaccharide ratio, biological activity and antigenicity. The highest biological activity and antigenicity were found in fraction A, which had the highest relative content of protein and highest molecular weight. The other fractions were weakly active or inactive.

7. The presence in factor E_i of haemolysin or NAD-ase, previously demonstrated by other authors as partial factors of *L. monocytogenes* virulence, was excluded by experiment.

RÉSUMÉ

Le complex E_i, renferme 60 p.100 de protéines, 18 p.100 de polysaccharides et 8 p.100 de lipides. Le rapport des protéines à des polysaccharides était, chez 25 charges du complexe E_i, en gros 4:1. La dose active minimum de la charge la plus efficace du complexe E_i était 0.02 mg. L'activité biologique du complexe E_i, déterminée par rapport au lapin et à des souries chargées d'actinomycine D, était détruite par chauffage de 30 min. à 100 °C. Du complexe E_i on a isolé 0.9 p.100 des acides gras. Leur spectre diffère quantitativement de celui des lipides totaux de listerias. On n'a trouvé aucune différence dans l'activité biologique ni dans la composition chimique des complexes E_i

isolés de la souche K_1 (le sérotype I) et K_2 (le sérotype V). A l'aide du Sephadex G-200 on a obtenu 4 fractions différentes entre elles par leur activité biologique, leur pouvoir antigénique et leur composition chimique. La fraction A, la plus efficace possède la plus grande quantité des protéines et la plus petite quantité des polysaccharides. Dans les préparations du complexe E_i étudiés, on n'a pas trouvé d'activité hémolytique ni celle de la NAD-ase.

ZUSAMMENFASSUNG

Das Komplex E_i enthält 60 % der Eiweisstoffe, 18 % Polysaccharide und 8 % Lipide. Das Verhältnis der Eiweisstoffe zu Polysachariden war bei 25 Chargen E_i ungefähr 4:1. Die minimale Reaktionsdosis bei der wirksamsten Charge E_i war 0,02 mg. An Kaninchen und Mäusen, die man mit Aktinomycin belastet hat, wurde die biologische Aktivität gemessen. Sie wurde durch Erhitzen auf 100 °C in 30 Minuten zerstört. Aus dem E_i Komplex wurden 0.9 % Fettsäuren isoliert. Das Spektrum ist nur kvantitativ unterschiedlich von dem Spektrum der Gesamtlipide bei Listerien.

Es wurde kein Unterschied ermittelt in der biologischen Aktivität und der chemischen Zusammensetzung des E_i, das aus dem Stamm K_1 (Serotyp I) und K_2 (Serotyp V) isoliert wurde. An Sephadex G-200 erhielten wir vier Fraktionen die verschieden waren in der biologischen Aktivität, Antigenität und in der chemischen Zusammensetzung. Die wirksamste Fraktion A hat am meisten Eiweisstoffe und am wenigsten Polysaccharide. In Präparate E_i wurde keine hemolytische und NAD$^+$ase Aktivität gefunden.

RESUMEN

El complejo E_i contiene 60 % de albúminas, 18 % de polisacáridos y 8 % de lípidos. La proporción de las albúminas a los polisacáridos encontramos en 25 series E_i más o menos 4:1. La dosis reactiva mínima de la más eficiente serie E_i fue 0,02 mg. La actividad biológica del E_i, determinada en el conejo y los ratones con la actinomicina D aplicada, es destruida por el calor de 100 °C después de 30 minutos. Del E_i aislamos 0,9 % de los ácidos grasos. Su espectro no se difievre del espectro en los lípidos conjuntos de L. monocytogenes cualitativamente jero se difiere cuantitativamente. No encontramos la diferencia en la actividad biológica y en la composición química de los E_i, aislados de la cepa K_1 suerotipo I.) y K_2 (suerotipo V.). Del Sephadex G-200 obtuvimos 4 fracciones con las diferencias en la actividad biológica, la antigenicidad y la composición química. La más eficiente fracción A tiene el más alto contenido de las albúminas y el más bajo contenido de los polisacáridos. En los preparados E_i no encontramos la actividad hemolítica y de NAD+.

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M. Mára, Laboratory for Special Medical Microbiology,
Faculty of Medicine,
Studničkova 7, Praha 2, Czechoslovakia

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